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295.	Angiotensin and drinking	Poster	Sun PM
89.	Biological rhythms I	Slide	Fri AM
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45.	Circuitry and pattern generation I	Slide	Thu PM
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159.	Feeding and drinking: cues for need state I	Slide	Sat AM
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92.	Feeding and drinking: neuropharmacology I	Poster	Fri AM
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97.	Human neuropsychology and behavioral neurobiology I	Poster	Fri AM

2 SYMPOSIUM. RECEPTOR BINDING RADIOAUTOGRAPHY: TECHNIQUES, LIMITATIONS AND RECENT DATA. C.A. Boast, CIBA-GEIGY Pharm. (Chairperson); M.J. Kuhar, Johns Hopkins Univ. Sch. Med.; T. C. Rainbow, Univ. Penn. Med. Sch.; E.W. Snowhill*, CIBA-GEIGY Pharm.; J.B. Penney, Univ. Mich.; K.A. Frey, Univ. Mich.

Radioautographic techniques have been used extensively to visualize specific receptor populations in various tissues. Applications of this approach have been increasing for several years. Dr. Kuhar will provide a general overview of the development of these techniques, in vitro and in vivo, and their use in positron emission tomography (PET) scanning, with emphases on the advantages and technical limitations of each. Dr. Rainbow will discuss the use of [^{125}I] labeled ligands to localize subtypes of monoamine receptors. These ligands have significant advantages in that they allow the generation of autoradiograms in a few hours and show no differential absorption by white matter. The use of [^{125}I]pindolol to localize the β_1 and β_2 subtypes of adrenergic receptors will be discussed, as will the use of [^{125}I]lysergic acid diethylamide (LSD) to localize S_2 serotonin and D_2 dopamine receptors. The assessment of benzodiazepine (BZ) binding kinetics in various brain regions will be discussed by Dr. Snowhill. Many compounds currently available have reportedly different affinities for proposed subtypes of BZ receptors. These include CL 218,872, CGS 8216, CGS 9896 and halazepam. Autoradiography offers an ideal method for localizing any regional differences in the K_d or B_{max} of BZ receptor binding, or in the concentrations of these drugs required for inhibition of [^{3}H]BZ ligand binding. Dr. Penney will describe studies of BZ and amino acid receptors in human brains with Huntington's, Parkinson's or Alzheimer's diseases. Significant changes seen in striatal BZ, gamma-aminobutyric acid and glutamate receptors in Huntington's disease, and cortical changes in glutamate receptors observed in Alzheimer's disease, demonstrate the utility of autoradiographic receptor analysis in the pathologic assessment of human disease. Dr. Frey will present muscarinic receptor binding data focusing on two important aspects of in vivo studies, selection of appropriate ligands and experimental models. The tentative selection of ligands is based on in vitro prediction of blood-brain barrier permeability and the molecular mechanism(s) of ligand-receptor interaction. A proposed model will be solved by using static equilibrium and tracer kinetic methods. Data using the latter technique is not autoradiographic, but has direct implications for the design and implementation of PET receptor imaging experiments.

3 SYMPOSIUM. NATURALLY-OCCURRING NEURONAL DEATH IN VERTEBRATES. R. W. Oppenheim, Bowman Gray Medical School (Chairperson); A. Lamb*, Neuromuscular Research Institute, Western Australia; C. Lance-Jones, Univ. of Pittsburgh; K. Herrup, Yale Univ.; E. Johnson, Washington Univ.; B. Finlay, Cornell Univ.; D. D. M. O'Leary, Salk Institute.

After a long period of neglect in the 50's and 60's, the phenomenon of naturally-occurring neuron death in vertebrates has, in the past 10 years, become a topic of general interest to neurobiologists. Although neuron death has been shown to be a normal developmental stage in the life history of most populations of central and peripheral neurons, both the mechanisms regulating this phenomenon and its biological (adaptive) value are still not well understood. Consequently, a major focus of this symposium will be the discussion of experimental models for elucidating these two major issues.

Alan Lamb will present data on motoneurons which challenge the idea that the survival of these cells is dependent on successful competition for limited amounts of some entity supplied by the targets. Instead, he will argue that some form of error correction is associated with cell death in this population.

By contrast, Cynthia Lance-Jones will present data from chick and mice embryos which indicates that the naturally-occurring death of spinal motoneurons is not due to target mismatch (error correction), at least at the level of individual limb muscles.

Karl Herrup will discuss observations and experiments on mouse mutants which provide new perspectives on the mechanisms and biological value of neuron death in the CNS.

Eugene Johnson will address the fundamental issue of the role of neurotrophic factors in the regulation of neuron survival in vivo, using evidence from the study of NGF as a model.

Barbara Finlay will describe studies in the mammalian CNS which indicate that both target size and functional activity are important for cell survival. She will also present data indicating that cell death is involved in the mapping of projections onto their targets.

Finally, Dennis O'Leary will discuss recent data on the relative roles of collateral elimination, cell death and electrical activity in the restriction of early extensive axonal projections in the mammalian CNS.

EPILEPSY I

4.1 THE EPILEPTOGENIC EFFECTS OF PENICILLIN AND STRYCHNINE ARE DIRECTED AGAINST DIFFERENT NEOCORTICAL LAYERS. J.S. Ebersole and A.B. Chatt*. Epilepsy Center, West Haven VA Medical Center and Dept. of Neurology, Yale University School of Medicine, New Haven, Ct. 06510.

We have previously shown that stellate layer 4 of cat striate cortex is most susceptible to the epileptogenic effects of penicillin, and its involvement facilitates evolution of epileptiform response abnormalities in adjacent pyramidal layers. Our hypothesis has been that such differences in sensitivity reflected the degree or type of inhibitory modulation operative within the laminar networks, rather than the presence of an unusual population of neurons with intrinsic pacemaker properties. If this were so, a convulsant with a different mechanism of action might preferentially affect other cortical layers.

Microelectrodes with three recording barrels, which had longitudinal tip separations, were positioned to span the layers of cat striate cortex. Laminar field potentials and extracellular unit activity were evoked by visual field-specific photic stimulation. In a series of randomized trials in each animal, comparable nanoliter volumes of strychnine sulfate (5-20 mM) were microinjected through a twin barrel at cortical depths corresponding to layers 2-3, 4, and 5-6. At the lowest concentration and volume, significant alterations in evoked activity (enhancement of the normal primary potential and development of a longer latency epileptiform potential) occurred only following injection into layers 2-3 and lasted approximately 15 minutes. Similar abnormalities could be induced in layer 4 with higher concentrations and volumes of convulsant, but they developed at a longer delay, were less exaggerated, and shorter lived. Layers 5-6 were least sensitive to strychnine alteration.

Epileptiform response alterations to strychnine, a blocker of glycine inhibition, occur most readily in the superficial pyramidal layers, rather than in the middle stellate layer as they do with penicillin, a blocker of GABA inhibition. These findings support the idea that inhibitory controls vary among the cortical laminae and may be preferentially disrupted by convulsant agents of appropriate antagonistic action. Given the necessary intralaminar circuitry, this disinhibition allows the excitatory interactions of epileptogenesis to develop within the neuronal population of that level.

4.2 ELEVATED EXTRACELLULAR POTASSIUM- AND 4-AMINOPYRIDINE-INDUCED EPILEPTIFORM ACTIVITY IN CA3 HIPPOCAMPAL NEURONS. P.A. Rutecki*, F.J. Lebeda, and D. Johnston (SPON: W. Strittmatter). Dept. of Neurol. & Prog. in Neurosci., Baylor Col. of Med., Houston, TX 77030.

In the hippocampal slice preparation, one class of convulsants (e.g. penicillin, bicuculline, picrotoxin) appears to produce spontaneous epileptiform activity by blocking the inhibitory action of GABA postsynaptically. A number of other agents (e.g. elevated extracellular potassium ($[K]_o$), 4-aminopyridine (4AP), and tetraethylammonium), which are not known to be inhibitors of GABA activity, also produce spontaneous epileptiform activity in vitro. In order to understand the mechanism of action involved in generating elevated $[K]_o$- and 4AP-induced epileptiform events, we have undertaken a study of spontaneous paroxysmal depolarizing shifts (PDSs) using current- and voltage-clamp techniques.

Rat hippocampal slices were prepared in a conventional manner. Intracellular recordings were made from CA3 pyramidal neurons using Cs-filled microelectrodes and a single-electrode current- and voltage-clamp system.

With $[K]_o$ between 6.5-10 mM, intracellular recordings revealed regularly occurring spontaneous PDSs. The frequency of occurrence of spontaneous PDSs was independent of membrane potential. At depolarized potentials (> -30 mV), a hyperpolarization often preceded the depolarization, and frequent spontaneous IPSPs were present. The reversal potential for the excitatory synaptic component was -9.85 ± 2.9 mV (N=10, ± SEM). Using voltage clamp techniques, the excitatory synaptic component reversed at -8.26 ± 6.38 mV and had a conductance of 59.2 ± 9.35 nS (N=7).

5 µm 4AP in 2.5 mM-$[K]_o$ saline produced spontaneous PDSs in CA3 neurons. There was a large excitatory synaptic component that accompanied the interictal discharge. The conductance associated with this excitatory synaptic event was large (> 100 nS) and reversed near 0 mV. There were numerous spontaneous outward currents at potentials greater than -40 mV. These were presumably inhibitory events.

Our experiments demonstrate that the high $[K]_o$- and 4AP-induced PDS results from a large EPSP (Johnston & Brown, Science, 211, 1981). However, unlike the GABA antagonist convulsants, elevated $[K]_o$ and 4AP do not block spontaneous IPSPs. Experiments are being done to quantify the inhibition present with high $[K]_o$ or 4AP. (Supported by the Grass Foundation and NIH grants NS18295, 15772 & 11535, and USAMRDC DAMD17-82-C-2254)

*Indicates nonmember of the Society for Neuroscience

PO indicates abstracts that are published only

4.3 Adenosine Receptor Agonists Elevate Seizure Threshold in Rats. P. Szot* and T.F. Murray. Oregon State University, College of Pharmacy and Hatfield Marine Science Center, Corvallis, Oregon 97331.

Considerable experimental evidence suggests that endogenous adenosine may function as a neuromodulator or cotransmitter substance in the peripheral and central nervous system. Adenosine and adenosine analogs have recently been shown to have anticonvulsant effects in mice. The purpose of the present investigation was to evaluate the effects of adenosine receptor agonists and antagonists on pentylenetetrazol (PTZ) seizure threshold in rats. The effects of adenosine analogs on seizure threshold were determined by measuring the dose of PTZ, infused through a tail vein, required to elicit a seizure. PTZ (5 mg/ml) was infused at a rate of 1.5 ml/min via a 25 gauge butterfly needle inserted into a lateral tail vein. The time to the first myoclonic twitch was recorded and the dose of PTZ required to elicit the seizure was calculated from the recorded time, concentration of PTZ and weight of the animal. The PTZ dose required to elicit a convulsion in saline pretreated rats was 19.8±0.4 mg/kg (mean±S.E.M.; n=81). The adenosine receptor agonist 2-chloroadenosine (2-CLA), raised the PTZ seizure threshold from 19.8 to 26.7 mg/kg when administered intravenously in a dose of 1.0 mg/kg. The peak effect of 2-CLA on seizure threshold occurred approximately 1 min after IV injection. A similar elevation of PTZ seizure threshold (30-35%) was elicited by L-N^6-phenylisopropyladenosine (L-PIA) and its D-diastereoisomer, D-PIA. The rank order of adenosine agonist potency in elevating PTZ seizure threshold was L-PIA>2-CLA>D-PIA with L-PIA being approximately 50 times more potent than D-PIA. In contrast to these effects of adenosine receptor agonists, theophylline (THEO) in doses of 15 to 75 mg/kg i.p. elicited a reduction in seizure threshold. The effects of THEO on lowering PTZ seizure threshold plateaued at 30 mg/kg, a dose which reduced the threshold dose by 25%. To determine whether the observed effects of 2-CLA on PTZ seizure threshold were mediated via an activation of adenosine receptors, we administered THEO (5 mg/kg, IV) 15 min before the IV injection of 2-CLA. This dose of THEO elicited a significant antagonism of the 2-CLA-induced elevation of seizure threshold. These results provide support for an involvement of adenosine receptors in the observed modulation of seizure threshold by adenosine analogs. (This investigation was supported by a research grant from the Epilepsy Foundation of America.)

4.4 KINDLING OF GENERALIZED CONVULSIONS BY INTRACEREBRALLY ADMINISTERED OPIOID PEPTIDES: REPLICATION AND MAPPING OF RESPONSIVE SITES IN THE RAT. D.P. CAIN and M.E. CORCORAN. Depts. of Psychology, U. Western Ontario, London, CANADA and U. Victoria, Victoria, B.C., CANADA.

Recently we reported the first evidence of the kindling of generalized convulsions by repeated small doses of B-endorphin (B-END) and met-enkephalin (M-ENK) given intracerebrally. Here we report the results of additional work designed to map the limbic forebrain extent of the response in the male hooded rat. B-END, M-ENK or morphine (MOR) were administered through a chronically indwelling chemitrode in a volume of 1 ul or less at a rate of .029 ul/sec using an infusion pump. All administrations were spaced at 48 hrs, and EEG was taken.

The results from sites in the amygdala, hippocampus and dentate gyrus indicate that B-END (10 ug) was the most potent opioid. It kindled generalized convulsions from a total of 13 sites, evoked epileptiform spiking without convulsions from 5 sites and was without effect in 6 sites. M=ENK (10 ug) similarly kindled convulsions from 10 sites, evoked only spiking from 14 sites and was without effect in 19 sites. In contrast MOR (10 ug) was completely without effect in 11 sites, and evoked weak spiking from 5 sites. The opposite pattern of response was obtained from sites in the anterior amygdaloid area. Here, MOR evoked strong spiking from 6 of 12 sites, whereas B-END and M-ENK were completely without effect in 19 sites. Most animals that were kindled by B-END or M-ENK did so after 2-4 administrations. After 2-4 administrtaions of MOR the spiking disappeared, suggesting tolerance. None of the drugs was effective in any thalamic (n=8) or striatal (n=7) site tested. Repeated injections of saline (1 ul) failed to cause any epileptiform effects in control rats. Naloxone (10 mg/kg) blocked the spiking and convulsions, suggesting that the drug effects were mediated by opioid receptors. Experiments in which the opioid-kindled rats were electrically rekindled demonstrate a highly significant savings (transfer) in rekindling rate.

These results confirm and extend our earlier observations, and provide evidence for a site-specificity within the rat brain for the epileptiform and kindling effects of the drugs. They also provide evidence for an opiate receptor specificity for the effects. The fact that only B-END and M-ENK were effective in kindling convulsions suggests that the delta and epsilon receptors may be of importance in the kindling response to these drugs, and that the mu receptor is not. The results suggest that opioid mechanisms might play a role in convulsive seizures.Supp. by NSERC (DPC) & MRC, NSERC (MEC).

4.5 DEVELOPMENT OF TOLERANCE TO THE PRO- AND ANTICONVULSANT EFFECTS OF MORPHINE. F. Foote* and K. Gale, Georgetown Univ. School of Medicine, Washington, D.C. 20007.

We recently reported that morphine (10-50 mg/kg i.p.) attenuated seizures induced by maximal electroshock (MES) and potentiated seizures induced by bicuculline (BCC) (Europ. J. Pharmacol. 95: 259-64). These effects were antagonized by naloxone. We now report that both effects of morphine exhibit tolerance following several days of daily morphine administration. Male Sprague-Dawley rats received injections of morphine (10-100 mg/kg s.c.) twice daily for a period of 13 days. Controls that received chronic saline injections were tested concurrently. Following chronic morphine treatment, animals showed significantly less potentiation by morphine of BCC seizures, and significantly less attenuation by morphine of MES seizures, when compared to chronic saline controls that received morphine for the first time. In controls the ED50 for potentiation of BCC seizures was 25 mg/kg of morphine i.p., whereas in tolerant animals, no significant effect was obtained with 30 mg/kg of morphine. The ED50 for blockade of tonic hindlimb extension (THE) component of MES seizures was 18 mg/kg of morphine i.p.; 50 mg/kg of morphine i.p. conferred complete protection from THE component of MES in nearly all controls tested. In morphine-tolerant animals, only 33% of animals showed blockade of THE even at doses of 75 or 100 mg/kg of morphine. In addition, we examined seizure responses in rats that had been withdrawn from morphine for 24-48 h. Rats in morphine withdrawal showed decreased severity of BCC seizures and an increase in THE duration in response to MES.

Thus, it appears that the systems mediating both the pro- and anticonvulsant effects of morphine can adapt to repeated exposure to morphine. This adaptation not only results in a decreased effectiveness of (i.e. tolerance to) morphine, but also causes an alteration in sensitivity to the seizure-inducing stimuli in the absence of morphine. This suggests that opiate-receptor-mediated transmission may participate in regulating seizure susceptibility; the direction of the opiate influence depends on the specific seizure type.

4.6 CHOLESTERYL ESTER HYPOTHESIS IN SEIZURE. Ingming Jeng* (SPON: D. Cash). Neurochemistry Research Unit, Missouri Institute of Psychiatry, Biochem. Dept., University of Missouri-Columbia, School of Medicine, 5400 Arsenal St., St. Louis, MO 63139.

Cholesterol represents the most rigid component in the biological membrane. Cholesteryl ester, the storage form of cholesterol, exists as droplets and does not associate with membrane. The interconversion between cholesterol and cholesteryl ester is expected to regulate the fluidity of membrane and will affect the functions of membrane. We have recently completed our study on the characterization of neuronal acyl-CoA cholesterol acyl-transferase (ACAT). This enzyme was solely responsible for esterifying cholesterol in the nervous system. Interestingly, U18666A, a hypocholesterolemic drug which induces experimental epilepsy, was found to be a potent inhibitor of ACAT activity in glial homogenates and of cellular cholesteryl ester formation in glioblastoma cells. The inhibition of cholesterol esterification by U18666A was cell dependent, partial, and reversible. Furthermore, the potency of U18666A inhibition was dependent on the presence of mevalonate or lipoprotein in the medium. The inhibition of cholesterol esterification in neuronal cells by an epileptigenic compound led us to propose that a deficiency in cholesterol esterification may cause seizure. Consistent with this hypothesis, lidocaine, a local anesthetic known to induce seizure, had a direct effect on ACAT activity. Lidocaine specifically inhibited cholesterol esterification since it failed to change the phosphatidyl choline formation. Interestingly, the inhibitory effect of lidocaine on cellular cholesterol esterification declined with time. Cells were capable of developing a compensatory mechanism to overcome the inhibition of this critical process by lidocaine. U18666A and lidocaine interfered with cholesterol esterification in different manners. Despite the apparent differences, they lead to similar symptoms. In summary, we have established a possible link between cholesteryl ester metabolism and seizure. Further experimentation is being conducted to substantiate the hypothesis that high cholesterol content in membrane is responsible for seizure.

4.7 LOCUS COERULEUS STIMULATION RETARDS THE DEVELOPMENT OF AMYGDALA KINDLING IN RATS. Carlos A. Jimenez-Rivera*, Anna Maria Voltura*, Gerald K. Weiss* (SPON: G. Ballam). Dept. of Physiology, University of New Mexico, School of Medicine, Albuquerque, New Mexico 87131

Kindling refers to the enhancement of convulsive excitability with repeated electric subconvulsive stimulus culminating in the production of motor seizures. Depletion of catecholamines, especially norepinephrine have been shown to markedly accelerate the development of amygdala kindling in rats. The majority of the cell bodies of noradrenergic neurons are situated in the locus coeruleus (L.C.). The L.C. projections are widely distributed into the cerebellum, spinal cord, and forebrain structures. However, the physiological significance of the L.C. activity in the development of kindled seizure has not been studied. We designed an experiment to electrically stimulate the L.C. during the development of amygdala kindled seizures. Bipolar electrodes were implanted in the right amygdala and in the right and left L.C. of male Sprague-Dawley rats. The rats were allowed to have one week of recovery post-surgery after which the kindling procedure was started. The protocol consisted of amygdaloid stimulation three times per day at 90 min. intervals (400 u Amp, 1 msec, 60HZ) until 3 consecutive stage 5 were achieved. Prior to each amygdala stimulation the L.C. was stimulated for 20 minutes (180-500uA, 0.2 msec, 10HZ). Control animals had L.C. electrodes but were not stimulated.

The results showed that L.C. stimulation prior to amygdala kindling retards the normal sequence of seizure development as compared to controls. This was due to a prolongation of the time spent in the early stages, especially before entering Stage 1. The after-discharge (AD) recorded from the stimulated amygdala during the prolonged early stages maintained durations characteristic of that stage and did not lengthen until the appearance of the next stage. In some animals, recordings from the L.C. showed discharges coincident with the AD produced in the amygdala. These data are consistent with the idea that enhanced activity of the noradrenergic system inhibits the early stages of seizure development and may be most important in preventing the first motor manifestations in seizure development.
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4.8 TRANSECTION OF THE CORPUS CALLOSUM FAILS TO PREVENT THE SPREAD OF SEIZURE ACTIVITY INDUCED BY AMYGDALOID STIMULATION IN RABBITS. J.A. Kusske, P.C. Rinaldi*, and P. Callahan*. Div. of Neurosurgery, Dept. of Surgery, UCI School of Medicine, Irvine, CA. 92717

Evidence from studies involving patients with generalized seizures suggests that transection of the corpus callosum (CC) abolishes or markedly diminishes these seizures where other means of control have failed. This work is being conducted as part of an ongoing series of studies investigating the role of the commissural pathways in development and spread of seizure activity. The patterns and spread of seizures were investigated in rabbits with an afterdischarge induced by electrical stimulation of the amygdala.

In the initial phase of the experiment under Rompun and Ketamine anesthesia, rabbits were placed in a stereotaxic frame, the skull marked for future electrode placement, and the bone removed to allow for transection of the CC. The dura above the exposed cortex was removed. In one group of rabbits (n=5) the CC was transected by suction. In a second group (n=7) the CC was left intact. Following these procedures, the bone flap was replaced, the overlying skin sutured, antibiotics administered, and the rabbit allowed to recover for two to three weeks.

In the second phase, following surgical procedures carried out under halothane anesthesia, rabbits were immobilized with gallamine triethiodide and ventilated to maintain normal PO_2 and PCO_2. Stereotaxically placed tungsten microelectrodes were implanted to record waves and extracellular unit activity from the dorsal medial thalamic nucleus, hippocampus, and cortex. The stimulation electrode was located in the amygdala contralateral to these structures. Each rabbit received a set of three stimuli, 3,6, and 9 sec. in duration, with 20 minutes between each stimulus. Data was collected during the 5 minutes preceding and following each stimulus and stored on FM tape for later analysis. Electrode placements and transection of CC were confirmed histologically.

Experiments completed to date suggest that transection of the CC fails to reduce the probability of occurrence or severity of the seizure activity following electrical stimulation of the amygdala. There is, in fact, indication of increased incidence of seizures characterized by full tonic/clonic development as assessed in the waves and spikes of the various structures. Further investigations of this and other epilepsy models are warranted to characterize the role of the commissural systems in intractable seizures.

4.9 POSTICTAL CEREBRAL METABOLISM-BLOODFLOW MISMATCHES OBSERVED IN HIPPOCAMPUS AND SUBSTANTIA NIGRA OF AMYGDALA-KINDLED RATS. Robert F. Ackermann, Harry T. Chugani, Sally Caldecott-Hazard and Jerome Engel, Jr. UCLA School of Medicine, Los Angeles, CA 90024.

Previously we reported apparent mismatches between metabolic and bloodflow rates in hippocampi of rats undergoing either amygdala-kindled or bicuculline induced seizures (Ackermann et al., J. Cereb. Blood Flow Metab., 3:supp 1, S238-S239 1983; Yan et al., Soc. Neurosci. Abstr., 9:1108, 1983). In the present experiments, we infused rats with either ^{14}C-iodoantipyrine (IAP) or ^{14}C-2-deoxyglucose for quantitatively measuring local cerebral bloodflow (BF) (Sakurada et al., 1978) or local cerebral metabolism (MET) (Sokoloff et al., 1977), respectively. All animals were fully amygdala-kindled (stage 5) beforehand, but no seizures were induced for at least 2 weeks before the tracer experiments. The tracer infusions then occurred under two seizure-related conditions: (1) postictal, in which tracer was infused 0-180 sec after termination of 1 additional kindled seizure; and (2) interictal, in which tracer was infused with no additional seizure. Comparisons between these two groups, and between each group and untreated controls, revealed two striking results: (1) an apparently profound postictal mismatch between MET and BF in the hippocampus (HIPP); and (2) an equally profound postictal mismatch between MET and BF in the substantia nigra (SN). In our previous studies, ictus rarely occupied the entire 40-45 sec IAP infusion duration. The present results suggest that the then-observed relative HIPP BF deficits were late-ictal to postictal in origin. By contrast, the presently observed SN mismatches were not evident in the previous ictal studies. Recently, the SN has been implicated by several groups in the mediation of seizure severity and duration. The presently reported MET-BF mismatches could signify posticitally-reduced SN function, which may in part account for the postictal seizure refractoriness characteristic of rat adults (Moshé et al., Dev. Brain Res., 7:81-85, 1983).

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4.10 PREFERENTIAL BLOOD FLOW TO BRAINSTEM DURING GENERALIZED SEIZURES IN THE NEWBORN MONKEY. D.G. Fujikawa*, B.E. Dwyer* and C.G. Wasterlain* (SPON: R. Nishimura). Epilepsy Res. Lab., V.A. Med. Ctr., Sepulveda, CA 91343 and Dept. of Neurology and Brain Res. Inst., UCLA Sch. of Med., Los Angeles, CA 90024.

Prolonged generalized seizures may be associated with irreversible neuronal injury in certain brain regions, and recent findings suggest that this may be at least partly related to changes in local cerebral blood flow (l-CBF). We studied the effect of generalized seizures on l-CBF by autoradiography in 24 marmoset monkeys 7 days to 8 weeks of age, using ^{123}I- or ^{131}I-isopropyliodoamphetamine or ^{14}C-iodoantipyrine as l-CBF indicators. Generalized convulsions were induced in ketamine-anesthetized and awake monkeys with I.M. bicuculline and continued for 4 to 71 minutes. During convulsions in marmosets 7 to 18 days of age (n=10), there was a striking rearrangement of l-CBF in favor of the pontomedullary region. Seizure animals 4 to 8 weeks of age (n=5) did not show this redistribution of l-CBF and did not differ significantly from controls (n=9). In the younger seizure animals, the ratios of blood flow in lower brainstem to blood flow in frontal cortex, putamen, dorsomedial thalamic nucleus, frontal white matter and cerebellum were 1.6 to 2.2 times those of the older seizure animals and controls ($p < 0.01$). Neither the duration of seizures nor ketamine affected the results. In three 14 to 16 day old marmosets with continuous seizures for 30 minutes in room air, PaO_2 was 83.5 ± 19.2 mm Hg (mean ± S.D.), $PaCO_2$ was 32.7 ± 13.7 mm Hg, HCO_3^- was 8.4 ± 1.7 mM, and pH was 7.04 ± 0.21; arterial blood pressure increased 1½ to 2 times. These results indicate that at least in some animals, factors other than hypoxemia, hypercapnia and hypotension must be sought to explain the altered blood flow pattern. The reason that only newborn animals show preferential blood flow to brainstem during seizures is unknown. It could be related to age-dependent differences in the vascular reactivity and/or vascular density of various brain regions, differences which could diminish as less mature areas develop. In any event, the redistribution of l-CBF during seizures in the neonatal monkey may enhance survival by increasing the delivery of oxygen and glucose to vital brainstem structures. However, this may be associated with a relative underperfusion of other brain regions that are functionally active during the seizures, which in turn could contribute to the production of neuronal damage. (Supported by NINCDS Grant NS-13515 and the Research Service of the Veterans Administration.)

4.11 POSITRON EMISSION TOMOGRAPHY IN EPILEPTIC PSYCHOSIS. S.Ohno*, T.Miyauchi*, H.Kishimoto, Y.Nomura*, S. Yokoi* and M.Iio*. Department of Psychiatry, Yokohama City University, Yokohama 232 and Nakano National Hospital, Nakano, Tokyo 165, JAPAN.

The development of positron emission tomography(PET) for the measurement of local cerebral glucose metabolism enables three-dimensional imaging and quantification of metabolic rates. Kuhl etal. reported interictal hypometabolism in regions of epileptic foci detected by EEG, using 18F-fluoro-deoxyglucose to measure local cerebral metabolism. In this study, local cerebral metabolic activities of the psychotic epileptic patients were analysed by PET using photosynthesysed 11C-glucose.

Five cases which showed paranoid-hallucinatory state were selected. Hysteria with seizures and schizophrenia-like state which showed flattening of affect were excluded. Two cases were generalized tonic-clonic seizure and two cases were complex partial seizure. The other one was combined generalized tonic-clonic seizure with complex partial seizure. The patients were laid down on the CT bed in a darkened room with eyes closed and given 20mCi 11c-glucose by per os. After the 11C-glucose administration, two to six horizontal brain scans parallel to the orbital-meatal line were done.

In two cases which showed generalized tonic-clonic seizure, there was no difference of local cerebral metabolic activity between in the right cerebral area and in the left. The other three cases had significantly lower metabolic activity in the temporal or front-temporal cortex ipsilateral to the site of the focus of the epilepsy as determined by surface EEG recordings. These five cases showed no reduction in cerebral metabolic activity in frontal area.

These results showed that focal epileptic patients with paranoid-hallucinatory state found a decrease of the regional cerebral metabolism in an epileptic focus and that epileptic patients with paranoid-hallucinatory stats did not find a reduction in metabolic activity of the frontal cortex which was seen in schizophrenic patients. Supported by Grant 83-10-11(NCNMMD) of the Ministry of Health and Wwlfare, Japan.

4.12 D-TUBOCURARINE CAUSES BRAIN DAMAGE AND SEIZURES. R. Dasheiff and M. Hirvonen.* Epilepsy Center, VAH and Univ. Wisconsin, Madison, WI 53705.

D-Tubocurarine (d-TC) is a drug commonly used to produce muscle paralysis. Although it has been demonstrated to produce seizures when injected directly into the cerebral ventricles, no lasting neurotoxic effects have been reported. D-TC, in doses of 1-3 ug in 1 ul, was stereotaxically injected into either the septal or temporal dentate gyrus of rat hippocampus. When sacrificed one week later, there was a selective loss of dentate granule cells (DGC) in a 100 micron radius from the injection site. No seizures were recorded from electrodes implanted in the hippocampus. Injections at these same sites of 5-10 ug of d-TC induced limbic and convulsive seizures, and destruction of hippocampal pyramidal cells. Region CA1 was consistently damaged, whereas CA2-4 and DGC were variably affected. A strong correlation was noted between increasing dose of d-TC, duration and severity of seizures, and extent of pyramidal cell damage. Pharmacologic approaches to block the seizures suggested that the pyramidal cell loss was consistent with the syndrome of epileptic induced neuronal damage. Chronic phenobarbital administration blocked seizures and pyramidal cell death in some rats. High dose valium (20 mg/kg, i.p.) was ineffective.

Seizures and local cell damage at the injection site could also be produced from injections of d-TC into entorhinal cortex, frontal cortex and cerebellum, less so in substantia nigra, but not in caudate, septum or olfactory bulb. No distant lesions were produced from any injection site.

The effects of d-TC in the hippocampus appeared to be mediated by a nicotinic mechanism as evidenced from experiments with chronic subcutaneous injections of either diisopropyl fluorophosphate or nicotine, or intradentate injections of nicotine. Intradentate injections of either atropine or baclofen were used to exclude muscarinic cholinergic and GABAergic mechanisms, respectively.

Thus, two patterns of brain damage appear to result from d-TC: DGC destruction in the absence of seizures, and pyramidal cell damage induced by seizures. D-Tubocurarine appears to be a unique neurotoxin which has some of the properties of both colchicine and the excitotoxins (e.g., kainic acid).

POSTSYNAPTIC MECHANISMS I

5.1 **Functional Effects of Spine-stem Changes in Honeybee Kenyon Cells.** Donald H. Perkel and Richard G. Coss*. Dept. of Psychobiology, U.C. Irvine, California 92717 and Dept. of Psychology, U.C. Davis, California 95616

Spines on Kenyon cells in the corpora pedunculata of the honeybee have been shown to shorten significantly after the bees take their first flight (Coss & Brandon, 1982). Using the extensive morphological data on pre- and post-flight bees, we have examined the effects of spine-stem shortening on postsynaptic potentials in these cells; in particular, we compared the responses to synaptic activation for spine heads having passive membrane with those having active voltage-dependent channels.

For simulation, the neuron was divided into isopotential compartments. Passive membrane was assumed to have a specific resistivity of 20 kΩ cm^2 and a capacitance of 1.0 μF/cm^2; membrane area included all observed spines. Cytoplasmic resistivity was 70 ohm/cm. Spine-stem resistance was 180 MΩ before flight and 90 MΩ after the flight; this represents the range of variation seen anatomically. Active membrane in the spine head was described by the Hodgkin-Huxley model of squid axon, with ten times its channel densities. Activation of a synapse on the spine head produced a conductance change following an alpha function with time constant 2.0 msec; maximum transient conductance was 0.92 nS, producing PSPs between 9 and 15 mv. The reversal potential was 75 mv above rest.

For passive membrane in the spine head, shortening the spine stem increased PSP amplitude in the spine head, but decreased it by about 10% in the dendrites. In contrast, with active membrane in the spine head, shortening the spine stem greatly increased the PSP in the spine head and not only decreased it somewhat in the dendrites, but also increased the latency to peak by nearly 2.0 msec.

These results are consistent with earlier theoretical studies demonstrating that when spine heads have active membrane there can be a clearly optimal stem resistance. In the honeybee example, that resistance was near the optimum before flight, and was reduced during flight to produce a smaller PSP. These examples also show that when spine morphology changes as a result of experience, the nature and amount of resulting functional change may depend strongly on the kinds and amounts of ionic conductances that are influenced by synaptic activation.

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5.2 REVERSAL PROPERTIES OF MONOSYNAPTIC EPSPs IN NEONATAL RAT MOTONEURONS STUDIED IN SPINAL CORD IN VITRO. K. Walton, Dept. Physiol. Biophys., New York Univ. Med. Ctr., 550 First Ave., New York, NY 10016.

The reversal properties of spinal motoneuron EPSPs have long been a subject of controversy. Most studies have been carried out in adult cat where the spinal motoneurons are quite large and have correspondingly large space constants. In order to obviate this difficulty and to have a preparation in which intracellular recordings remained stable for many hours, a hemisected spinal cord from neonatal rat (4 - 11 days) was used in these studies. Motoneurons were activated synaptically using suction electrodes on the appropriate dorsal root. Polysynaptic activation was minimized by adding 1 mM Mephenesin to the bathing solution.

EPSPs were reversed by injecting current through the recording electrode. Relatively low levels of direct current were required to reverse the sign of the EPSP (about 2 nA). This was expected from the rather high input resistance of the cells (6-30 MΩ, depending on animal age) as compared with adult cat (1 - 3.6 MΩ; Barrett & Crill: J. Physiol. 239: 301, 1964). The EPSPs could be classified into three groups according to their reversal properties. In the first group the synaptic potential reversal was monophasic, while in the second the reversal was biphasic. In the latter type the early portion of the EPSP reversed at a lower level of membrane depolarization than did the late portion. This type of reversal resembled that described in "distributed" synaptic junctions such as between climbing fiber and Purkinje cell. These two types of reversals may reflect two patterns of distribution of synaptic boutons, with the afferent terminals relatively close to site of electode impalement (presumably the soma) in the monophasic reversal and distributed over the cell, some electrically close to electrode site and some further away (presumably on dendrites), in the case of a biphasic reversal. In order to rule out possible contamination by IPSPs, reversals were repeated after blockage of GABA and glycine with bicuculline and strychnine respectively. No change in reversal properties was observed under these circumstances. The presence of dendritic calcium spikes in neonatal motoneurons (Walton & Fulton: Soc. Neurosci. Abst. 7: 246, 1981) may be a contributing factor to a third group of EPSPs that failed to reverse. Supported by Grant NS13742 from NINCDS.

5.3 FREE CALCIUM DYNAMICS IN DENDRITIC SPINES: A BIOPHYSICAL MECHANISM FOR RAPID MEMORY. H.P.C. Robinson* and C. Koch. (Spon: Tomaso Poggio) Physiological Laboratory, Cambridge, U.K. CB2 3EG, and Center for Biological Information Processing, MIT, Cambridge MA 02139.

Dendritic spines in cortical structures have been implicated, both experimentally (Fifkova & van Harreveld 1977 J. Neurocyt.6,211) and theoretically (Koch & Poggio 1983 Proc. Roy. Soc. Lond.B 218,455) in post-synaptic plasticity. Moreover Crick (1982 Trends Neurosci. 5,44) has postulated that the dendritic spine might be the site of very rapid modification of synaptic efficiency as a function of presynaptic activity. Using immunocytochemical and biochemical evidence concerning the intracellular distribution of calcium and the major calcium-binding proteins (calmodulin, calcineurin) we propose the following scheme: (1) Presynaptic electrical activity results in influx of Ca through Ca-channels, into the spine head. (2) This Ca binds to calmodulin, calcineurin, and other buffers. Taking diffusion into account, we model a spine as four compartments, containing differing amounts of Ca buffers. (3) The high buffering capability of the spine cytoplasm together with pumping and diffusion into the dendrite work to keep Ca concentration low in response to moderate presynaptic activity. However, if presynaptic activity exceeds a certain critical amount, the buffers saturate. (4) Subsequently, and with continued presynaptic input, intracellular free Ca rises rapidly, causing a very fast contraction of the actin/myosin in the spine neck: this is functionally equivalent to a short-lasting change in synaptic efficiency. (5) In addition we propose that Ca activates calmodulin associated with the cytoskeleton, causing microtubule disassembly through binding to tau proteins by a "flip-flop" mechanism (Kakiuchi & Sobue 1983 Trends Biochem. 8,59) and interacting with the fodrin-actin network by binding to fodrin. This may lead to loosening of the cytoskeleton, and may allow for more permanent spine shape changes, implementing a long-lasting modification of synaptic weight.

The proteins involved in this scheme are all known to be present in mammalian brain, most of them in particularly high concentrations in post-synaptic structures. We will present a computer model of this mechanism and discuss its possible relevance to memory in cortical structures, with particular emphasis on LTP in hippocampus.

5.4 PHOSPHOPROTEINS ASSOCIATED WITH THE REGULATION OF A K^+ CONDUCTANCE IN APLYSIA CELL R15. J.R. Lemos*, I. Novak-Hofer* and I.B. Levitan (SPON: H. Gillary). Friedrich Miescher Institut, P.O. Box 2543, CH-4002 Basel, Switzerland.

We have established a method that allows us to measure protein phosphorylation in an individual neuron. Using this technique we have performed a series of pharmacological experiments to identify specific phosphoproteins involved in the regulation of K^+ conductance in Aplysia neuron R15. Serotonin (5HT) activates a specific K^+ conductance in this cell and this response has been shown to be mediated by cAMP and cAMP-dependent protein phosphorylation. 5HT changes the phosphorylation state of a number of proteins in R15. Five of these phosphoproteins are dependent upon 5HT-treatment for detectability. The use of different 5HT-receptor ligands has led to the conclusion that this alteration in phosphoprotein pattern is receptor mediated since whenever the receptor specific for 5HT is occupied the 5HT-dependent changes in phosphoprotein pattern are evident. The use of cAMP analogs and the stimulation or inhibition of adenylate cyclase by specific agents has shown that some, but not all, of the phosphorylation changes evoked by 5HT in R15 are cAMP-mediated. It is impossible to dissociate the increase in K^+ conductance from certain of the cAMP-dependent phosphorylation changes. Experiments which effectively blocked changes in intracellular K^+ concentrations, either by holding the cell at the K^+ equilibrium potential or using Cs^+ to block the anomalously-rectifying channel, revealed that net K^+ movement is not necessary for the 5HT-induced changes in phosphoprotein pattern to occur. One would expect that phosphoprotein changes responsible for the regulation of channel properties must necessarily accompany the increase in K^+ conductance. Kinetic analysis has revealed that two phosphoproteins are most closely associated, in time, with the change in K^+ conductance. In conclusion, using the probes and manipulations available to us, we were never able to dissociate the appearance of two phosphoproteins of M_r = 29,000 and of M_r = 70,000 from the 5HT-evoked increase in K^+ conductance. One or both of these phosphoproteins may be involved in the regulation of this specific K^+ channel in R15.

5.5 SEROTONIN ENHANCES THE RESPONSE OF EXCITATORY ACETYLCHOLINE RECEPTORS IN THE RB CELL CLUSTER OF APLYSIA CALIFORNICA. L.K. Simmons. Center for Neurobiology and Behavior, Columbia University and NY State Psychiatric Institute, New York, NY 10032.

The RB cells of the abdominal ganglion of Aplysia have acetylcholine (ACh) receptors which exhibit an excitatory response when driven by identified cholinergic cell L10 (Kandel et al., J. Neurophys., 30:1352, 1967). Characterization of the RB cells' ACh receptor/channel complexes using the patch clamp technique (Simmons, Neurosc. Abst. #135.8, 1983) and iontopheretic application of ACh onto voltage-clamped cellbodies (Ascher et al., J. Physiol., 278:177, 1978) indicate that these channels have a relatively non-selective permeability to cations, a mean open time that is voltage dependent and a mean close time that depends upon the ACh concentration, and an elementary conductance of approximately 31 pS.

The RB cellbodies also have serotonergic receptors which respond to iontophoretic serotonin (5-HT). I have now demonstrated that 5-HT also modulates the ACh response in these cells.

Individual RB cells were voltage clamped (-55 to -70 m V) with two electrodes. The ganglion was maintained in a constantly perfusing bath of artificial sea water (ASW). Acetylcholine was iontophoresed onto RB cellbodies with constant current pulses; 5-HT (and other putative transmitters) were added to ASW, mixed and then perfused into the bath.

When ASW containing concentrations of 10^{-7} to 10^{-3} M 5-HT was perfused into the bath the response to the constant current pulses of ACh was enhanced by a factor of 1.2 to 2.8 in a dose-dependent fashion. The 5-HT modulation was observed in tied off RB cells and in cells maintained in high divalent cation ASW, indicating that the effect is not being mediated by an intervening interneuron.

The RB cells are responsive to dopamine and histamine; however bath application of these putative transmitters did not enhance the ACh response. Serotonin did not enhance the response of two other types of ACh receptors in Aplysia; the inhibitory response to ACh in L11 and unidentified LB cells (mediated by Cl^- specific channels) or in left upper quadrant cells (which have both the K^+ and Cl^- selective channels). It also appears that the 5-HT-induced enhancement of the ACh response is not a common property of all excitatory ACh channels in Aplysia. The identified R15 neuron has excitatory ACh channels similar to those observed in the RB cluster, but these ACh responses are not modulated by 5-HT. Supported by NIH Grants NS07038 and NS19328 and a grant from the Muscular Dystrophy Association.

5.6 NEUROTRANSMITTER-INDUCED [^{3}H]-INOSITOL-1-PHOSPHATE ACCUMULATION IN HIPPOCAMPUS: EFFECTS OF DENERVATION, ADRENALECTOMY AND PHORBOL ESTERS
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We have recently characterized the lithium-amplified agonist-induced accumulation of [^{3}H]-myo-inositol-1-phosphate (MIP) in rat hippocampal slices (Janowsky et al., Life Sciences, 1984). The accumulation of MIP is stimulated by carbachol and this response is potently blocked by atropine, a muscarinic-cholinergic antagonist. Norepinephrine (NE)-induced MIP accumulation is mediated by α_1-adrenoreceptors since prazosin, a selective α_1-adrenoreceptor antagonist blocked the response at low concentrations whereas RX 781094, a specific α_2-adrenoreceptor antagonist, was without effect. The MIP accumulation elicited by serotonin appears to be mediated by $5HT_1$ receptors since metergoline but not mianserin inhibited 5HT's effect.

Following acute (4 days) and chronic (28 days) denervation of NE-containing afferents to the hippocampus by intracerebroventricular administration of 6-hydroxydopamine or unilateral surgical lesions of the ascending medial forebrain bundle, a marked increase in the maximal response to both carbachol and NE was observed, suggesting that the effects of denervation may be due to changes either proximal or distal to the receptors' recognition site.

Four weeks following adrenalectomy the carbachol and NE induced MIP accumulation was significantly reduced compared to sham controls, indicating that the integrity of the adrenal glands is required for physiological agonist-induced phosphatidylinositol hydrolysis in hippocampal slices.

The potent tumor-promoting agents, 4β-phorbol 12β-myristate 13α-acetate and 4β-phorbol 12β,13α-dibutyrate, significantly inhibited the carbachol and NE responses whereas the inactive 4-α-phorbol had no effect. Whether these results indicate that the stimulation of protein kinase C by diacylglycerol represents a negative feed-back mechanism regulating PI hydrolysis, remains to be determined.

5.7 AN ELECTROPHYSIOLOGICAL ASSESSMENT OF THE EFFECTS OF SYSTEMICALLY AND IONTOPHORETICALLY APPLIED PHENCYCLIDINE (PCP) ON VENTRAL TEGMENTAL AREA (VTA)(A10) DOPAMINE NEURONS. G.Brush* and E.D. French, Maryland Psychiatric Research Center, Baltimore, MD 21228.

Although PCP's CNS effects have been linked to a variety of transmitter systems, considerably more evidence has centered around a PCP-DA interaction. It has been hypothesized that PCP's behavioral actions are mediated through presynaptic catecholaminergic mechanisms. Because PCP's effects on midbrain DA systems may be particularly relevant for understanding its behavioral consequences, the response of VTA DA neurons to PCP was evaluated.

Standard extracellular recording and iontophoretic procedures were used in anesthetized rats. Only neurons found 6.3-7.8 mm below dura with biphasic action potentials of >2 msec and firing rates of 1-9 spikes/sec were accepted for testing.

The response of 50 presumptive A10 DA neurons was measured following systemic PCP: 31 were slowed, 14 excited, and 5 unaffected. 15 of the inhibitions either spontaneously recovered(n=2) or were reversed by an injection of haloperidol (n=13). The other inhibitions showed no recovery or reversal. Of the 14 cells speeded half showed a progressive decrease in spike size concomitant with a greatly augmented firing rate and an eventual cessation of detectable activity.This apparent depolarization block could be related to PCP's reported effects on Na/K channels. When the spontaneous or glutamate-induced activity of 21 A10 neurons was tested with iontophoretically applied PCP, 19 were inhibited and 2 unaffected. Importantly, excitations were never observed during local PCP application.

Our results show that, unlike amphetamine, VTA DA neurons are inhibited as well as excited by systemically administered PCP. The fact that a number of inhibitions were reversed by haloperidol indicates that PCP may affect some A10 neurons via a DA interaction. Whether this effect is mediated through negative feedback or local autoregulatory mechanisms remains to be resolved. The iontophoretic results seem to suggest that the effects are mediated within the VTA. Additional studies employing specific pharmacological and anatomical manipulations of DA content will be necessary to more accurately define a PCP-DA mode of action.

(Supported by a Pharmaceutical Manufacturers Foundation Research Starter Grant).

5.8 WITHDRAWN

5.9 DECREASES IN TEMPERATURE ALTER INTRINSIC AND SYNAPTIC PROPERTIES OF HIPPOCAMPAL CA1 PYRAMIDAL CELLS. L.M. Masukawa, S.M. Thompson and D.A. Prince. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Temperature is one important variable in studies which employ the in vitro brain slice technique. Different laboratories have used slices maintained at from 23-37°C. We therefore performed experiments to examine the effects of cooling on membrane properties and synaptic responses of CA1 guinea pig hippocampal pyramidal neurons maintained in vitro. Slices were kept at the air-fluid interface of an Andersen type chamber. In some experiments populations of neurons were examined either before or after a temperature change between 37° and 27°C. In other experiments, single cells were held during comparable temperature changes produced by using a Peltier device to alter the perfusate temperature. Similar results were seen under both conditions. The effects of brief cooling were reversible.

Cooling did not affect the resting membrane potential, however the input resistance (R_N) increased by about a factor of 2 from 26.8±6.2 MΩ (37°) to 50.4±11.9 MΩ (27°). Action potentials increased in duration at 27° with slowing of both rising and falling phases. Amplitude of the first spike in a train was unchanged by cooling however subsequent spikes were more attenuated at 27° than at 37°C. The afterhyperpolarization (AHP) which followed a train of spikes was significantly increased in amplitude and duration at lower temperatures. Spike frequency accomodation, tested during long depolarizing current pulses, was much more pronounced at cool temperatures. At 37° the orthodromic synaptic response consisted of an EPSP-IPSP sequence, however, at lower temperatures a second longer latency slow hyperpolarizing potential was evoked. Early and late synaptically evoked hyperpolarizations had different reversal potentials which were near E_{Cl}- and E_K+ respectively. We propose that the major effects of cooling are to augment the AHP and either enhance or initiate a synaptically evoked, possibly Ca^{++}-activated, K^+-mediated potential. We speculate that Ca^{++} buffering is depressed at lower temperatures because of alterations in active pump mechanisms which lead to the development of prolonged AHPs and Ca^{++} activated, K^+-mediated late hyperpolarizing synaptic potentials. The temperature chosen for slice experiments may thus significantly affect both intrinsic and synaptic properties of hippocampal neurons. Supported by NIH grants NS 12151 and NS 06477 from the NINCDS.

5.10

WITHDRAWN

5.11 CAUDATE INDUCED CORTICAL INHIBITON IN THE RAT. PRELIMINARY EVIDENCE SUGGESTING A GLYCINE-MEDIATED PHENOMENON. P. Ente* G.T. Golden and R.G. Fariello (SPON: C. Bianchi). Department of Neurology, Thomas Jefferson University Hospital, 1025 Walnut St., Philadelphia, PA 19107 and Service of Neurology, VA Medical Center, Coatesville, PA 19320

In several animal species, stimulation of the caudate nucleus inhibits cortical neuronal activity for approximately 250 msec. This inhibition corresponds to a delay in the performance of learned motor tasks. At the end of the inhibitory period, a brief rebound excitation is usually seen, followed by a 10 Hz spindle activity on surface EEG. The neuroanatomical pathways of this inhibition are speculative, and we are not aware of previous attempts to identify the inhibitory transmitter involved. Long Evans Hooded rats were anesthetized with urethane (1.2 g/kg) and unit and multiunit activity were recorded from the somatosensory cortex with standard electrophysiological techniques, using metal microelectrodes. Square wave pulses of .1 msec duration at variable intensities were delivered to the head of the caudate nucleus, ipsilateral to the site of cortical recording (AP + 2.4, L 2.8, DV -5, according to Pellegrino and Cushman). Poststimulus histograms were obtained with a Neurolog system. 58 units from 14 animals were examined. Each caudate stimulus was followed by a 150-280 msec suppression of unit and multiunit activity. Once this pattern was established, solutions of various epileptogenic agents known to act through selective antagonism of a specific putative inhibitory neurotransmitter were superfused on the cortex in subconvulsant and convulsant doses. TAG, a taurine antagonist, and bicuculline, a GABA antagonist, did not suppress inhibition even at convulsant doses as revealed by recording of EEG spikes and unit bursting activity. Strychnine produced a reversible loss of the inhibitory period in 15 of 24 units tested from the 14 animals. In 6 of the 9 units that failed to show a breakdown of inhibition after strychnine application, a severe suppression of activity with firing, occurring mainly in the rebound excitation period, was noted. When glycine, GABA, and taurine were superfused onto the cortex, only glycine enhanced the inhibition, restored inhibition when suppressed, and sharpened postinhibitory rebounds of neuronal firing. These preliminary data strongly suggest that glycinergic mechanisms are primarily responsible for the powerful cortical inhibitory effect seen after application of single shocks to the head of the caudate nucleus in rats.

5.12 MONOCLONAL ANTIBODIES TO PURIFIED GLYCINE RECEPTOR BIND TO GLYCINE RECEPTORS ON MOUSE SPINAL NEURONS IN CULTURE.
P.A. St.John, D.G. Owen, & J.L. Barker, and F. Pfeisser & H. Betz; Lab. of Neurophysiology, NINCDS, NIH, Bethesda, MD., and Univ. of Heidelberg, GDR.

Studies of neurotransmitter receptors are greatly advanced by high-affinity, specific probes that bind to those receptors. Glycine, an amino acid generally accepted to mediate synaptic inhibition in the spinal cord, has been shown to have pronounced inhibitory effects on mouse spinal cord neurons in culture; responses to glycine are present on virtually all these neurons. We now report that monoclonal antibodies against the glycine receptor bind to the surfaces of mouse spinal neurons in culture and that one of them appears to interact with the glycine receptor/channel complex to activate a conductance in these cells.

Nine monoclonal antibodies were prepared against the detergent-solubilized and affinity-purified glycine receptor of the rat spinal cord (H. Betz et al., this volume). We tested whether any of the antibodies binds to "native" glycine receptors on live neurons. Immunofluorescence (IF) microscopy was used to localize antibody binding in mature cultures of mouse spinal cord neurons. Binding is confined to neurons, is present on almost all neurons, and is found only on cell surfaces, as expected for glycine receptors. The binding has a nonuniform, "patchy" distribution over the cell surface. Clusters of binding sites are not simply induced by post-binding aggregation, since the same distribution is found on neurons that were fixed prior to antibody binding. Evidence that antibodies are binding to glycine receptor/channel complexes comes from intracellular recordings of the effects of one antibody. An initial depression of responses to both glycine and GABA lasts only about 10 sec. A second phase, lasting over 60 min., shows a potentiation of glycine responses. During the second phase, there is an increase in conductance and membrane current variance. Noise analysis suggests kinetics characteristic of glycine-activated channels.

Our results suggest that these monoclonal antibodies bind tightly and specifically to glycine receptor/channel complexes on live spinal neurons, that these complexes are distributed in clusters on these neurons, and that one antibody directly activates these complexes. Fluorescence-activated cell sorting and IF microscopy suggest that receptors appear on all cells more or less in synchrony fairly late in embryonic development in the intact animal or within a few days in culture.

5.13 CURRENT- AND VOLTAGE-CLAMP ANALYSES OF PUTATIVE MOTONEURONES CULTURED FROM THE EMBRYONIC MOUSE SPINAL CORD.
D.G. Owen*, A.E. Schaffner, P.A. St. John and J.L.Barker. (SPON: D.L. Gilbert). LNP-NINCDS, NIH, Bethesda, MD.

Putative motoneurones were isolated from neonatal mouse spinal cord using FACS techniques previously described (1) and grown in culture for a period of 4-5 weeks. Electrophysiological recordings were made at this time in modified Hank's Balanced Salt Solutions using microelectrodes containing 3M KCl. Cells viewed with Hoffman-Modulation and phase-contrast optics had sizeable (30-50 micron diam.), multipolar somas and extensive neuritic arborizations. Typically, resting membrane potentials lay between -40mV and -60mV, input resistances were 35-100 Mohms and membrane time constant was about 10ms. Rapidly rising action potentials (3-5ms duration) were triggered with brief depolarizing current injections and were reversibly blocked by 1uM TTX. Outward rectification occurred at depolarized potentials and inward rectification was apparent at negative membrane potentials. Slow after-depolarizations occasionally seen following TTX-sensitive spikes, were abolished by both TTX and Cd. 10mM TEA enhanced after-depolarizations and promoted paroxysmal depolarizing events. In the presence of TTX, TEA reduced outward rectification and sometimes resulted in the expression of slow regenerative potentials (presumably Ca-dependent). Voltage-clamp analyses confirmed the presence of an outward rectifier current carried by K ions. Activation occurred at potentials more depolarized than -40mV, was extremely rapid and was blocked by TEA but not 10uM apamin. Inward currents activated at potentials more negative than -80mV, and were abolished by extracellular Cs (5mM). Transient outward currents activated by depolarizing voltage steps were rarely observed. Glycine (Gly) and GABA both activated Cl^- conductances. In particular, Gly receptors (Gly-R) may be concentrated in neurites as evidenced by responses to Gly applied to different areas of the cell and by discrete binding of Gly-R-channel antibodies to neuritic elements (2). These findings are consistent with the putative motoneuronal character of this purified population of spinal cord cells. Refs: 1) Schaffner et al., Soc. Neurosci. Abs., 9, p7.(1983). 2) St. John et al., this meeting.

BASAL GANGLIA: ANATOMY AND PHYSIOLOGY I

6.1 LIGHT MICROSCOPIC EVIDENCE FOR STRIATAL AND AMYGDALOID INPUT TO CHOLINERGIC CELL GROUP CH4 IN THE RAT.
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In the rat, part of the cholinergic cell group denoted Ch4 by Mesulam et al. (Neurosci. 11,'84), and known to innervate cerebral cortex and amygdala, lies within an antero-ventro-medial region of the globus pallidus, where its strongly AChE-positive cells mingle with typically pallidal, AChE-negative neurons that project to the subthalamic nucleus and substantia nigra (unpublished) as well as to various limbic system-associated subcortical structures (Grove et al., Neurosci. Abst. 9,'83). In this location both AChE-positive and negative neurons lie embedded in an extremely dense plexus of striatopallidal fibers from a large antero-ventro-medial striatal region that includes the nucleus accumbens. The incursion of Ch4 neurons into the path of striatal outflow prompts the question whether they, like neighboring pallidal cells, receive a direct innervation from the striatum.

To approach this question, the anterograde tracer, red kidney bean lectin (PHA-L) (Gerfen and Sawchenko, Brain Res. 290,'84), was injected into various medial striatal loci; Ch4 neurons were marked in the same material by staining for AChE or by retrograde WGA-HRP labeling from anteromedial cortex.

A profusion of PHA-L-filled axons ending in terminal swellings or displaying regularly spaced, bead-like varicosities appeared in the medial globus pallidus. PHA-L-labeled fibers encapsulating pallidal neurons often formed 'empty baskets' of terminal labeling. Moreover, fibers displaying varicosities frequently converged upon and entwined strongly AChE-positive, or HRP-positive,perikarya and their proximal dendrites. Similar observations were made in a parallel study employing enkephalin-like immunoreactivity instead of PHA-L-labeling (Haber, this volume).

Striatal injections of PHA-L produced no terminal labeling in the cholinergic magnocellular nuclei ventral to the pallidum. Labeling in these nuclei, by contrast, followed PHA-L injections into the amygdala. Dense terminal labeling in the substantia innominata, for example, followed an injection of PHA-L into the central nucleus. Varicose fibers again frequently entwined strongly AChE-positive neurons.

If ultrastructural analyses of similar material show that such juxtapositions represent synaptic contact, then Ch4 neurons intruding into the pallidum and substantia innominata may be strategically located to share the afferents of local non-cholinergic neurons. Supported by PHS grant 5 P01 MH 31154 and NSF grant BNS83-06284.

6.2 STRIATAL INPUT TO A BASAL FOREBRAIN ACHE-POSITIVE CELL GROUP SETS IT APART FROM THE REST OF THE FOREBRAIN CHOLINERGIC NEURONS.
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Enkephalin-like immunoreactivity (ELI) appears in striatal efferent fibers projecting to the globus pallidus in a unique pattern called woolly fibers. These fibers are found throughout the rat globus pallidus, ventral pallidum and pallidal regions of the olfactory tubercle. Acetylcholinesterase (ACHE)-positive cell bodies, used as a marker for the basal nucleus of Meynert, are also found scattered in these regions. As previously reported (Haber et al., Neurosci. Abstr., 1983), these ACHE-positive cells seem to be located within the peptide-rich areas, and thus lying within the path of striatal efferents. The cells may represent a different group of cholinergic neurons from those lying outside striatal circuitry. The purpose of this study was to determine, at the light microscopic level, whether ACHE-positive cells might receive enkephalin-positive striatal efference.

After fixation, tissue from normal adult rats, was sectioned at 50 u and then processed, first for ACHE staining (Jensen-Blackstad technique) followed by enkephalin immunohistofluorescence (primary antisera donated by Dr. Robert Elde). A second group of rats received striatal lesions (either suction or ibotenic acid). These animals were then processed as above.

Results from the first set of animals indicated that the ACHE-positive cells which lie within the ventral pallidum, and medial globus pallidus are in the direct path of enkephalin-positive striatal efference. It appears as if the enkephalin-woolly fibers make direct contact with the ACHE-positive cells. There are a number of more ventrally located ACHE-positive neurons which are not in the striatal efferent path. Results indicating striatal input to ACHE neurons are supported by other techniques (Groove and Nauta, Neurosci. Abstr., 1984). In the lesioned animals, ELI is depleted in regions of the pallidum which correspond to its striatal input. ACHE-positive neurons remain unaffected. Comparing the lesioned with non-lesioned animals, a regional assessment of enkephalin striatal input to ACHE-positive neurons can be made.

ACHE-positive neurons which receive peptide striatal input may also be unique with respect to their output. This group of ACHE neurons may represent a subgroup of basal forebrain cholinergic neurons whose circuitry is different from other forebrain cholinergic neurons.

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6.3 CHOLINERGIC CELLS OF THE VENTRAL PALLIDUM: A COMBINED ELECTRON MICROSCOPIC IMMUNOCYTOCHEMICAL, DEGENERATION AND HRP STUDY. L.Záborszky[1], F.Eckenstein[*2], Cs.Léránth[*3], W.Oertel[4], D.Schmechel[*5], V.Alones[*1], and L.Heimer[1]. 1. Dept. of Neurol., Univ. Med. Ctr., Charlottesville, VA 22908; 2. Dept. of Neurobiol., Harvard Med. Sch., Boston, MA; 3. Dept. of Obst., Yale Univ. Sch. of Med., New Haven, CT; 4. Dept. of Neurol., Tech. Univ. of Munich, FRG; 5. Duke Univ. Med. Ctr., Durham, NC.

The ventral pallidum (VP) is a ventral, subcommissural extension of the main body of the globus pallidus, and it is rich in glutamic acid decarboxylase (GAD) containing terminals (Zaborszky et al. 1982). Although cholinacetyltransferase (ChAT) containing cell bodies are randomly distributed in the VP, they are especially numerous in its more caudal part. The cell bodies show an oval, fusiform or triangular shape (23x11.6µm), and they usually emit three main dendrites, which can be followed as far as 150 µm in thick sections. The dendrites do not branch frequently. The karyoplasm contains characteristic parallel arrays of ER cisternae, and the nucleus has 2-4 small invaginations.

In order to characterize the synaptic relationships of the ChAT neurons, lesions were made in the n. accumbens or olfactory tubercle. In addition, the animals received an HRP injection in the basolateral amygdala and survived for 30-48 hours. The VP was processed for ChAT or combined ChAT-GAD immunocytochemistry. The following observations were made: 1) Half of the retrogradely labeled cells were also ChAT-positive. 2) Some of the ChAT cells (mostly those located close to the ventral border of the caudate nucleus) were contacted by boutons containing anterogradely transported HRP. These axo-dendritic boutons established asymmetric contacts. 3) Degenerated complexes were seen in close proximity to the retrogradely labeled (ChAT and non-ChAT-positive) cells. 4) GAD-containing boutons made symmetric contacts with both the dendrites, the soma and the axon-hillock of the ChAT cells. The results suggest that only one synaptic link may be inserted between the ventral striatum (accumbens and olfactory tubercle) and the basolateral amygdala. Furthermore, amygdalofugal axons seem to reciprocate the pallido-amygdaloid projection. Direct GABA-ergic-cholinergic interactions seem to be involved in the ventral pallidal circuits.

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6.4 THE VENTRAL PALLIDO-THALAMIC PROJECTION: A LIGHT AND EM IMMUNOHISTOCHEMICAL STUDY USING FLUORESCENT TRACERS, HORSERADISH PEROXIDASE AND ANTIBODIES AGAINST GLUTAMATE DECARBOXYLASE AND CHOLINE ACETYLTRANSFERASE. D.S. Zahm*, L. Zaborszky, W.H. Oertel, D.E. Schmechel, and L. Heimer. (SPON: W. Scott Young, III) 1. Department of Neurology, University of Virginia School of Medicine, 2. Department of Neurology, Technical University, Munich, FRG., 3. Division of Neurology, Duke University Medical Center, Durham, N.C.

A subset of ventral pallidal neurons (VP) projects to the mediodorsal nucleus (MD) of the thalamus (see Young et al., '84 for references). We marked VP cells by retrograde transport of fluorescent tracer substances or horseradish peroxidase (HRP) and examined their glutamate decarboxylase (GAD) and choline acetyltransferase (CAT) immunoreactivities with light and electron microscopy. Whereas in the light microscope the dense GAD terminal staining of VP prevented unambiguous assessment of the GAD immunoreactivity of retrogradely labeled perikarya (Young et al., '84), it could clearly be concluded that no labeled cells were CAT positive. In electron micrographs of HRP labeled cells, the perikarya and dendrites were lined by boutons of which 80% to 90% were strongly GAD positive. Following incubation with anti-GAD in the absence of colchicine a small amount of reaction product was usually present on the endoplasmic reticulum of cells which recieved the dense gabaergic input, suggesting that the cells also are gabaergic. To verify this impression, we are continuing our studies using rats which have been exposed to colchicine following retrograde transport of HRP from MD injections. (Supported by USPHS-NIH, NINCDS #T32 NS07199 and NS 17743)

6.5 CHOLINERGIC INNERVATION OF RAT AMYGDALA AS DETERMINED BY COMBINED RETROGRADE FLUORESCENT TRACING AND NEUROTRANSMITTER HISTOCHEMISTRY. J. Carlsen*, L. Zaborszky, and L. Heimer. Department of Neurology and The Clinical Neuroscience Research Center, University of Virginia School of Medicine, Charlottesville, Va. 22908.

In order to identify the origin of cholinergic innervation of the amygdala, retrograde fluorescent tracers were injected in different amygdaloid nuclei, and sections processed for concomitant immunofluorescent demonstration of cholinergic neurons with anti-choline acetyltransferase. Since many tracer- and ChAT-positive neurons were identified in basal forebrain areas including the ventral pallidum, the same sections were also incubated with antibody towards glutamate decarboxylase to delineate pallidal areas (Young et al., 1984).

Following Fast Blue injections in the basolateral amygdaloid nucleus (BL), retrogradely labeled neurons were found in the cerebral cortex, dorsal pallidum, midline thalamic nuclei, upper brainstem and ventral forebrain areas. Combined tracer and ChAT-positive neurons were observed only in the ventral forebrain, specifically in a continuum stretching from the caudo-dorsal part of ventral pallidum through the subpallidal part of the substantia innominata to the most ventral part of dorsal pallidum and peripallidal areas. No labeled neurons were found in the medial septal nucleus or the nucleus of the horizontal limb of the diagonal band. These structures, however, were labeled after control injections involving the pyriform and entorhinal cortices.

Since populations of cholinergic neurons in the basal forebrain have been implicated as origin for both neocortical and amygdaloid cholinergic input, injections of different fluorescent tracers were placed in these structures. However, no double labeled cells were observed, suggesting that the cholinergic input to neocortex and amygdala originates in two separate subsets of cholinergic cells.

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6.6 A SUBSET OF CORTICALLY PROJECTING NEURONS IN THE RAT GLOBUS PALLIDUS ARE CHRONICALLY LABELED AFTER A SINGLE PERIPHERAL INJECTION OF A HEAVY METAL. G F. Alheid[1], J. Carlsen[2*] and L. Heimer[2]. 1. Dept. Behav. Med. and Psychiatry, and 2. Dept. Neurology, Univ. Virginia Sch. of Med., Charlottesville, Va. 22908.

The corticopetal cholinergic cell group extending from the medial septum through the diagonal band nuclei and into the globus pallidus is implicated in the etiology of Alzheimer's disease. It was recently found (Rungby and Danscher, 1983) that peripheral injection of silver compounds result in labeling of CNS neurons. Forebrain neurons with the highest concentrations of silver are found in the medial septum, diagonal band, and the dorsal and ventral pallidum. Since the silver labeled neurons are coextensive with the cholinergic corticopetal cell groups, we investigated the possibility that some of the cortically projecting cells also concentrate heavy metals. A single subcutaneous injection (100 mg/kg) of Protargol$_R$ (silver coupled to albumin) was given to adult male rats. One month later, these same animals received large neocortical injections of Fast Blue. After 1-2 weeks, the animals were anesthetized, perfused, and their brains sectioned on a cryostat. The sections were examined for the presence of retrogradely labeled neurons, photographed, and subsequently treated to physical development in order to visualize silver containing neurons.

Most retrogradely labeled ventral forebrain neurons were not double labeled with silver although these two types of neurons were intermingled as adjacent clusters throughout their range. In the globus pallidus, however, double labeled neurons were found in significant numbers. These occurred most commonly in the more medial aspects of the anterior pallidum. We are extending these observations to retrograde labeling after allocortical injections and will attempt to double-label silver neurons with antibodies to choline acetyltransferase. The uptake of silver into basal forebrain neurons suggests that these cells may be susceptable to more toxic metals. Their location and size is similar to that of the cholinergic cell groups, but not identical, so that pathological studies based on cell size and location might easily confuse these two groups. Since some of the neurons that concentrate silver also project to neocortex, a subset of the corticopetal cell group may be directly affected by trace metal toxicity. NS #17743.

6.7 RESPONSES OF FRONTAL CORTEX SINGLE UNITS TO MAGNOCELLULAR BASAL FOREBRAIN STIMULATION IN THE RAT. W.C. Stern and W.W. Pugh. North Carolina Foundation for Mental Health Research, Raleigh, NC 27611 and Burroughs Wellcome Co., Research Triangle, NC 27709.

While recent neuroanatomical and neurochemical investigations have shown a major ascending cholinergic projection to cortical structures arising from neurons of the basal forebrain (BF) in several mammalian species, the physiology of this system is, as yet, poorly understood. We have studied BF-FC stimulus-response relationships by recording neuronal activity of post-synaptic frontal cortex (FC) neurons while stimulating BF in 17 urethane anesthetized adult rats. First, a monopolar macroelectrode was placed 1 mm deep in FC. A concentric bipolar stimulating electrode was advanced through the BF region in 1 mm steps while recording the FC evoked potential at each site. The BF stimulation site which yielded the greatest FC evoked potential was selected for the remainder of the experiment. Then, the FC macroelectrode was replaced by a glass micropipette for extracellular single unit recording from the cortical surface to a depth of 2 mm. Baseline, stimulation and post-stimulation neuronal activity for 133 FC cells was analyzed by computer. Results indicate that BF stimulation (0.2 msec pulses, 0.1-10 Hz, 0.1-1.0 mA for 1-30 seconds) influenced activity of FC neurons by: excitation, usually seen as a brief discharge (latency 8-20 msec) followed by an inhibitory gap of 30-200 msec and then increased firing; inhibition, usually seen as a silent or inhibitory period lasting 40-500 msec following a stimulus; or no effect on firing rate or pattern of discharge. In many cases, the stimulation produced excitation or inhibition far outlasted these averages, often persisting for seconds or even minutes. BF stimulation sites confined to BF areas which project to FC (Pugh and Stern, unpublished observations) produced excitation in 76% and inhibition in 51% of 37 FC cells studied. Stimulation sites adjacent (<1 mm) to FC projecting BF areas excited 46% and inhibited 59% of 70 FC cells; and distant (> 1 mm) BF stimulation sites excited only 37% while inhibiting 81% of 26 FC neurons. The BF, along with n. raphe dorsalis and n. locus coeruleus, appear to constitute an important triad of subcortical structures which influence ongoing activity of cortical neurons.

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6.8 HISTOGENESIS OF THE ENTOPEDUNCULAR NUCLEUS AND SUBSTANTIA NIGRA IN THE RAT. R.G. Marchand and L. Lajoie*. Centre de recherche en Neurobiologie, Hôpital de l'Enfant-Jésus, 1401, 18e Rue, Québec G1J 1Z4.

In the past, various authors have proposed that the pallidum and the substantia nigra (SN) share a close embryological relationship. Spatz suggested that the pallidum and the SN originate from a common diencephalic matrix. Mirto also implied that the neurons of the pars reticulata of the substantia nigra are pallidal neurons that have migrated caudalwards. More recently, it was shown that the non-dopaminergic neurons and more especially the reticulata type neurons had cytological and histochemical characteristics, as well as related nervous connections with the entopeduncular nucleus. It thus appeared reasonable to investigate systematically the development of the SN and of the entopeduncular nucleus.

We studied the neurogenesis and the histogenesis of these two nuclei with the aid of [^{3}H]-thymidine autoradiography. The results of our study showed that although both nuclei have a similar time of origin (from day 12 to day 15 of gestation for the substantia nigra compared to day 11 to day 14 for the entopeduncular nucleus), their embryological development is different. Cell migration and site of origin along the germinal layer are very important aspects of the embryological development and should be further investigated. The neurons of the entopeduncular nucleus apparently generate from the subthalamic longitudinal zone. On the other hand, the neurons of the substantia nigra originate from two different points of the basal plate at the level of the fovea isthmi at the mesencephalo-isthmic junction. These neurons migrated radially from their site of origin and then rostrally toward their adult site.

Our study did not disclose any direct developmental relationship between the substantia nigra and the entopeduncular nucleus despite numerous evidences or suggestions given by various neuroembryologists and neuroanatomists during the last century. During development however, the substantia nigra and the entopeduncular nucleus are both derived from the basal plate or from a rostral prolongation of the basal plate within the diencephalon. Since both nuclei share numerous characteristics in the adult, they might differentiate on a parallel path under the influence of common or similar factors. (Supported by the MRC of Canada and FRSQ).

6.9 THE ANATOMICAL ORGANIZATION OF THE EFFERENT PROJECTIONS OF THE A8 DOPAMINE CELL GROUP. A.Y. Deutch, [1]M. Goldstein, B.S. Bunney, and R.H. Roth. Neuropsychopharmacology Research Unit, Yale School of Medicine, New Haven, and [1]NYU Medical Center.

Recent preliminary anatomical data and behavioral studies indicate that the A8 dopamine cell group and its efferent projections are closely allied to the A10-derived mesolimbic system. We have therefore examined the anatomical organization of the efferent projections of the A8 cell group using both anterograde and retrograde histochemical techniques.

Anterograde projections were examined using immunohistochemical demonstration of PHA-L transport. Bilateral terminal labeling was noted in the striatum; the crossed striatal projection was sparse. Dense unilateral labeling of the nuc. accumbens was observed. The striatal and accumbens inputs occured in patches. Other mesolimbic terminal fields also exhibited PHA-L labeling: the olfactory tubercle, central amygdala, and bed nuc. of the stria terminalis all exhibited terminal labeling. Very few fibers were observed in either the medial or suprarhinal prefrontal cortices. The agranular insular cortex lateral to the claustrum was consistently labelled. A8 injections of PHA-L resulted in labeling of a broad band across the ventral pallidal region, ranging medially from the lateral preoptic area to the substantia innominata and extending laterally to the pyriform cortex; a globus pallidus input was also noted. Among diencephalic sites labeled were the lateral and anterior hypothalamic regions, and the nuc. reuniens and mediodorsalis of the thalamus. A number of descending projections originating from the A8 area were also observed; these fibers were typically distributed bilaterally. Areas labeled included the periaqueductal gray, nuc. pedunculopontis tegmentalis, dorsal tegmental nuc., nuc. cuneiformis, locus coeruleus, and the dorsal and ventral parabrachial area. The dopaminergic nature of various efferent projections originating from A8 were determined by means of combined retrograde tracer-monoamine histoflourescence or HRP-TH immunohistochemical techniqes. Among the confirmed DA projections from A8 are those to the nuc. accumbens, olfactory tubercle, striatum, agranular cortex, and amygdala.

These data suggest that the efferent projections of the A8 cell group are unexpectedly extensive, and may occupy a key intermediate position between the projections of the A9 and A10 dopamine cell groups. Thus, A8 neurons may represent a nodal point from which activity within the nigrostriatal and mesolimbic/cortical systems may be modulated. Supported by MH-14092, MH-14276, and the State of Conneticut.

6.10 DIFFERENCES BETWEEN THE MORPHOLOGY AND DISTRIBUTION OF DOPAMINERGIC AND NON-DOPAMINERGIC NIGROSTRIATAL AFFERENTS. C.R. Gerfen, Lab of Neurophysiology, NIMH, Bethesda, MD

A method that allows the co-localization, within the same fibers and terminals, of the anterogradely transported axonal tracer, phaseolus vulgaris-leucoagglutinin (PHA-L) and immunohistochemically identifiable neurochemical markers was used to examine the nigrostriatal pathway in the rat. Injections of PHA-L were made into the substantia nigra using the previously described method of Gerfen and Sawchenko, '84. Following a 10 day survival period the animals were sacrificed and the brains were processed for immunohistochemical localization of PHA-L. One series of sections was processed sequentially through guinea pig antisera directed against PHA-L (1:1000 for 24 hours) and then rabbit antisera directed against the dopamine synthetic pathway enzyme tyrosine hydroxylase (TH, 1:1500 for 24 hours). After rinsing, the sections were incubated in a solution containing rhodamine labeled goat anti-guinea pig (1:100, Cappel) and fluorescein labeled goat anti-rabbit (1:100) for 45 min, rinsed, mounted and examined with a fluorescent microscope that was equipped with filters to view rhodamine and fluorescein separately. Alternate series were processed for immunoperoxidase localization of PHA-L or for acetylcholinesterase staining to distinguish the striatal neurochemical matrix and patch compartments.

PHA-L injections that filled both TH-positive (dopaminergic) and TH-negative neurons in the pars compacta and pars reticulata of the substantia nigra, respectively, gave labeling of both TH-positive and negative fibers in the striatum. The great majority of TH-positive/PHA-L labeled nigrostriatal fibers were distributed as a reticulum of very fine fibers (0.1 μm) on which varicosities of only slightly larger diameter were seen. A second TH/PHA-L labeled fiber type was of a larger diameter and possessed more prominent flattened varicosities. TH-negative/PHA-L labeled nigrostriatal fibers were by contrast much thicker than either TH-type and possessed very prominent, bulbous varicosities that were over 1.5 μm in diameter. The non-TH nigrostriatal fibers were distributed in patches throughout the striatum. On the other hand the TH/PHA-L positive nigrostriatal fibers were distributed in a very complex pattern; to the striatal patches in some areas and to both compartments in other areas with the non-TH fibers intermixed.

6.11 REGIONAL CATECHOLAMINE DISTRIBUTION AND TURNOVER IN RAT STRIATUM. M.F. Beal and J.B. Martin. Dept. of Neurology, Massachusetts General Hospital, Boston, MA 02114.

Neurochemical heterogeneities in the striatum have been noted by several authors and they may provide clues to functional organization. In the present study rat brains were sectioned at 1 mm intervals and 1 mm diameter punches were obtained from four quadrants of the striatum at 4 anterior-posterior levels. Punches were then analyzed for concentrations of dopamine (DA), serotonin (5-HT), hydroxyindoleacetic acid (HIAA) and dihydroxyphenylacetic acid (DOPAC) using HPLC with electrochemical detection. The ratios of DOPAC/DA and HIAA/5-HT were calculated as an index of turnover of DA and 5-HT. As noted by others (Ternaux, J.P. et al., Brain Res. 121:311, 1977, Strong, R. et al., J. Neurochem. 39:831, 1982) DA concentrations were highest in rostral striatum while 5-HT was highest in caudal striatum ($p<.01$). No consistent right-left differences were seen. DA concentrations were slightly higher in the dorsolateral striatum but otherwise were homogeneous throughout the striatum. DOPAC concentrations were highest in the nucleus accumbens while DOPAC/DA concentrations were greatest in the nucleus accumbens and ventromedial striatum ($p<.01$). Serotonin concentrations were 2-3 fold higher in ventral and ventromedial portions of the striatum ($p<.01$). HIAA was highest in the pallidum but otherwise no marked heterogeneities were seen. The ratio of HIAA/5HT was consistently highest in the dorsolateral striatum ($p<.01$). Dopamine turnover was therefore highest in limbic innervated (ventromedial) striatum while serotonin turnover was highest in sensorimotor innervated (dorsolateral) striatum. These findings support the concept that the striatum is organized in a medial-lateral topography as well as along its anterior-posterior axis and provide further evidence that there is functional compartmentalization within the striatum.

6.12 MORPHOLOGY AND ELECTROPHYSIOLOGY OF SUBSTANTIA NIGRA NERVE CELL CULTURES FROM FETAL MICE. J. H. Peacock and K. J. Futamachi*. Div. of Neurology, University of Nevada School of Medicine, Reno, Nevada 89557.

Neurons obtained from the lower third of the mesencephalon of 17-18 day old Swiss Webster mouse fetuses were mechanically dissociated and cultured in DMEM containing 5% horse serum and 5% fetal bovine serum. These cultures characteristically survived longer than 2 months, during which time neurons developed multiple, highly branched processes and soma diameters increased from about 10 uM to 30 uM.

To determine if these cells were dopaminergic, a combined glyoxylic acid/paraformaldehyde procedure was used to detect dopamine. We found most of the neurons produced bright green fluorescence after loading with dopamine in the presence of the monoamine oxidase inhibitor, pargyline.

Electrophysiologic features of these neurons included an age related decrease in resting membrane potential (RMP) from about -80 mV to -60 mV between 13 and 67 days in culture with an overall average RMP of 65.4 $\pm$ 10.6 mV ($\pm$ S.D., n=32). Input resistances ranged between 100 and 300 Mohms.

Abundant postsynaptic (PSP) activity was recorded with excitatory PSPs attaining amplitudes of 30 mV and inhibitory PSPs of 10 mV. Simultaneous intracellular recording between pairs of cells demonstrated reciprocal innervation. In some neurons, haloperidol blocked spontaneously occurring excitatory PSPs and action potentials (APs).

Both Na^+ and Ca^{++} mediated APs were present in these cells. Amplitudes for Na^+ APs were between 60-90 mV with 1-3 msec durations. When cultures were exposed to tetrodotoxin (1 ug/ml) or Na^+ free medium, most of the cells generated Ca^{++} APs. Ca^{++} AP generation was stable and highly reproducible; repetitive stimulation sometimes produced broadening of AP duration.

In conclusion, large neuronal size, catecholamine histofluorescence, and block of synaptic activity by the dopamine antagonist haloperidol provide strong evidence for identification of substantia nigra neurons in dissociated ventral mesencephalic cultures.

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MEMBRANE BIOPHYSICS I

7.1 PROLONGED INACTIVATION OF A Ca^{++}-DEPENDENT K^+ CURRENT BUT NOT Ca^{++} CURRENT BY LIGHT INDUCED ELEVATION OF INTRACELLULAR CALCIUM. D.L. Alkon and M. Sakakibara*. Section on Neural Systems, Lab. of Biophysics, NINCDS at MBL, Woods Hole,MA 02543.

Voltage clamp of Type B photoreceptor somata (isolated by axotomy) previously revealed a voltage-dependent Ca^{++} current, $I_{Ca^{++}}$, which in 10 mM Ca_o^{++} does not inactivate and a Ca-dependent K^+ current, I_C, which shows marked and prolonged inactivation (Alkon et al., Biophys. J., 1984), an early K^+ current, I_A, and a delayed rectifier (Shoukimas and Alkon, 1980). We report here on light-induced currents measured for Type B somata. In ASW at -60 mV, light elicits an early transient inward Na^+ current which progressively decreases with more positive holding potentials. Light also elicits a slow inward current which increases during progressively more positive command depolarizations. In 0 Na^+-ASW light elicits an early outward K^+ current as well as the delayed inward current. The magnitude of both light-elicited currents in 0 Na^+-ASW depends on $[K_o^+]$ and both currents are absent at E_{K^+}. Both currents are reduced by intracellular iontophoresis of EGTA. Neither current is markedly affected by the presence of 2-5 mM 4-aminopyridine or 100 mM tetraethylammonium ion in the bathing medium. Substitution of 10 mM Ba_o^{++} for Ca_o^{++} eliminates the light-induced delayed inward current without affecting the early outward current. Ca^{++}-current blockers (e.g. 10 mM Cd^{++} or 20 mM Co^{++}) reduce the delayed inward current but not the early outward current. These data indicate that light releases intracellular Ca^{++} (c.f. Connor and Alkon, J. Neurophysiol., 1984) which causes a transient increase of a Ca^{++}-dependent K^+ current, I_C, and a slower prolonged decrease of I_C. Light does not alter the voltage-dependent Ca^{++} current. The light-induced rise of Ca^{++} inactivates I_C directly rather than by inactivating $I_{Ca^{++}}$ as described for other neurons (Tillotson,1979; Eckert et al.,1981). At -10 mV the light-induced decrease (lasting many seconds) of I_C follows light steps (e.g. 2 sec) of low intensity ($\leq$ 10-50 ergs/cm^2·sec). Low intensity light steps (predominantly red wavelengths) did not elicit either I_{Na^+} or an early increase of I_C, both of which appeared with increasing intensity of predominantly blue-green wavelength light. Less elevation of Ca_i^{++} is necessary therefore to cause decrease of I_C than an increase when a steady-state I_C is present (e.g. at -10 mV). Also, light-induced elevation of Ca^{++} apparently utilizes a visual pigment (in the red region) distinct from that (rhodopsin) which effects the light-induced I_{Na^+}.

7.2 TRANSDUCTION CURRENT IN SACCULAR HAIR CELLS EXAMINED WITH THE WHOLE-CELL VOLTAGE-CLAMP TECHNIQUE. T. Holton and A.J. Hudspeth. University of California School of Medicine, San Francisco, CA 94143.

Vertebrate hair cells transduce mechanical stimuli incident on their hair bundles into electrical responses. The transduction process involves the rapid gating of ion channels by mechanical stimulation of the hair bundle. The channels' poor cation selectivity (Corey, D.P. and Hudspeth, A.J., *Nature 281*: 675, 1979) suggests a unitary conductance large enough that currents through single channels or small ensembles of channels can be recorded.

In the present experiments the transduction channel was characterized by measuring displacement-modulated receptor current in single hair cells with the whole-cell voltage-clamp technique. Pipettes were lowered onto the apical surface of hair cells in an *in vitro* preparation of the bullfrog's saccular macula to form seals with shunt resistances exceeding 1 GΩ; the membrane under the pipette tip was then ruptured by slight suction. With K^+ aspartate in the pipette, depolarizing voltage steps elicited a voltage-dependent Ca^{2+} current and a Ca^{2+}-activated K^+ current qualitatively similar to those studied in solitary saccular hair cells (Lewis, R.S. and Hudspeth, A.J., *Nature 304*: 538, 1983). These currents were blocked by replacing K^+ by Cs^+ in the pipette and by holding the cell's membrane potential at -73 mV. When the hair bundle was then stimulated with a piezoelectrically driven microprobe at 12°C, a receptor current was elicited. Responses showed saturation in response to 1 μm stimuli and had peak-to-peak amplitudes that could exceed 150 pA. Fluctuations in the receptor current were observed whose root-mean-square amplitude varied with the mean current amplitude. The results suggest that the transduction current arises from the summation of currents of 50 to 100 mechanically gated channels with a unitary conductance of about 30 pS.

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7.3 A BIOPHYSICAL MODEL FOR ELECTRICAL RESONANCE IN HAIR CELLS OF THE BULLFROG'S SACCULUS. R.S. Lewis. Division of Biology, California Institute of Technology, Pasadena, CA 91125, and Dept. of Physiology, Univ. Cal. San Francisco Sch. of Med., San Francisco, CA 94143.

Vertebrate hair cells, the receptor cells of the auditory and vestibular systems, are highly selective for mechanical stimuli of particular frequencies. In several species this selectivity results from electrical resonance in the cell's membrane; the resonance can be detected as damped oscillations in membrane potential elicited by injection of depolarizing current steps. We have previously described inward Ca (I_{Ca}) and outward Ca-activated K (I_C) currents in voltage-clamped hair cells from the bullfrog's sacculus, and have proposed that these two currents produce resonance properties (Lewis, R.S. and Hudspeth, A.J., *Nature 304*:538, 1983). To test this hypothesis we have modeled the cell's behavior under current-clamp conditions in terms of I_{Ca} and I_C. This model can account for the character of voltage oscillations produced by extrinsic current steps under a variety of conditions.

The model consists of four parts: (1) Voltage-dependent I_{Ca} activation is approximated by a third-order Hodgkin-Huxley scheme. (2) Ca entering the cell is assumed to be confined to a small submembrane compartment which contains an excess of Ca buffer; Ca leaves this space with first-order kinetics. (3) C-channel activation is Ca- and voltage-dependent, and is described by the kinetic scheme $C \rightleftharpoons Ca_1C \rightleftharpoons Ca_2C \rightleftharpoons Ca_2O \rightleftharpoons Ca_3O$, where Ca_nC and Ca_nO denote closed and open states with n Ca bound. Results of single-channel recordings support the general form of this C-channel gating scheme. (4) Leakage conductance and membrane capacitance are constant.

Using the gigohm-seal technique, whole-cell I_{Ca} and I_C were recorded in solitary hair cells under voltage-clamp conditions (Lewis, R.S. and Hudspeth, A.J., *idem.*). For each cell, values of model parameters were chosen to provide the best fits to these two currents in the voltage range of -50 to -30 mV. After rates in the model were thus specified, the model was used to predict the cell's response under current-clamp conditions. The model correctly predicts the character of oscillations produced by current injection, including the dependence of oscillation frequency and damping on membrane voltage, both under normal conditions and under conditions in which I_{Ca} or I_C is decreased pharmacologically. (Supported by NIH grant GM-07737 and a System Development Foundation grant to A.J. Hudspeth.)

7.4 SEARCH FOR AN EARLY CA CHANNEL IN TWITCH MUSCLE FIBERS OF THE FROG. G. Cota* and E. Stefani. Dept. Physiology, CIEA of IPN, Mexico DF 07000, AP 14-740.

In intact muscle fibers Ca currents (ICa) are conventionally recorded in hypertonic sucrose solutions to avoid contractions. In the present experiments we have studied the effects of hypertonicity on Ca channel properties. To this end we recorded Ca action potentials and ICa as a function of the time to exposure to hypertonic sucrose (350 mM). Experiments were performed in cutaneous pectoris muscle fibers of Rana pipiens and moctezuma by using two microelectrode current-clamp or three microlectrode voltage-clamp techniques. The recording solution contained 120 mM-Tea-methanesulphonate and 10 mM-Ca. The records obtained during the first 5 minutes of exposure to the hypertonic solutions showed two components of ICa that were easily distinguishable by their voltage dependence, amplitude and time course. Two components were also observed in the Ca action potential. At 18 °C the first component was detected at -60 mV and reached a maximum peak value of -10 to -25 uA/cm^2 at about -20 mV. This current has a relatively fast time course of activation. In these experiments the onset was not resolved since it was masked by the capacity transients. An upper limit value for the peak time at -45 mV was 40 to 60 msec. The corresponding time constant of decay was 1.5 to 2.0 sec. The second component of ICa was detected at -30 mV; at ca 0 mV reached a maximum peak value of 40 to 60 uA/cm^2 in 250 to 400 msec and had a time constant of decay of 1.5 sec. After 10 to 15 minutes in hypertonic solution, the early component disappeared and only the already reported slow ICa was recorded. In order to avoid the damage induced by hypertonicity we attempted to record voltage responses after replacing 10 mM-Ca by 10 mM-Ba, since in this condition contraction induced by depolarization was less conspicuous. The maximum rate of rise of Ba action potential was larger in isotonic than in hypertonic solutions suggesting a reduction of Ca channel conductance by hypertonicity. Furthermore, in isotonic conditions the rising phase of Ba action potentials frequently showed a biphasic time course. These results are consistent with the existence of two populations of Ca channels in intact muscle fibers in isotonic solutions. The early Ca channel has a low threshold and a fast time course of activation, a slow time course of decay and it tends to disappear in hypertonic sucrose. Supported by CONACyT of Mexico, grants PCCBBNA-020187 and PCCBNAL-790022.

7.5 THE APPLICABILITY AND ADVANTAGES OF THE USE OF CONTINUUM DIFFUSION THEORY WITH MEMBRANE SYSTEMS COMPOSED OF DISCRETE CHANNELS. T.L. Schwartz*. Biological Sciences Group, The University of Connecticut, Storrs, CT 06268.

The reasons for the failure of the independence principle in an ensemble of K^+ selective, cholinergic channels in the neurons of Aplysia californica are examined. A modern, but simple formulation of thermodynamic continuum diffusion theory that has no need for the classical but restrictive assumptions of constant field and equal and opposite phase boundary potentials - which thus yields a theory which is much more general than that used classically is used here to analyze the data. The investigation exposes the fact that the independence principle actually depends on the applicability of three physical assumptions. They are: that no coupling between flows exists; that the intracellular concentration of the permeant species is invariant in the face of its extracellular manipulation; and that the permeability remains unaltered when the extracellular concentration of the permeant species is changed. The first of these constraints is met in these experiments. The second is not but the error that this introduces is easily corrected and proves to be irrelevant to this discussion. The new theory predicts that the third will, in general, not be satisfied. The experimental results confirm this prediction. The miscarriage of the independence principle is thus due to oversimplified physical approximations inherent in its derivation; but not to flaws imbedded in all continuum theory, which actually anticipates the failure of this principle. It is shown that a similar conclusion applies also to problems that emerge with the use of both the Goldman-Hodgkin-Katz equations and the Ussing unidirectional flux ratio. More modern continuum theory which is free of the problems peculiar to the older and necessarily oversimplified approaches is also shown to be capable of revealing previously inaccessible channel properties. Philosophical problems connected with the use of continuum theories for work on systems containing discrete diffusion channels as well as discrete intrachannel sites are discussed. They are shown not to be of concern with regard to ionic diffusion through membranes.

7.6 IF MEMBRANE CURRENTS ARE TO BE RELATED QUANTITATIVELY TO COMPLEX MOLECULAR EVENTS, NONLINEAR SYSTEMS ANALYTIC TECHNIQUES MUST BE USED. G.D. Lange. Lab. of Neurophysiology, NINCDS NIH, Bethesda MD 20205

Standard time-series statistics (auto- and cross-correlation) and their Fourier equivalents (power and coherence spectra) are useful in describing systems where linear models apply. An example is the use of a Lorentzian power spectrum to quantify the parameters of a simple chemical equilibrium model for membrane channel behavior. Often, the spectra do not conform to theory and one is tempted to construct complicated linear models in order to improve the fit to the data. In many cases however, such as, for example, with calcium-activated potassium channels, we should expect that models relating transmembrane voltage to ionic currents will be nonlinear. In particular, if the concentration of one ion (Ca) can influence the voltage dependent flux of another ion (K), then behavior formally equivalent to time-dependent amplitude or frequency modulation may occur. A type of analysis associated with Wiener, Volterra, and others deals directly with such problems. These methods reduce to construction of high-order correlation functions in time or of their Fourier equivalents in frequency. Unlike the autocorrelation function, which relates pairs of points in a time series, these functions relate triplets or higher order n-tuplets of times. It is both convenient and instructive to do the analysis in the Fourier domain. The resulting "spectra" are multidimensional.

An example of the squared amplitude portion of a non-normalized second order spectrum is S in the following equation:

$$S(f',f'') = s(f')s(f'')s(f'+f'').$$

The function s is the ordinary power spectrum; f' and f'' are any two frequencies. S has high points where two frequencies and associated sum or difference frequencies all have significant power. This condition will occur when nonlinear modulations are important. The phase portion of the second order spectrum has further information on temporal properties of these nonlinearities.

7.7 A CYCLIC KINETIC MODEL FOR ACETYLCHOLINE ACTIVATED CHANNELS. S. Hestrin*, J.I. Korenbrot and A.V. Maricq*. Dept. of Physiology, UCSF, San Francisco, CA 94143.

Records of acetylcholine (ACh) induced single channel currents have been shown to consist of separated groups of closely spaced opening that are termed bursts. Even in the presence of a low concentration of agonist that produces an overall low frequency of activity, the burst behavior persists and many openings are interrupted by brief closures. Colquhoun & Sakmann (1981) first suggested that during the brief closures and also right after the end of the burst the channels occupy a unique kinetic state from which immediate reopening can occur. We performed experiments that were designed to test the adequacy of this model in a mouse muscle cell line: C2. The kinetics of ACh activated channels was studied using the patch clamp technique in the presence of both low and high concentration of ACh. It was found that the inferred rate of opening under low concentration of ACh, assuming a sequential activation, was considerably slower than the rate that was estimated directly from the dwell time between openings under high agonist concentration. Thus the linear sequential model provides an inconsistent estimate of the opening rate since the rate of transition to the open state should be independent of the agonist concentration. An alternative model, one in which channels are activated in a cyclic scheme will be presented. In this cyclic model agonist molecules can unbind from the open channel leading to a transition to a closed state from which channels cannot readily reopen. If this interpretation is correct then calculations of the opening rate that are based exclusively on observations under low concentration of agonist may have to be reexamined. Furthermore, the open channel life time appears to be more complex than previously believed since it is controlled not only by the closing rate but also by steps involving agonist dissociation.

7.8 NEITHER MONOLIGANDED NOR DESENSITIZED RECEPTORS ACCOUNT FOR EXCESS BRIEF ACETYLCHOLINE CHANNELS IN CULTURED RAT MUSCLE. H. A. Lester and L. D. Chabala. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Several investigators have reported that single acetylcholine receptor channels show two lifetimes. One component equals that expected from macroscopic relaxations and fluctuations; there is a second, much briefer component (<1 msec) with the same conductance. We have investigated two hypotheses for the origin of the excess brief channels in patch-clamp measurements on cultured rat myoballs (-100 mV, 15°). We first studied the covalently bound ("tethered") agonists, QBr and bromoacetylcholine (BrACH). In recordings with better frequency response (4 kHz) than previously used, we now find that single channels induced by tethered agonists have two exponentially distributed lifetimes, <1 msec and either 5 msec (QBr) or 10 msec (BrACH). The relative numbers resemble those seen with reversibly bound agonists and with d-tubocurarine. The longer component corresponds to the single time constant of macroscopic voltage-jump relaxations and, with QBr, of light-flash relaxations. Light-flash experiments with tethered QBr suggest that the predominant open state is monoliganded, in agreement with published reports on the stoichiometry of BrACH binding under our conditions. We conclude that the brief channels do not arise exclusively from monoliganded receptors.

We then used flashes to produce cis → trans photoisomerizations of Bis-Q molecules within a spherical region (15-50 μm diam.) centered on the pipette tip. The newly created agonist concentration induces only small fractional receptor activation; agonist then diffuses away over the next few sec. Whole-cell recordings superimpose from one flash to the next (10 sec intervals); thus there is no residual desensitization between flashes. With excised outside-out patches, we measured the fraction of channel durations less than 2 msec that opened within 300 msec after the concentration-jump of agonist (there were too few channels for complete histograms with individual patches). Such brief channels accounted for 45% of the openings, a large excess over the 20% expected from a single component with a time constant of 9 msec (the longer component seen with bath-applied Bis-Q). Therefore, the excess brief channels do not manifest desensitized receptors. The experiments do not yet point to an explanation for this component. Supported: MDA Fellowship (L.D.C.), NS-11756.

7.9 CHOLESTEROL ATTENUATES HALOTHANE-INDUCED OPENTIME REDUCTIONS OF ACETYLCHOLINE RECEPTOR CHANNELS. J. Lechleiter and R. Gruener. Department of Physiology, College of Medicine, University of Arizona, Tucson, AZ 85724.

Using the patch-clamp single channel technique, we have recently shown that the volatile anesthetic halothane reduces the burst duration (opentime) of nicotinic acetylcholine receptor (AChR) channels (Lechleiter, J. and Gruener, R., PNAS, 81, 1984). In order to further investigate the action of general anesthetics, we examined the effects of halothane on AChR channels in Xenopus myocytes pretreated with cholesterol-enriched liposomes.

Liposomes were prepared according to Shinitzky and Inbar (J. Mol. Biol., 85:603, 1974) with or without exogenous cholesterol (40 mg/10 ml). Cultured myocytes were incubated with cholesterol-rich (CR) or cholesterol-free (CF) liposomes for 12 hours prior to use. Inside-out cell-free patches were obtained with patch electrodes containing 0.4 μM ACh. Patches were superfused with recording medium, bubbled with halothane, as previously described.

Consistent with our previous reports, exposure to halothane resulted in a concentration-dependent reduction in burst duration for both low- (LC) and high-conductance (HC) channels in all myocytes. However, the halothane-induced reduction of burst durations was smaller in CR- than in CF-treated myocytes. A 2% halothane concentration reduced the burst duration to 84%±5 (% of control ±SE) and 82%±11, for LC and HC channels in CR-treated myocytes. In CF-treated myocytes, exposure to the same concentration of halothane resulted in burst duration reductions of 63%±6 and 59%±6. Similarly, at 4% halothane, CR-treated myocytes had burst duration reductions of 55%±6 and 61%±6 and CF-treated myocytes had burst duration reductions of 31%±11 and 29%±5 for LC and HC channels, respectively. Consistent with our previous data, halothane did not affect channel conductance in either CR- or CF-treated myocytes. Cholesterol analysis, by gas chromatography, showed a significant increase in CR-treated myocytes and no change in CF-treated myocytes when compared to untreated cells.

We report here that halothane-induced reductions in burst duration are attenuated in CR-treated myocytes and enhanced in CF-treated myocytes. Our analysis indicates that attenuation of the effects of halothane is coincident with an increase in membrane cholesterol. Although we cannot exclude the possibility that the increase in cholesterol may be due, in part, to the adhesion of CR-liposomes to the cells, these data provide further evidence for the role of membrane lipids, and thus possibly membrane fluidity, in the mechanism of action of general anesthetics.

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7.10 **5α-DIHYDROTESTOSTERONE MODULATES ACETYLCHOLINE-ACTIVATED SINGLE CHANNELS IN MYOTUBES CULTURED FROM ADULT MALE XENOPUS LAEVIS LARYNX.** S.D. Erulkar, D.M. Wetzel, L. Kilgren,* J. Rendt,* T. Parsons and S. Yang,* Dept. of Pharmacol., Univ. of Pennsylvania, Philadelphia, PA 19104.

The androgen, 5α-dihydrotestosterone (DHT) restores species-typical sex behavior (clasping and mate-calling) in castrated adult male Xenopus laevis (Wetzel & Kelley, Hormones and Behavior 17:388, 1983). Some of the central neurons involved in these behaviors concentrate androgens (Kelley, JCN 199:221, 1981). Furthermore, the muscles of the larynx which produce calls have high affinity androgen receptors (Segil et al., Abs. Soc. Neurosci. 9:1093, 1983). DHT influences the patterns of firing of spinal motor neurons which project to clasp muscles (Erulkar et al., PNAS 78:5876, 1981). In other androgen-sensitive muscles, androgens influence acetylcholine receptor (AChR) density (Bleisch et al., J. Neurosci. 4:786, 1984). Using freeze-dry autoradiography, it was found that myotubes grown in long-term cell culture after dissociation of adult male laryngeal muscle concentrate ^{3}H-DHT in cytoplasmic and nuclear compartments. This demonstrates the presence of androgen receptors in these myotubes. Cell attached, inside-out, and outside-out configurations of the patch clamp were used to determine how DHT might modulate membrane activity of these myotubes grown with and without DHT. In myotubes raised in normal media without DHT, addition of DHT ($2 \times 10^{-8} - 10^{-6}$ M) to the bath elicited burst activity in the presence of ACh ($2 \times 10^{-8} - 10^{-6}$ M) in all three patch configurations. These effects occurred within 10 minutes. The conductance of these channels was 33-80 pS. Burst activity often became progressively longer in duration, but no desensitization was observed at any ACh concentration tested for up to 3 hours. Continued exposure to DHT caused a modest increase in conductance (90-100 pS). The effects of DHT were seen with excised patches, suggesting a direct action of DHT on the membrane. The burst activity was similar to that seen during the blocking of AChR with some local anesthetics (Ogden et al., Nature 289:596, 1981). These short-term effects were also seen in patches from thigh muscle myotubes. In laryngeal myotubes exposed to 25 nM DHT for greater than 6 days, ACh channels with fast burst activity, and large conductances (250-380 pS) were observed; furthermore, patches with at least two to three channels were often recorded. The latter two effects were not observed in patches from thigh muscle myotubes exposed to DHT for more than 6 days. The higher conductance and also the greater density of AChR suggest that DHT may be affecting the synthesis and distribution of AChR, perhaps via genomic action. These effects were not observed when similar experiments were done with 5β-DHT, estriol, or cholesterol. (Supported by NS 02211, MH 46554-06, and BRSG S07-RR-05415-22).

7.11 MEMBRANE CURRENT OF APLYSIA NEURONS RECORDED FROM A LARGE PATCH. J. W. Johnson* and S. H. Thompson*(SPON: J. K. Ono). Hopkins Marine Station, Stanford Univ., Pacific Grove, CA 93950.

Voltage clamp analysis of whole unisolated neurons is hindered by recording of large currents flowing across unclamped membrane. Cell isolation greatly reduces this problem, but often results in cell damage or some membrane that is not very well clamped. Current recordings from sub-micron diameter patches allows good voltage control and excellent resolution for single channel recording, but data concerning channel distribution and densities are difficult to obtain. An intermediate approach which allows recording of current from a large area of well clamped membrane is through use of a large patch pipette (2 to 50 u) for current recording (similar to Almers et al, J. Physiol., 336:261,1983) while voltage clamping the whole cell (similar to Frank & Tauc, Cellular Function of Membrane Transport:113,1963 and Neher & Lux, Pflugers Arch.,311:272,1969). The major difference in the technique described here is the development of a relatively high resistance seal (5 to 1000 Mohm) on Aplysia neurons following enzymatic treatment. Patch pipette voltage errors due to shank resistance can be kept to under 1 mV during maximum ionic current flow, and patch current loss across the seal resistance can be under 5%. Thus a simple patch current recording circuit can be used. If the bath voltage is clamped or is added to patch voltage, rejection of current from unclamped membrane is possible to the limit of resolution. Current noise is greatly reduced by the high seal resistance.

Currents recorded from large patches differ from whole cell currents. During an action potential, capacitative currents recorded from a patch on the soma are far greater than ionic currents there, as has been previously described. Consistent with this observation, under voltage clamp there is a much lower ionic current density (normalized to membrane capacitance) in soma patches than in whole cell. The relative magnitudes of membrane currents and their apparent kinetics vary significantly between patch and whole cell.

DEVELOPMENT AND PLASTICITY: TRANSMITTER PHENOTYPIC PLASTICITY I

8.1 ENVIRONMENTAL REGULATION OF PEPTIDE NEUROTRANSMITTER PHENOTYPIC EXPRESSION. John A. Kessler, Depts. of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y. 10461

Regulation of peptide neurotransmitter metabolism was examined in dissociated cell cultures of neonatal rat sympathetic and sensory ganglia. Sympathetic neurons of the superior cervical ganglion cultured in the presence of ganglion non-neuronal cells expressed both substance P (SP) and somatostatin (SS). By contrast, pure neuron cultures had higher levels of SS but virtually no SP, suggesting that ganglion non-neuronal cells foster SP and inhibit SS development. Peptide expression was also influenced by soluble factors produced by sympathetic target tissues. Pineal gland "conditioned" medium (PCM) treatment increased SP levels and cholineacetyltransferase (CHAC) activity and decreased tyrosine hydroxylase (TOH) activity and SS content of sympathetic neurons cultured in the presence of ganglion non-neuronal cells. Conversely, treatment of pure neuron cultures resulted in a dose dependent increase in SS and TOH, while SP and CHAC were virtually undetectable at all doses. These observations suggest that there is a reciprocal relationship between SP and SS expression by sympathetic neurons analogous to previous observations regarding cholinergic-noradrenergic expression. Moreover, in sympathetic neurons SS may be linked with noradrenergic expression, while SP may be associated with cholinergic development. PCM treatment increased peptide development in other neuronal populations as well. Dorsal root ganglion, trigeminal ganglion, and nodose ganglion sensory neurons contained SP both in the presence and absence of ganglion non-neuronal cells. Moreover, in each of these neuronal populations treatment with PCM increased SP levels both in the presence and in the absence of ganglion non-neuronal cells. These observations suggest that ganglion non-neuronal cells are necessary for sympathetic but not sensory neuron expression of SP. Moreover, PCM apparently stimulates SP in neurons which already contain the peptide, but the factor cannot foster de novo expression of the phenotype. The large number and the heterogeneity of neuronal populations affected by PCM suggest that similar mechanisms may regulate peptide metabolism throughout the nervous system.

8.2 DIFFERENTIAL EXPRESSION AND REGULATION OF NORADRENERGIC TRAITS BY THE NUCLEUS LOCUS COERULEUS IN VIVO AND IN CULTURE. C.F. Dreyfus, K.A. Markey and I.B. Black. Cornell Univ. Med. Coll. N.Y., NY 10021.

To begin defining mechanisms governing brain development, we have been studying ontogeny of noradrenergic traits in the mouse nucleus locus coeruleus (l.c.) in vivo and in explant culture. Previous work indicated that tyrosine hydroxylase (TH) and dopamine-ß-hydroxylase, noradrenergic biosynthetic enzymes, exhibit striking developmental increases in the embryonic l.c. cultured for 3 weeks. To determine whether expression and development of different noradrenergic traits are similarly regulated, we now compare ontogeny of TH with the high-affinity uptake system for norepinephrine (NE).

Uptake was monitored by incubating the l.c.with 0.5 µM NE (Km for brain), and by following the saturable, temperature-dependent, desmethylimipramine-inhibited process. Combined ^{3}H-NE radioautography and TH immunocytochemistry ensured that uptake and enzyme were localized to the same neurons.

In vivo, uptake was initially detected on embryonic day 12 (E12), a full day before initial TH detection, suggesting that expression of the 2 characters may be independently regulated. In culture, specific NE uptake into TH-immunoreactive neurons increased progressively with age, as did the rise in TH activity. Membrane depolarization in culture has been shown to increase TH activity in l.c. To determine whether depolarization similarly increases uptake, cultures were exposed to veratridine. Although veratridine significantly increased TH, ^{3}H-NE uptake, localized to the same cells, did not rise. Consequently, these two traits, associated with the same neuron, appear to be differentially regulated. In aggregate, our studies suggest the expression and regulation of 2 noradrenergic characters exhibited by the same neuron are independently expressed and regulated. We are now able to characterize the influence of specific epigenetic factors on expression and development of individual phenotypic characters in defined brain neurons.

(Supported by NSF Grant BNS 80-24081, NIH grants NS 10259, HD 12108 and the American Parkinson Disease Association. C.F.D.is the recipient of a Teacher-Scientist Award from the Andrew W. Mellon Fdn.)

8.3 SYMPATHETIC NEURONS RETAIN DUAL TRANSMITTER FUNCTION AND PREDOMINANCE OF ADRENERGIC VESICLES WHEN CO-CULTURED WITH PINEALCYTES. C. E. Phillips, J. E. Freschi, and A. G. Parfitt. Physiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814.

Sympathetic neurons in culture exhibit considerable plasticity in expression of neurotransmitter properties. Non-neuronal "background" cells can induce in the neurons a shift from adrenergic to cholinergic transmitter metabolism (Patterson, Annu. Rev. Neurosci. 1:1, 1978). Growth factors and hormones can inhibit this cholinergic induction (Fukada, Nature, 287:553, 1980), and may directly promote adrenergic differentiation. It is less clear how neuronal contact with target cells influences subsequent transmitter development. Most studies have noted that cholinergic shift occurs regardless of the receptor profile on the target tissue. In this study, we examined the effect of the pineal, an adrenergic target tissue, on the synaptic structure of neonatal rat superior cervical ganglion (SCG) neurons developing in culture. we previously reported that, in co-culture with pinealcytes, SCG neurons form functional cholinergic synapses onto each other and release a β-adrenergic agonist that induces an increase in pineal N-acetyltransferase activity (Parfitt et al., Melatonin Rhythm Generating System, ed. Klein, Karger, 1982).

Sets of co-cultures were incubated with and without noradrenaline, fixed in potassium permanganate, and processed for electron microscopy. Adrenergic vesicles were identified by their dense granular precipitate. We found that the rate of decline in the number of small granular vesicles (SGV) was considerably slower than the drop-out of these vesicles and concomitant rise in clear vesicles seen in SCG neurons cultured without pinealcytes (cf, Landis, Develop. Biol. 77:349, 1980). In addition, the varicosities or terminals remained of one predominant vesicle type rather than the mixed vesicle populations seen in SCG neurons co-cultured with cardiac myocytes (Landis, Proc. Natl. Acad. Sci. USA 73:4220, 1976). Although release at neuron-neuron synapses in these cultures causes cholinergic excitatory potentials, somal synapses contained numerous SGV throughout the 6 weeks of study.

Thus, we found that, although they enhance cholinergic metabolism in co-cultured sympathetic neurons (Rowe & Parr, J. Neurobiol. 11:547, 1980), pinealcytes also appear to influence the conservation of adrenergic vesicles in these neurons. We are now comparing the vesicle content of synapses onto SCG somata with those in varicosities (e.g., axonal swellings without synaptic contact). Similary, we are studying 5-hydroxydopamine-loaded vesicles in horseradish peroxidase-filled neurons so that, from the same cell, we can compare vesicle content in somal synapses and axonal varicosities.

8.4 EXPRESSION AND REGULATION OF TYROSINE HYDROXYLASE IN MAMMALIAN PRIMARY SENSORY NEURONS IN VITRO. D.M. Katz, J.E. Adler, K.A. Markey and I.B. Black. Cornell Univ. Med. Coll., N.Y., NY 10021.

Studies in this laboratory have recently documented expression of catecholaminergic (CA) phenotypic characteristics in primary sensory neurons of the normal adult rat, in vivo (Katz, et al., 1983, PNAS, 80:3526). These cells, localized to the vagal nodose and glossopharyngeal petrosal ganglia, express catalytically active tyrosine hydroxylase (TOH), catecholamine fluorescence, and increased catecholamine levels after inhibition of monoamine oxidase. Initial studies demonstrated that expression of sensory CA characteristics is mutable in adult animals, and may be subject to regulation by cues in the periphery.

To define molecular mechanisms of sensory CA regulation, we have been studying expression of TOH in explant cultures of petrosal ganglion neurons from fetal and adult rats. CA sensory neurons in tissue culture express phenotype characteristics typical of these cells in vivo, including 1) functional TOH, measured by catalytic enzyme assay, and 2) immunoreactivity to a highly specific anti-TOH antiserum. Therefore, explant cultures provide an opportunity to define regulation of sensory CA traits, and to compare regulatory mechanisms with those expressed by other CA populations, such as sympathetic neurons.

Explants were grown alone, or attached to their normal carotid body targets, for one week in serum-containing medium with added NGF (10u/ml). Ganglion neurons exhibited abundant TOH immunoreactivity in the presence or absence of the carotid body, indicating that normal targets are not required for expression of sensory TOH in vitro. Moreover, in the absence of normal target tissues, ganglion cells expressed TOH in a catalytically active form.

To determine whether Nerve Growth Factor (NGF) plays a role in expression and/or regulation of sensory TOH, explants of fetal ganglia were grown for one week, without targets, in the absence of added NGF and in the presence of high concentrations of antiserum against the β-subunit of NGF (A-NGF). Under these conditions, sensory ganglion cells exhibited TOH immunoreactivity; consequently, NGF may not be required for expression of sensory TOH in culture. On the other hand, preliminary experiments suggest that NGF may play a role in quantitative regulation of sensory TOH because enzyme levels in ganglion explants appear to be depressed in the presence of A-NGF. (Supported by Dysautonomia Fdn.Inc. and NIH HD 12108 & NS 10259).

8.5 PLASTICITY OF SYNAPTIC PHENOTYPE: INSULIN AND c-AMP INDEPENDENTLY INITIATE FORMATION OF ELECTROTONIC SYNAPSES IN CULTURED SYMPATHETIC NEURONS. D.C. Spray, J.C. Saez*, M.V.L. Bennett, and J.A. Kessler+, Depts. Neuroscience & Neurology+, Albert Einstein Coll. Med., Bronx, N.Y. 10461.

Electrotonic coupling between pairs of sympathetic neurons dissociated from superior cervical ganglia (SCG) of neonatal rats is rare when cells are cultured in a serum containing medium (Ham's nutrient solution F12 plus 10% fetal calf serum) but is common when cells are cultured for the same period in a serum free, defined medium; this phenomenon was described previously for embryonic SCG neurons by Higgins & Burton (Neuroscience 7:2241, 1982). Our defined medium was F12 with five added factors (progesterone, transferrin, putrescine, insulin and selenium). When added singly to serum containing medium, insulin and, to a lesser extent, selenium promote the formation of electrotonic coupling. The insulin effect is obtained with doses as low as 0.01 ug/ml and is maximal after exposures from 3-5 days. The incidence of electrotonic coupling is also enhanced by exposure of cells to dibutyryl cyclic adenosine 3'5' monophosphate (db-cAMP). This effect is obtained with doses as low as 0.1 mM, is faster, being maximal at about 12 hrs exposure, and is prolonged in the presence of the phosphodiesterase inhibitor caffeine. Butyrate itself promotes coupling to a small extent, but cAMP involvement is confirmed by similar effects of other membrane permeant cAMP analogues. Endogenous levels of $cAMP^{p}$ are significantly elevated in cultures grown in the defined medium but not in those in serum containing medium to which insulin or selenium are added. We conclude that the promotion of electrotonic synapse formation by cAMP and by insulin or selenium are independent. The formation of electrotonic connections in defined medium thus seems to be a consequence of the addition of promoting substances (insulin, selenium) and the removal of an inhibitory effect of serum on cAMP levels. Supported in part by NIH grants NS 14830 & 20013. DCS is recipient of a McKnight Development Award, JAK of a George Cotzius Award.

8.6 DEVELOPMENT OF CATECHOLAMINE- AND PEPTIDE-CONTAINING CELLS IN NEURAL CREST CULTURES: RELATIONSHIP OF DNA SYNTHESIS TO PHENOTYPIC EXPRESSION. G.D. Maxwell and P.D. Sietz*. Dept. of Anatomy, Univ. of Conn. Health Ctr., Farmington, CT 06032.

Neural crest cultures prepared from quail embryos give rise to catecholamine (CA)-containing cells, and about one-fifth of these CA cells also contain somatostatin-like immunoreactivity (SLI) (Maxwell et al., 1984 Dev. Biol. 101: 357-366). These cells begin to be detectable at 4-5 days in vitro. In the embryo, cells containing SLI are observed early in the development of quail lumbosacral paravertebral sympathetic ganglia (Maxwell et al., 1984 J. Neurosci. 4: 576-584). We wish to understand the mechanisms which control the differentiation of these CA and SLI cell populations.

Neural crest cultures were grown in the presence of [^{3}H]thymidine for the times indicated below and then, in separate experiments, immediately processed to visualize either SLI-positive cells, by indirect immunofluorescence, or CA-positive cells, by a histochemical procedure which results in a water-stable fluorophore. The cultures were then processed for autoradiography. The percentage of CA- or SLI-positive cells with nuclei labeled with silver grains was then determined.

Days in vitro [^{3}H]thymidine present	Percent of cells labeled CA-positive	SLI-positive
2-7	89.9 ± 1.9 (6)	94.0 ± 0.4 (7)
4-7	48.3 ± 2.9 (6)	54.8 ± 1.0 (5)
7-8	11.0 ± 0.9 (5)	12.4 ± 2.5 (5)
7 (1 hr)	0.5 ± 0.1 (12)	3.8 ± 0.9 (5)
8 (3 hr)	not done	2.6 ± 1.8 (5)
9 (1 hr)	1.8 (2)	not done

Values represent mean ± SEM (number of cultures).

These results demonstrate that 90% or more of the CA and SLI precursors undergo DNA synthesis at least once during the period of 2-7 days in vitro. The ability of cells to undergo DNA synthesis decreases gradually with time in culture. A small percentage of cells appear to undergo DNA synthesis after they become CA- or SLI-positive. These data also suggest that there is no dramatic difference in the developmental pattern of DNA synthesis in CA-positive cells with or without SLI.

Supported by NIH grants NS 16115 and Research Career Development Award NS 00696 (GDM).

8.7 SYMPATHETIC NEURONS MAINTAINED WITHIN THE PERITONEAL CAVITY FORM CHOLINERGIC SYNAPSES. D. Higgins, Dept. of Pharmacology, School of Medicine, State University of New York, Buffalo, N.Y. 14214.

To determine whether endogenous macromolecules capable of inducing cholinergic function are present in adult mammals, I have examined the synaptic interactions of fetal sympathetic neurons maintained within the peritoneal cavity of adult rats. Neurons dissociated from the superior cervical ganglia of rat fetuses (21d) were plated in small chambers and maintained for 1 day in a serum-free medium that does not promote cholinergic function. On the morning of the second day in vitro, some cultures were irradiated (4000 rad, ^{60}Co) to prevent the growth of non-neuronal cells. On that afternoon, cultures with or without non-neuronal cells were sealed inside of dialysis tubing with either a lower (12-14,000 dalton) or a higher (50,000) molecular weight cutoff (MWCO) and they were placed in the peritoneal cavity of adult rats ($\geq$3 months old). (The use of dialysis tubing was necessary to allow the unambiguous identification of implanted cells). After 9 to 11 days, the implants were removed and simultaneous intracellular recordings were obtained from pairs of neurons to examine the nature of the synaptic interactions. With both types of dialysis tubing, nicotinic cholinergic synapses were frequently observed among neurons (~50 of all pairs tested) in cultures in which non-neuronal cells were allowed to proliferate. Cholinergic synapses were rare (~3% of all pairs tested) among neurons maintained in the absence of non-neuronal cells in dialysis tubing with a 12-14,000 MWCO; however, they were 10 to 15 times more frequent in cultures without non-neuronal cells that had been sealed within dialysis tubing with a 50,000 MWCO. These data indicate: (1) that a factor(s) capable of inducing cholinergic function in sympathetic neurons is present or can be generated within the peritoneal cavity of adult rats and (2) that this factor(s) more readily crosses dialysis tubing with a 50,000 MWCO than with a 12-14,000 MWCO. Thus this implantation technique appears to be useful for assaying the presence of factors altering the differentiation of sympathetic neurons; it is possible it may also be useful for the detection of agents promoting neuronal suvival and growth. (Supported by the Moir P. Tanner Fund and by a Research Development Fund Award from the State University of New York Research Foundation.)

8.8 ONTOGENY OF CO-LOCALIZATION OF TYROSINE HYDROXYLASE- AND NEUROPEPTIDE Y-LIKE IMMUNOREACTIVITY OF THE FOETAL RAT BRAIN. G.A. Foster*, M. Schultzberg*, M. Goldstein and T.Hökfelt* (SPON:G. Grant). Dept. of Histology, Karolinska Institute,PO Box 60400, S-104 01 Stockholm.

It has recently been shown that adrenergic and noradrenergic neurones in the adult rat medulla oblongata and pons may also store neuropeptide Y (NPY), a novel 36 amino acid peptide isolated from porcine brain which may possess a messenger role. Although the appearance during foetal brain development of both NPY and tyrosine hydroxylase (TH), a marker for catecholamine neurones, has been separately documented by immunohistochemical analysis, the ontogeny of their co-localization within the same neurones has not. The aim of the study was to fill this lacuna, and simultaneously to discover if the neuro-transmitter/modulator complement of central nervous system neurones is ineradicably programmed.

Antibodies to TH and NPY were raised in rabbits and were used in the indirect immunofluorescence technique to localize TH- and NPY-like immunoreactivity (LI) in foetal rat brain. In the locus coeruleus, where in the adult over 40% of the TH-immunoreactive (TH-I) cells are also NPY-I, no NPY-LI was observed until day 21 of gestation. In contrast, a very densely packed and extensive group of TH-I cells appeared in the region as early as day 13. In the rostral part of the nucleus of the solitary tract in the mature animal, most of the NPY-I neurones also store TH-LI. In the foetal rat, NPY-I cells were present in the nucleus from day 13 onwards, whereas TH-LI could not be detected in the same cells until after birth. Adrenergic and noradrenergic neurones of the ventrolateral medulla oblongata exhibit extensive co-localization of TH- and NPY-LI in the adult rat. In the caudal part of the embryonic rat ventrolateral medulla, neurones displaying both NPY- and TH-LI were identifiable as early as day 17 of gestation. By days 20/21 the rostral limit of this group of co-localizing cells now extended as far anterior as the facial nucleus, to include most of the C1 group of cells.

It is apparent from the results that neurones that will eventually store both TH- and NPY-LI in the adult may start producing TH before, after, or simultaneously with the onset of NPY synthesis. It would seem, therefore, that the production of two of the putative messengers in these neurones is not inextricably linked.

8.9 REGULATION OF PROENKEPHALIN mRNA AND LEU-ENKEPHALIN IN EXPLANTED RAT ADRENAL MEDULLAE. E.F. La Gamma., J.E. Krause, J.E. Adler, J.D. White, J.F. McKelvy and I.B. Black. Cornell Univ. Med. Coll., N.Y., NY 10021 and SUNY, Stony Brook, NY 11794.

Impulse activity differentially regulates enkephalinergic and catecholaminergic (CA) transmitter phenotypic characteristics in rat adrenal medulla in vivo and in vitro (LaGamma, Adler and Black, Science-In Press). Adult male rat adrenal medullae grown as explants for 4 days show a 50-fold rise in Leu-enkephalin-like immunoreactivity (LEU), following a 2 day plateau period. Tyrosine hydroxylase, the rate-limiting enzyme in CA biosynthesis, and phenylethanolamine-N-methyltransferase, the enzyme which converts norepinephrine to epinephrine, do not increase. To further characterize molecular mechanisms governing the rise in LEU, 2 1/2 day cultured medullae were treated with inhibitors of DNA (Ara-C, 10^{-5}M), RNA (Act-D, 1 mcg/ml), or protein (cycloheximide, 2 mcg/ml) synthesis. Cycloheximide completely prevented the rise in LEU while Act-D prevented 50% of the increase. Ara-C had no effect. This suggests that both ongoing RNA and protein synthesis are required for the rise in LEU occuring from days 2 to 3. To begin characterizing the molecular level of regulation, proenkephalin mRNA was measured in this culture system using a 918 base pair, ^{32}P nick translated cDNA probe complementary to human pheochromocytoma proenkephalin mRNA (Herbert et al, PNAS 79:360, 1982). Dot blot analysis was performed on zero time and 4 day explants. After 24 hour hybridization, autoradiography was performed for 1 and 3 days. Proenkephalin mRNA levels from zero time medullae do not differ from background or nonneuronal poly A^+ RNA standards. In contrast, 4 day explants revealed a striking increase in proenkephalin mRNA which paralleled the rise in LEU. These data suggest that the rise in LEU correlates with increased proenkephalin mRNA and that ongoing synthesis of both mRNA and enkephalin prohormone is required to increase LEU. This culture system may permit characterization of the genomic processes involved in differential expression of CA characters and the putative peptide transmitter, leu-enkephalin, in denervated, explanted adrenal medullary cells. (Supported by NIH Grants HL00756, NS10259 and HD12108).

9.1 CHRONIC EFFECTS OF FLUOXETINE, AN ANTIDEPRESSANT AND A SELECTIVE INHIBITOR OF SEROTONIN UPTAKE, ON TRANSMITTER RECEPTORS. D. T. Wong, L. R. Reid*, F. P. Bymaster* and P. G. Threlkeld*. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

Fluoxetine (F) exhibits little or no affinity toward receptors of various transmitters in vitro[1]. However, chronic administration of F for 2-6 weeks lowered the number of binding sites (B_{max}) of serotonin ($5HT_1$) receptors[2,3] without changing the sensitivity of norepinephrine-activated adenylate cyclase[4] or radioligand binding to beta-adrenergic or $5HT_2$ receptors[5]. We have now found that chronic F treatment for 12 days did not change the K_D or B_{max} in saturable binding of radioligands to respective receptors: ^{3}H-dihydroalprenolol, ^{3}H-WB4101 and ^{3}H-clonidine (beta-, $alpha_1$- and $alpha_2$-adrenergic receptors), ^{3}H-quinuclidinyl benzilate (muscarinic acetylcholine receptor), ^{3}H-pyrilamine (histamine H_1 receptor) and ^{3}H-naloxone (opiate receptor), whereas down-regulation (DR) of $5HT_1$ receptor persists. Thus, F as a selective inhibitor of 5HT uptake does not modify receptors other than $5HT_1$ receptor even after chronic administration. Further, DR of $5HT_1$ receptors in frontal cortex was induced within 1 week in rats fed an F-incorporated diet (equivalent dose of 10 mg F/kg/day). In fact, detectable DR occurred within 49 hr during 3 daily injections of F at 10 mg/kg i.p. In rats pretreated for 3 days with p-chlorophenylalanine, an inhibitor of 5HT synthesis, F treatment for 4 days had no effect, but F produced DR of $5HT_1$ receptor in control rats. Thus a dependence on synaptic concentrations of 5HT is suggested. These studies provide further evidence of the selectivity of F as a 5HT reuptake inhibitor.

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9.2 SEROTONIN-2 (S-2) BINDING SITES DECREASE IN OLD MICE, BUT ARE DOWN-REGULATED TO THE SAME EXTENT BY SUBCHRONIC AMITRYPTALINE AT ALL AGES. C.E. Finch and D.G. Morgan (SPON: C.A. Kasal). Andrus Gerontology Center, Univ. of Southern California, Los Angeles, CA, 90089-0191.

S-2 binding sites are reported by several groups to decrease in density following tricyclic antidepressant treatment in rats. In Study 1, we administered amitryptaline in the drinking water at 0, 5, 15, 45, or 5-15-45 (1st wk-2nd wk-3rd wk) mg/kg/d to 8 mo. old male C57BL/6J mice for 21 days. After a 3d drug free period, mice were sacrificed and the cortex was removed and frozen. S-2 and alpha-1 adrenergic binding sites were measured with 2 nM (3H)spiperone. Binding displaced by 10 nM prazosin was designated alpha-1 specific binding. S-2 specific binding was the difference between prazosin displaceable and nonspecific binding defined with 500 nM ketanserin. Amitryptaline significantly reduced S-2 binding at all doses (Table 1). No effect on alpha-1 adrenergic binding was observed.

TABLE 1.

S-2 binding (fmol/mg prot.)	Dose of Amitryptaline (mg/kg/d) 0	5	15	45	5/15/45
Mean	70.6	58.1	52.5	47.9	40.0
±sem	4.2	5.2	5.3	1.4	2.2
% 0 dose	100%	82%	74%	68%	57%

In Study 2, mice of 4 age groups were administered 0 or 15 mg/kg/d of amitryptaline in the drinking water for 21 days. Cortex was collected 2 days after removing the drug. S-2 binding sites were measured in cortical membranes by saturation analysis using 6 concentrations of (3H)spiperone (0.25 to 8 nM) with prazosin added to all tubes to occlude the alpha-1 adrenergic site. Nonspecific binding was estimated with 500 nM ketanserin. There was a significant reduction in S-2 binding sites with age and with amitryptaline treatment, but no age by drug interaction (Table 2). Neither variable affected Kd (0.85 ±.06 nM). We conclude that fewer S-2 receptors are present in aged mouse cortex (as in human cortex), but these receptors retain their capacity for down-regulation by intermediate doses of amitryptaline.

TABLE 2.

	AGE 4-6 mo.	9-11 mo.	15-20 mo.	24-26 mo.
Bmax 0 mg/kg	174 ±6	158 ±9	154 ±6	138 ±8
Bmax 15 mg/kg	129 ±5	117 ±7	110 ±7	104 ±5
Percent	74%	75%	72%	76%

Supported by Potamkin-Lerner Fellowship (DGM) and AG-00117, AG03272 (CEF).

9.3 MODIFICATION OF SEROTONERGIC AND NORADRENERGIC NEUROTRANSMISSION BY LONG-TERM ADMINISTRATION OF MONOAMINE OXIDASE INHIBITORS I. ACTIVITY OF PRESYNAPTIC NEURONS. C. de Montigny and P. Blier (SPON: L. Descarries), Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec, Canada, H3C 3J7.

Monoamine oxidase exists in two forms: type A deaminates serotonin (5-HT) and norepinephrine (NE), and type B, β-phenylethylamine. The antidepressant effect of monoamine oxidase inhibitors (MAOI) is generally assumed to result from an increased availability of 5-HT and/or NE since clorgyline, a selective MAOI-A, is as effective an antidepressant as non-selective MAOI. Deprenyl, a preferential MAOI-B, has also been reported to have antidepressant activity but has been administered at regimens which also inhibit MAO-A.

Male Sprague-Dawley rats received daily injections of clorgyline (1 mg/kg, s.c.), deprenyl (0.25 mg/kg, s.c.), or phenelzine (2.5 mg/kg, i.p.) for 2, 7 or 21 days. Unitary extracellular recordings of 5-HT and NE neurons were obtained from the nucleus raphe dorsalis and the locus coeruleus under chloral hydrate anesthesia (400 mg/kg, i.p.), 24 h after the last injection. In the 21-day treatment groups, the sensitivity of 5-HT or NE autoreceptors was assessed by determining the effects of LSD or clonidine on the firing rate of 5-HT or NE neurons, respectively.

Two-day treatments with clorgyline or phenelzine markedly decreased the firing rate of 5-HT neurons; there was a partial recovery after 7 days of treatment and a return to normal firing rate after 21 days of treatment. By this time, there was also a desensitization of the 5-HT autoreceptor as indicated by a decreased effectiveness of LSD. In contrast, the 21-day deprenyl treatment failed to modify the sensitivity of the 5-HT autoreceptor. In the case of NE neurons, clorgyline and phenelzine produced a reduction of firing rate after 2, 7 and 21 days of treatment. Deprenyl had no such effect. The ED_{50} of clonidine was not modified by any of the treatments, indicating that the NE autoreceptor was not desensitized.

Given the delayed antidepressant activity of MAOI and their rapid and sustained effect on NE neurons, it appears unlikely that the therapeutic effect of these drugs is mediated by the NE system. However, the gradual recovery of firing activity of 5-HT neurons, attributable to the progressive desensitization of their autoreceptor, correlates well with the delayed onset of the antidepressant action of MAOI.

9.4 MODIFICATION OF SEROTONERGIC AND NORADRENERGIC NEUROTRANSMISSION BY LONG-TERM ADMINISTRATION OF MONOAMINE OXIDASE INHIBITORS II. RESPONSIVENESS OF POSTSYNAPTIC NEURONS. P. Blier, C. de Montigny and A.J. Azzaro. Université de Montréal, Canada, and University of West Virginia, Morgantown, USA.

Serotonin (5-HT) but not norepinephrine (NE) neurons regain their normal firing rate during long-term administration of antidepressant monoamine oxidase inhibitors (MAOI). The present study was undertaken to determine if these presynaptic modifications result in altered neurotransmission.

Male Sprague-Dawley rats received daily injections of clorgyline (1 mg/kg, s.c.), deprenyl (0.1 mg/kg, s.c.) or phenelzine (2.5 mg/kg, i.p.) for 21 days. Activity of MAO-A and B was assessed using subsaturating concentrations of 5-HT and β-phenylethylamine as substrates. Clorgyline and deprenyl inhibited very selectively MAO-A and B, respectively, whereas phenelzine inhibited both forms of the enzyme. Five-barrelled micropipettes were used to record from hippocampal pyramidal neurons and to assess their responsiveness to microiontophoretically-applied 5-HT and NE using the IT_{50} method (current X time required to obtain a 50% decrease of firing rate) in control and 21-day treated rats under chloral hydrate anesthesia (400 mg/kg, i.p.), 24 h after the last injection. The responses of the same neurons to the electrical stimulation (0.5 ms pulses delivered at 0.8 Hz with currents of 80 to 640 μA) of the ventromedial 5-HT pathway and to that of the dorsal NE bundle was estimated from peristimulus time histograms.

The IT_{50} values for 5-HT were increased by the clorgyline, but unmodified by deprenyl and phenelzine, whereas those for NE were not altered by any of the treatments. The suppression of firing induced by the stimulation of the 5-HT pathway was increased by phenelzine and clorgyline, but not by deprenyl. Phenelzine and clorgyline, but not deprenyl, reduced the effect of the stimulation of the dorsal NE bundle.

These data show that prolonged inhibition of MAO-A, but not that of MAO-B, results in an enhanced 5-HT and a reduced NE neurotransmission. Taken together with our finding that 5-HT, but not NE neurons, progressively recover their normal firing rate during long-term MAO-A inhibition, this suggests that the enhancement of 5-HT neurotransmission is more likely than the reduction of NE neurotransmission to mediate the delayed antidepressant effect of MAO-A inhibition.

9.5 PRE- AND POSTSYNAPTIC EFFECTS OF TRAZODONE ON SEROTONIN NEUROTRANSMISSION: SINGLE CELL STUDIES IN THE RAT. M. Dowdall and C. de Montigny. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Canada, H3C 3J7.

Several clinically effective antidepressant treatments have been shown electrophysiologically to enhance serotonin (5-HT) neurotransmission in the rat forebrain. Biochemical and behavioral studies have suggested that trazodone, an antidepressant triazolopyridine derivative, might have 5-HT agonist and/or antagonist properties. The present study was undertaken to determine whether long-term trazodone treatment modifies 5-HT neurotransmission.

Male Sprague-Dawley rats were treated with trazodone (10 mg/kg, i.p.) for 2, 7 or 14 days. Twenty-four hours after the last dose, rats were anesthetized with urethane (1.25 g/kg, i.p.). In a first series of experiments, extracellular unitary recordings were obtained from pyramidal cells of the dorsal hippocampus with a five-barrelled microiontophoretic pipette; side barrels contained acetylcholine chloride (ACh) (20 mM in 200 mM NaCl; pH 4), norepinephrine (NE) (100 mM in 50 mM NaCl; pH 4), 5-HT creatinine sulfate (0.5 mM in 200 mM NaCl; pH 4) and dopamine (DA) (100 mM; pH 4). Silent or slowly-discharging neurons were activated to 8-12 Hz with a small current (1-5 nA) of ACh. Responsiveness to microiontophoretically-applied 5-HT, NE, and DA was assessed using the IT_{50} method (i.e. current X time required to obtain a 50% inhibition of firing rate from baseline). In a second series of experiments, systematic descents into the nucleus raphe dorsalis were carried out using a single-barrelled micropipette; the number of spontaneously active 5-HT neurons per trajectory and their rate of discharge were recorded.

A 14-day treatment with trazodone did not modify the responsiveness of pyramidal neurons of the hippocampus to 5-HT, NE, or DA. In rats treated for 2 days, there was a reduction in the firing rate of dorsal raphe 5-HT neurons. There was a partial recovery of their firing activity after 7 days of treatment, and a complete recovery after 14 days of treatment. At this time, there was a marked reduction of the response of these neurons to intravenous LSD, indicating a desensitization of the 5-HT autoreceptor.

These results suggest that long-term trazodone treatment might enhance 5-HT neurotransmission via a presynaptic action resulting in a desensitization of the 5-HT autoreceptor. The time course of this phenomenon is compatible with the delayed antidepressant effect of this drug.

9.6 THE EFFECTS OF ACUTE AND CHRONIC ADMINISTRATION OF CITALOPRAM ON SEROTONERGIC NEUROTRANSMISSION: ELECTROPHYSIOLOGICAL STUDIES IN THE RAT. Y. Chaput*, P. Blier and C. de Montigny (SPON: J. Stewart). Neuroscience Research Center, Université de Montréal, Montréal, Canada, H3C 3J7.

Citalopram (CIT), a specific 5-HT reuptake blocker, has been reported to be effective in the treatment of major depression. CIT blocks in vitro the action of LSD on 5-HT release suggesting that it could modify the properties of the 5-HT autoreceptor (Langer et al., J. Pharmacol. exp. Ther. 222: 220, 1982). Hence, the present studies were undertaken to investigate in vivo the effects of CIT on 5-HT neurotransmission.

Male Sprague-Dawley rats (240-270 g) were treated with CIT (20 mg/kg/day, i.p.) for 14 days. The responsiveness of hippocampal pyramidal neurons to microiontophoretically-applied 5-HT (0.5 mM in 0.2 M NaCl, pH: 4) and NE (0.1 M in 0.2 M NaCl, pH: 4) was assessed using the $I.T_{50}$ method [i.e. current (in nA) X time (in sec) required to obtain a 50% depression of firing rate] under chloral hydrate anesthesia (400 mg/kg, i.p.). The effect of the electrical stimulation of the ascending 5-HT pathway in the ventromedial tegmentum on these same neurons was determined from peristimulus time histograms.

Long-term treatment with CIT did not modify the responsiveness of the hippocampal pyramidal neurons to microiontophoretically-applied 5-HT or NE; however, the effect of electrical stimulation of the ascending 5-HT pathway on the same neurons was markedly enhanced. To determine if 5-HT reuptake blockade itself could be responsible for this enhancement, CIT (1 mg/kg) was injected intravenously in naive rats while stimulating the ascending 5-HT pathway; the effect of the stimulation was not increased. To assess the involvement of the 5-HT autoreceptor, methiothepin, a 5-HT autoreceptor antagonist, was injected intravenously (1 mg/kg) in naive and in CIT-treated animals while stimulating the ascending 5-HT pathway; methiothepin markedly enhanced the effect of the stimulation in naive rats but failed to do so in rats chronically treated with CIT.

These results indicate that long-term CIT administration enhances 5-HT neurotransmission via a presynaptic mechanism. The markedly reduced effect of methiothepin in rats chronically treated with CIT suggests that the enhancement of 5-HT neurotransmission by CIT might be due to a desensitization of the 5-HT autoreceptor located on the 5-HT terminals, presumably resulting in an increased release of 5-HT.

9.7 SEPARATE MECHANISMS FOR DIAZEPAM'S EFFECTS ON AGGRESSION AND PUNISHED DRINKING. K. A. Miczek. Dept. of Psychology, Tufts Univ., Medford, MA 02155.

Benzodiazepines increase behavior that is suppressed by aversive consequences in animals, including humans, and this effect correlates with the drugs' clinical anxiolytic effects. Benzodiazepine receptor blockers antagonize the punishment-attenuating as well as anxiolytic effects. Benzodiazepines also exert a "taming" effect on aggressive animals, although under certain conditions these drugs may actually enhance aggressive behavior. We investigated the aggression-modulating and punishment-attenuating effects of diazepam in rats (1) by comparing the most effective doses for both types of effects, (2) by antagonizing both effects with Ro15-1788 and (3) by enhancing the effects of diazepam with concurrent administration of ethanol. Punishment-attenuating drug effects were studied according to a protocol in which rats, after water deprivation, were given access to water and every 20th lick was followed by delivery of a 0.5 μA electric shock. Aggressive behavior was engendered in resident-intruder confrontations quantitatively recorded. Low doses of diazepam (0.6, 1.0, 3.0 mg/kg, IP) increased the incidence of several aggressive acts and postures, whereas 10 mg/kg decreased these behaviors. We confirmed the already known punishment-attenuating effects of diazepam. At the 10.0 mg/kg dose sedative and muscle-relaxant effects became evident. Ro15-1788 (10 mg/kg, IP) effectively antagonized the aggression-decreasing and punishment-attenuating effects of diazepam. However, the aggression-enhancing effects of low diazepam doses failed to be antagonized by Ro-15-1788. The anxiolytic, sedative, and antiaggressive effects of diazepam appear to be based on interaction with distinct receptor complexes in the CNS, which differ from the drug's mechanism of action for the aggression-enhancing effects.

9.8 REGIONAL LOCALIZATION OF THE ANXIOSELECTIVE DRUG BUSPIRONE IN RAT BRAIN. D. P. Taylor, R. E. Gammans,* D. K. Hyslop, G. K. Matheson,[1] R. F. Mayol,* and S. Moon Edley[2]. Pharmaceutical Research and Development Division, Bristol-Myers Company, Evansville, IN, 47721, and [1]Indiana University School of Medicine, Evansville, IN, 47732, and [2]Laboratory of Neurophysiology, NIMH, Bethesda, MD, 20205.

Clinical trials have demonstrated that buspirone (Buspar™) is effective in the treatment of anxiety neurosis with efficacy and dosage comparable to diazepam or clorazepate. However, buspirone is not only chemically distinct from the benzodiazepines and other psychotropic agents, but it also presents a clinical pharmacologic profile which is "anxioselective". We have used a specific radioimmunoassay to determine that following anxiolytically-relevant doses (10 mg/kg), levels of buspirone are highest in cortex, followed in descending order by hippocampus, midbrain, thalamus, medulla-pons, striatum, hypothalamus and cerebellum. The levels of buspirone in the cortex were 50 percent higher than those in the cerebellum and twice as high as circulating plasma levels of the drug. Recently, we have obtained [^{3}H]buspirone of high specific activity (68 Ci/mmol) and carried out preliminary autoradiographic studies. Animals were dosed with saline or unlabelled buspirone (10 mg/kg, i.p.) fifteen minutes prior to i.v. injection of 150 μCi [^{3}H]buspirone. Frozen brain slices (20 μm) were exposed to LKB Ultrofilm for 14 weeks, and the developed autoradiograms were analyzed by computerized densitometry. Liquid scintillation counting of adjacent slices revealed that pretreatment with unlabeled buspirone resulted in a 70 percent decrease in radioactivity present. Specific localization (representing binding and/or uptake processes) of radioactivity was high in pyriform cortex, anterior cingulate gyrus, somesthetic cortex, parietal cortex, basolateral and corticomedial amygdaloid nucleus, and hippocampus, among other regions. Radioactivity was less densely localized in fiber tracts, zona incerta, thalamus, the spinal nucleus of cranial nerve V, and the paraflocculus of the cerebellar cortex. The relatively low degree of localization in some nuclear regions and fibers of buspirone may be taken as an indication of specificity. Pharmacologic experiments to illustrate this will be presented. Results of further experiments designed to satisfy requirements for stability of ligand and saturability are in progress. Finally, because of the position of the tritium label, the localized radioactivity can not be considered to be a metabolite of buspirone, 1-(2-pyrimidinyl)piperazine.

9.9 THE ANTIPANIC/ANTIPHOBIC DRUG,ALPRAZOLAM,INHIBITS THE AGGREGATION OF HUMAN PLATELETS BY PLATELET ACTIVATING FACTOR (PAF). E. Kornecki*, Y.H. Ehrlich, and R.H. Lenox (SPON: E. Hendley). Dept. of Psychiatry, University of Vermont, Burlington, Vermont 05405.

Panic attacks with debilitating agoraphobia is a chronic,disabling disorder which afflicts 2 to 4% of the general population in the USA and 10 to 14% of patients in cardiology practices. Recently it was shown that the triazolobenzodiazepine,alprazolam, produces clinical improvement in patients with severe agoraphobia and panic attacks (Sheehan et al. J.Clin.Psychopharm.4:66,1984). They demonstrated that in these patients, plasma concentrations of platelet factor 4 and beta-thromboglobulin were significantly elevated,and normalized during treatment with alprazolam. These results suggest that increased platelet activation may be associated with panic/agoraphobic state and this activation is reversed during treatment. In the present study we tested whether activation of platelets by various agonists can be blocked by alprazolam.

PAF(1-O-alkyl,2-acetyl,glyceryl-3-phosphorylcholine) is a potent platelet activator. We determined the effects of alprazolam on PAF-induced human platelet aggregation both in platelet-rich plasma(PRP) and in a washed platelet system. Blood was obtained from healthy donors who had fasted for at least 10 hrs prior to blood collection. The blood was collected in 3.8% sodium citrate,layered over Ficoll-Hypaque,and centrifuged at room temperature to obtain PRP. Washed platelet suspensions were prepared by filtering PRP through a Sepharose 2B column equilibrated in Tyrode solution containing 0.5mM $Ca^{++}$and 0.35%BSA,pH 7.35. Aliquots(0.45ml) of PRP and washed platelets ($2\text{-}4X10^8$/ml) were preincubated for 1 min at 37°C under stirring conditions in a Payton aggregometer in the presence or absence of alprazolam. Platelet aggregation was initiated by the addition of various agonists. Alprazolam was found to completely inhibit PAF-induced aggregation of platelets both in PRP and in washed platelet preparations. On the other hand,alprazolam had no effect on ADP(1-10uM) & thrombin(0.1-1U/ml)-induced platelet aggregations,however, slight inhibitory effects on epinephrine(10uM),A23187(1-10uM) and collagen(200ug/ml)-induced platelet aggregations were observed. In PRP,an IC_{50} of 12uM for alprazolam was obtained at a PAF concentration of 2nM. Investigation of the process by which alprazolam specifically inhibits PAF mediated cellular responses may shed light on the biochemical mechanisms of action of triazolobenzodiazepines. (Supported in part by a grant from the Upjohn Company).

9.10 HALOPERIDOL-INDUCED INCREASE IN DOPAMINE RECEPTORS IS PREVENTED BY INSULIN. D. Lozovsky, I.J. Kopin and C.F. Saller.* Exp. Therapeutics Branch, NINCDS, and Lab. Clin Science, NIMH, NIH, Bethesda, MD 20205.

Dopamine (DA) receptor supersensitivity in rats made diabetic with alloxan can be prevented by chronic insulin or lithium (Science 214:1031, 1980; Am. J. Psychiat. 140: 673, 1983). We now report the effects of long-term insulin treatment on haloperidol-induced DA receptor supersensitivity. Rats were treated as follows: Group 1 (controls): 21 days of i.p. vehicle (1.7% tartaric acid, pH 5.5) followed by five days of saline (s.c.); Group 2: 21 days of i.p. haloperidol (1mg/kg) followed by five days of saline; Group 3: 21 days of haloperidol followed by five days of increasing doses (s.c.) of regular insulin (2.5, 4, 6, 6 and 8 U/kg); Group 4: 21 days of haloperidol and 12 days of insulin covering the last seven days of haloperidol and five days thereafter (2.5, 4, 6, 6, 7, 8, 9, 10, 11, 12, 13, and 14 U/kg); Group 5: 21 days of haloperidol and 26 days of insulin covering all 21 days of haloperidol and five days thereafter (2.5, 4, 6, 6, 7, 8, 9, 10, 11, 12, 13, 14, 13, 13, 10, 10, 8, 8 U/kg, and 7 U/kg for the last eight days); Group 6: 21 days of vehicle, followed by five days of insulin (as in group 3); Group 7: 21 days of vehicle and 12 days of insulin (as in group 4); Group 8: 21 days of vehicle and 26 days of insulin (as in group 5). Food was withdrawn from all the rats for six hours after the injections. All the rats were decapitated on the 27th day. Specific binding of 1.2 nM [^{3}H]spiperone to striatal membranes was used as a measure of DA receptors (pmoles/g tissue). Haloperidol induced a 38% increase in the number of DA receptors (26.3 ±0.9 and 19 ±0.8, $P<0.01$, for groups 2 and 1). Haloperidol-induced supersensitivity was not affected by five days of insulin treatment (26.3 ±0.9 and 24.7 ±0.8 for groups 2 and 3), whereas administration of insulin for 12 or 26 days in higher doses resulted in a lack of DA receptor sensitization in rats treated with haloperidol (20.1 ±0.9, $P<0.01$, and 20.4 ±0.8, $P<0.05$, for groups 4 and 5 vs. group 2). Insulin alone, however, had no effect on striatal [^{3}H]spiperone binding in vehicle-treated rats (20.4 ±1.5, 18.8 ±2.1, and 16.0 ±2.8 for groups 6, 7, and 8). The affinity of the [^{3}H]spiperone binding was not affected by any of the treatments. These findings may be relevant to the mechanism of the therapeutic effect of insulin in the treatment of schizophrenia since DA receptor supersensitivity has been implicated in the pathogenesis of this disorder.

9.11 PHENYLMETHANESULFONYL FLUORIDE AND METHANESULFONYL FLUORIDE: CNS SELECTIVE CHOLINESTERASE INHIBITORS. L.A. Rodriguez*, D.E. Moss and M.L. Camarena*. Department of Psychology, University of Texas at El Paso, El Paso, Texas 79968.

Several CNS diseases such as senile dementia of the Alzheimer type and some hyperkinetic motor disorders could theoretically benefit from enhancment of brain cholinergic activity. One treatment strategy designed to facilitate CNS cholinergic function has involved the use of ChE inhibitors. One serious problem is that ChE inhibitors usually also affect cholinergic neurotransmission in peripheral tissues (skeletal motor system, autonomic ganglia, and parasympathetic synapses) and produce toxic side effects. The present experiments, however, demonstrate that some sulfonyl fluorides (irreversible ChE inhibitors of the same general character as organic phosphates) have a high inherent selectivity for inhibition of CNS ChE and remarkably low general toxicity. Inhibition of brain, ileum, heart, and pectoral muscle ChE was assayed after in vivo administration of several sulfonyl fluorides. Repeated administration of 85 mg/kg phenylmethanesulfonyl fluoride (PMSF) produced up to 90% inhibition of CNS enzyme with not more than 30% inhibition in peripheral tissues. Methanesulfonyl fluoride (MSF) also has excellent selectivity toward CNS enzyme. Under similar conditions, but at 0.5 mg/kg, MSF produced 81% CNS inhibition with a maximum of 23% peripheral effect. The unique CNS selectivity of PMSF and MSF is not demonstrated by several other structurally related sulfonyl fluorides. Because the sulfonyl fluorides are long-lasting ChE inhibitors with unusually low general toxicity, these compounds may prove to have clinical value in the treatment of chronic CNS disorders.

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10.1 MELATONIN AND PHOTORECEPTOR METABOLISM: REGULATION OF CONE RETINOMOTOR MOVEMENT BY MELATONIN AND DOPAMINE. M.E. Pierce*, P.M. Iuvone, J.C. Besharse*. Dept. of Anatomy, Emory Sch. of Med., Atlanta, GA 30322

The retinas of some vertebrates adjust to changing light conditions by movements of the photoreceptors, presumably to reposition the outer segments for optimal exposure to light. In light, the myoid of the cone inner segment contracts; in dark, it elongates. This retinomotor movement is influenced by a circadian clock.[1] The following experiments were conducted to test the hypothesis that melatonin is involved in the regulation of cone retinomotor movement. This hypothesis was based on the following observations: 1) the activity of retinal serotonin N-acetyltransferase (NAT), a key enzyme in the synthesis of melatonin, is expressed as a circadian rhythm with peak activity in the dark;[2] 2) melatonin stimulates photoreceptor disc shedding, another circadian process.[3] Eye cups were prepared from Xenopus laevis that had been maintained in constant light at 26°C for 4 days, a process that blocks the circadian rhythm of NAT activity.[2] Eye cups were incubated in vitro in either light or dark for 3 hours. Addition of 0.5μM melatonin to the incubation medium stimulated cone elongation in light to an extent that was comparable to that elicited by darkness; i.e. melatonin mimicked the effect of darkness.

Retinal dopamine biosynthesis occurs in a rhythmic fashion with peak activity in the light.[4] We, therefore, examined the effects of dopamine on cone elongation and investigated a possible interaction between melatonin and dopamine metabolism. Addition of 50μM dopamine 1) blocked dark-induced elongation, 2) blocked melatonin-induced elongation, 3) caused contraction of dark-adapted cones, and 4) inhibited the stimulation of NAT activity by darkness. In addition, 0.5μM melatonin decreased the concentration of the dopamine metabolite 3,4-dihydroxyphenylacetic acid in light-exposed retinas, suggesting that melatonin inhibits dopamine release. These data suggest that the regulation of cone movement may involve an interaction of melatonin and dopamine, a putative neurotransmitter in the inner retina.

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10.2 DOSE-RESPONSE RELATIONSHIP BETWEEN LIGHT IRRADIANCE AND RETINAL DOPA AND DOPAMINE IN THE DARK ADAPTED RAT. G. C. Brainard and W. W. Morgan. Dept. Neurology, Thomas Jefferson Univ., Philadelphia, PA 19107 and Dept. Cellular and Structural Biology, Univ. Texas Health Science Center, San Antonio, TX 78284

Light exposure produces an increase of dopamine (DA) synthesis and a decrease of dihydroxyphenylalanine (DOPA) accumulation in rat retina (Morgan and Kamp, J. Neurochem. 39:1982). The purpose of the following study was to characterize how graded light irradiances influence changes in DOPA and DA levels in the rat retina.

Adult male Sprague-Dawley rats, weighing 200-250 grams, were dark adapted for a minimum of 12 hours. Under dim red light, each rat was anesthetized with sodium pentobarbital (25 mg/kg) and then given m-hydroxybenzylhydrazine (NSD 1015, 50 mg/kg) by tail vein injection. The rat was immediately positioned in a stereotaxic frame and exposed to a 15 minute pulse of white light. The experimental light was produced by a 500 watt tungsten bulb (Sylvania), collimated by a set of condensing lenses and passed through a glass infrared filter. Light intensity was adjusted by one or more glass neutral density filters. The resultant light beam was centered directly in front of the rat at eye level. Groups of 8 rats each were exposed to an irradiance (incident on the cornea) of 0, 1, 3, 5, 10, 25, 50, 100 or 1000 uW/cm^2. Immediately following light exposure, the rats were sacrificed by rapid decapitation and the retinas were collected. DOPA and DA levels were quantified by liquid chromatography with electrochemical detection. The data were analyzed by one-way ANOVA, Newman-Keuls and nonlinear regression.

Both the DOPA and DA levels responded to light irradiance in a dose-response fashion. Mean DOPA accumulation was observed to increase progressively in response to increasing light irradiance. Mean retinal DA was observed to decrease progressively in response to increasing light irradiance. Both DOPA and DA data fit hyperbolic curves with respective coefficients of correlation of 0.70 ($P<0.01$) and 0.79 ($P<0.01$). These results indicate that retinal DA synthesis and presumably DA neuron activity shows a graded response to increasing irradiances of white light. Exposure to white light at 25 uW/cm^2 or greater appears to elicit the maximum response of these neurons. (Supported by DA 00755 and DA00083 to WWM.)

10.3 DOPAMINERCIC EFFECTS IN HUMAN VISION: SPATIO-TEMPORAL CONTRAST SENSITIVITY FLUCTUATES BETWEEN "ON" AND "OFF" PERIODS IN PARKINSON'S DISEASE. I. Bodis-Wollner[1,2] and S. Mitra*[2] (SPON: G. Lehrer[1]). Departments of Neurology[1] and Ophthalmology[2], The Mount Sinai School of Medicine of the City University of New York, One Gustave L. Levy Place, New York, NY 10029.

Several reports suggest abnormal visual evoked potentials in Parkinson's disease (PD) (see Bodis-Wollner et al., J Neural Transmisison, Suppl. 19, 1983). Our psychophysical studies in 76 PD patients suggested spatial contrast sensitivity losses centered on 4-9 cycles per degree and temporal losses in the range of 4-10 Hz. These regions represent peak sensitivities of normals. In PD, dopaminergic deficiency is known to be localized to the basal ganglia but it is not known whether or not there is a generalized deficiency also affecting retinal dopaminergic neurons (Frederick et al., J Comp Neurol, 210:65-79, 1982) in the human. We studied spatio-temporal contrast sensitivity in 10 PD patients undergoing dopaminergic therapy and showing "on" and "off" periods in their symptoms. It is believed that such "on"-"off" changes correlate with the availability and/or effectiveness of postsynaptic dopamine receptors. We found that visual changes accompany oscillations in motor symptoms. Patients studied had normal ophthalmological exam and normal acuity. From "on" to "off" one observes a flattening of spatio-temporal peak sensitivity and relative elevation of the low frequency region without high frequency changes. In several patients there are interocular differences. These frequency-specific contrast sensitivity changes in parallel with motor symptoms suggest a role for dopamine in tuning and amplification in human vision.

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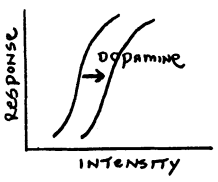
10.4 EFFECTS OF DOPAMINE ON RABBIT RETINAL GANGLION CELLS. Ralph J. Jensen. Department of Physiology & Biophysics, Washington University School of Medicine, St. Louis, MO 63110.

Using an isolated, perfused rabbit eyecup preparation, I have recently begun to examine the effects of dopamine on extracellular recordings from retinal ganglion cells. Results so far have been obtained primarily from OFF-center brisk ganglion cells, the majority of which have been "large field units". Dopamine (20-100 uM) was found to diminish the sensitivity of these ganglion cells to light stimuli (spots and annuli). Absolute thresholds of both center OFF responses and surround ON responses were elevated. The elevated thresholds were not the consequence of a tonic inhibitory input acting directly on the ganglion cells since dopamine did not decrease the spontaneous activity of these cells; spontaneous activity either increased or remained the same. With increased stimulus intensity, the responses of ganglion cells under the influence of exogenous dopamine were very similar to control, drug-free responses at lower light intensity. Plotting the size of ganglion cell response against stimulus intensity (intensity-response curve), I found that dopamine caused a parallel shift of the curve to the right. Shifts up to 1 log unit were observed on a few occasions for both center and surround responses. Whether dopamine causes similar shifts in the intensity-response curves for other ganglion cell types is currently being investigated.



The results suggest that dopamine may play a role in control of the state of adaptation of ganglion cells in the retina.

Supported by NIH Training Grant EY07057.

10.5 WHOLE-CELL CURRENT ANALYSIS OF ISOLATED HORIZONTAL CELL RESPONSES TO L-GLUTAMATE, KAINATE, AND QUISQUALATE. A. T. Ishida* and J. Neyton* (SPON:C. Sotelo). Lab. Neurobiologie, Ecole Normale Supérieure, 46 rue d'Ulm, 75005 Paris France.

Synaptic input from retinal photoreceptors to horizontal cells (HCs) is thought to be mediated by L-glutamate (L-GLU) since L-GLU and related analogs depolarize HCs in situ and in tissue culture. To study HC amino acid responses apart from non-synaptic voltage-dependent conductances and to estimate channel mean lifetime (τ) and conductance (γ) prior to single-channel analysis, we measured responses to L-GLU, kainate (KA), and quisqualate (QA) of HCs dissociated from goldfish retinas, under voltage clamp in the whole cell configuration of Hamill et al (Pflügers Arch. 391:85, 1981).

1) At HC resting potential (E_r, -65 to -70 mV), L-GLU, KA, and QA produce net inward currents accompanied by relatively small increases in noise. These currents reverse near zero mV, and rectify slightly in the outward direction. The reversal potentials for each agonist shift reversibly by approx. -15 mV when 50% of the external NaCl is replaced isosmotically by sucrose. Therefore these agonists appear to produce an increase in HC cationic conductance.

2) KA and QA are both effective down to 3 μM; L-GLU is 10X less effective. Responses to 3-30 μM KA, 3 μM QA, or 30 μM L-GLU show no change in amplitude for applications as long as a few minutes, and decay monotonically after wash-out. Responses to either 30 μM QA or 300 μM L-GLU reach a peak and decay within seconds to a plateau level; during wash-out these responses increase in amplitude before decaying completely. Steady-state amplitudes of KA responses at E_r increase with KA dose over a one log unit range, whereas those of L-GLU and QA responses increase with 0.5 log unit increases in dose above threshold and decrease at higher doses.

3) Both QA and L-GLU antagonize responses to KA. Even low doses of QA (3 μM) or L-GLU (30 μM) produce responses which neither sum linearly with, nor potentiate, responses to low doses of KA.

4) Power spectra of Fourier-analyzed noise in the L-GLU, QA, and KA responses can be fit by double Lorentzian functions. τ estimates from these spectra are approx. 1 and 6 msec for the fast and slow components, respectively, for each agonist. There is no pronounced dose-dependence in these values. Variance analysis of the noise suggests that γ is approx. 2 pS for each of the 3 agonists. Possible bandwidth limitations compel us to confirm these estimates in outside-out patches.

10.6 FUNCTIONAL ORGANIZATION OF GABA AND GLYCINE INPUT TO CAT RETINAL GANGLION CELLS. T. E. Frumkes, T. VOIGT* and H. Wässle*. Max-Planck Institut für Hirnforschung, 6000 Frankfurt 71, W. Germany

Ganglion cells from optically intact cat eyes were studied by extracellular recording and iontophoretic drug application. Results are from cells unequivocally defined as brisk sustained (X) or brisk transient (Y), and as On- or Off-center. 1). All units show similar decreases in activity to GABA and glycine, and all respond to their selective antagonists, bicuculline (BCC) and strychnine (STR). 2). Alone or in combination, STR and BCC can not eliminate center-surround RF (receptive field) antagonism. Furthermore, the responses produced by adding a center or removing a surround light are similarly influenced by STR and BCC. Apparently, GABA and glycine can play but a small role in spatial properties of ganglion cell RFs. 3). Intense stimulation of the RF center often results in the response sequence: transient response; a brief period of relative inactivity; a following period of sustained activity; and decreased firing at stimulus offset. Most evident in On-center cells, both periods of decreased firing are reduced by STR or BCC, and both drugs together generally produce greater reduction than increasing either alone. We suggest most ganglion cells receive transient inhibition at both light onset and offset from both GABAergic and glycinergic neurons, and this inhibition determines transient vs. sustained response characteristics. 4). In On-center cells, responses are similarly increased by STR and BCC, and STR similarly increases Off-center cell responses. However, providing the ratio of illumination between RF center and surround is less than 1 log unit, BCC usually decreases the overall activity of off-center cells. But in the presence of STR, BCC increases the overall activity of off-center cells. This suggests disinhibition: in addition to direct influences, GABAergic neurons inhibit the glycinergic input to Off-center cells. 5). The above results are independent of prevailing light adaptation level and whether rods and/or cones mediate photic responses.

10.7 PHARMACOLOGICAL MODIFICATION OF CENTER-SURROUND ORGANIZATION OF HORIZONTAL CELLS IN XENOPUS RETINA. S. Stone and P. Witkovsky. Dept. Ophthal., NYU Med. Ctr. New York, New York, 10016

In a previous report we described responses of the axon-bearing horizontal cell in the Xenopus retina (Hassin and Witkovsky, Vision Res. 23:921, 1983). These cells receive synaptic input from both rods and cones. In the present study we elicited responses from the same class of horizontal cell in eyecup preparations in which rod input was suppressed by continuous exposure to a diffuse, green adapting light. Under such conditions, the response is mediated by red sensitive (612 nm) cones.

Illumination of the surround with large spot or annular flashes evoked a rapid hyperpolarizing ON-transient followed by a prominent "rollback" to a more depolarized plateau potential for the duration of the stimulus. Occasionally the rollback exhibited small depolarizing transients. An additional feature of the light adapted horizontal cell response was a pronounced spike-like depolarization at light OFF. In contrast, equivalent amplitude responses to small central sports showed a square shaped waveform in which the rollback was reduced or absent; the OFF-transient was still apparent. When annular flashes were superimposed upon steady central illumination the depolarizing components of the horizontal cell response were enhanced and in some cells, a pure sustained depolarization in response to annular stimuli was observed. However intense central illumination tended to inhibit the rollback.

Superfusing the retina with GABA (5-7 mM) reduced the rollback and the OFF-transient. It also reduced the depolarizing component of the response to annuli superimposed on a steady central light. The effects of GABA were reversed by the GABA antagonist, picrotoxin (0.5 mM). Sr^{++} ions, which readily pass through Ca^{++} channels, greatly increased the amplitude of the OFF-transient and induced or increased the magnitude of the spike-like transients during the rollback.

Our findings support the view that in Xenopus horizontal cells, the depolarizing responses to surround illumination may be due to sign-inverting, GABA-mediated, synaptic feedback from horizontal cell axons onto cones. Accordingly, central cones would be depolarized by annular stimulation, resulting in a depolarizing response in the horizontal cell body. In addition, the effects of Sr^{++} suggest that these depolarizing responses in horizontal cells involve a calcium channel. Supported by EY 05527 to S.S. and EY 03570 to P.W.

10.8 ACTION OF THYROTROPIN-RELEASING HORMONE ON CAT RETINAL GANGLION CELLS. J. Bolz* and P. Thier* (SPON:C.D.Gilbert). Max-Planck Institut für Hirnforschung,6000 Frankfurt 71, West Germany.Present address:Lab.Neurobiology,Rockefeller University,New York,NY 10021 (J.B.) & Neurologische Universitätsklinik,7400 Tübingen,West Germany (P.T.).

A number of neuropeptides have been localized in particular classes of retinal neurons,but little is known concerning the functional role of these peptides. We attempted to ascertain the role of one peptide, thyrotropin-releasing hormone (TRH), by determining its effect on the light response and maintained activity of ganglion cells. A total of 62 cells of the cat retina were recorded extracellularly with multibarreled electrodes from the intact eye in vivo. Before application of TRH the ganglion cells were physiologically classified as brisk-sustained(X) or brisk-transient(Y); other classes were not analysed.

Under photopic stimulus conditions we found a differential action of TRH on ON- and OFF-centre cells which was independent of the brisk-sustained(X)/brisk-transient(Y) dichotomy. Application of TRH suppressed the light response and maintained activity of ON-centre cells. In contrast the firing of OFF-centre cells was enhanced by iontophoresis of TRH. The time course of the TRH response was similar to that seen after application of transmitters such as GABA,glycine, dopamine and serotonin.

Under scotopic stimulus conditions there was no influence of TRH on the ganglion cell discharge. The lowest background illumination at which a notable action of TRH could be observed was between 5-20 cd/m^2. This level of light luminance, when using a 4 mm artifical pupil, is in the mesopic range. The findings suggest, therefore, that TRH action requires activation of cone pathways in the retina.

Our results indicate that TRH does not act directly on ganglion cells, since under scotopic illumination exogenously applied TRH did not influence the ganglion cell activity, suggesting that TRH acts on neurons which are presynaptic to ganglion cells and are only active under photopic conditions.

A possible functinal role of TRH suggested by the reciprocal modulation of ON- and OFF-centre ganglion cells is in light adaptation. Ordinarily one might expect a rise in background illumination to increase the resting discharge of ON-centre cells and decrease that of OFF-centre cells, but the maintained activity of ganglion cells is largely independent of background illumination. The action of TRH is appropriate for this adaptive property.

10.9 CHOLECYSTOKININ IN THE CAT RETINA: ACTION OF EXOGENOUS CCK8 AND LOCALIZATION OF CHOLECYSTOKININ-LIKE IMMUNOREACTIVITY. P.Thier* and J.Bolz* (SPON: K.P.Hoffmann): Max-Planck-Institut für Hirnforschung, 6000 Frankfurt 71, F.R.G. Present address: Neurologische Universitätsklinik, 7400 Tübingen, F.R.G. (P.T.) and the Rockefeller University, Dept. Neurobiology, New York, NY 10021 (J.B.).

We tested the effects of iontophoretically applied cholecystokinin-octapeptide amide sulfated (CCK8) on the light response and the maintained discharge of cat retinal ganglion cells. Ganglion cells were recorded extracellularly with multibarreled electrodes in the optically intact eye in vivo. Only cells classified physiologically as brisk-sustained (X) and brisk-transient (Y) were analyzed. CCK8 suppressed the light evoked and the maintained discharge of X and Y cells without exception. This action of CCK8 was independent of the state of light adaptation and the drug did not alter the antagonistic organization of the receptive fields.

Using an antiserum directed against the C-terminal tetrapeptide of cholecystokinin, we studied the localization of CCK-like immunoreactivity in cryostat sections of the cat retina. CCK-like IR was found in a population of the larger amacrine cells. In the ganglion cell layer some small cells of approximately the same size as the amacrine cells were labelled. In addition CCK-like IR could be detected in horizontal cells and inconsistently also in ganglion cell somata and fibres.

An inhibitory action of CCK8 on cat retinal ganglion cells contrasts with its excitatory action in other parts of the brain. However, we cannot exclude that the action of exogenous CCK8 is mediated by excitatory receptors on interneurons, which in turn inhibit ganglion cells. The localization of CCK-like IR in a population of amacrine cells is in accordance with its localization in the retinae of other vertebrate species. CCK-like IR in horizontal cells and in ganglion cells could be attributed to a possible cross-reactivity with neurofilaments as was recently demonstrated for alpha-MSH-like IR found in these cells (Dräger et al., 1983).

10.10 CHEMICAL SYNAPSES OF CONE HORIZONTAL CELL AXONS IN THE GOLDFISH RETINA D.W. Marshak and J.E. Dowling. The Biological Laboratories, Harvard University, Cambridge, MA 02138

Cone horizontal cells in the retinas of cyprinid fish are among the most thoroughly-studied interneurons in the central nervous system, but we do not understand the roles of their axons (CHA) in visual information processing. Except for one preliminary report, no information concerning synapses made onto or by these elements is presently available. We report here that we have found that CHA's form conventional chemical synapses with at least 3 types of postsynaptic elements and 2 types of presynaptic elements. We have also demonstrated that processes of the dopaminergic interplexiform cell are among those presynaptic and postsynaptic to CHA's. Eyes were removed from light-adapted, 3" goldfish, hemisected, and immersed in a mixed aldehyde fixative. For immunocytochemistry, we used antiserum to tyrosine hydroxylase (kindly provided by Dr. Tong Joh). We sampled central and peripheral retina from all 4 quadrants of 1 eye and surveyed 2 other eyes. CHA's were identified by their abundant longitudinally-oriented microtubules and their high cytoplasmic electron density. The synapses were typical of the symmetric type observed elsewhere in the CNS. In 2 instances, small synaptic specializations were separated by a region of unspecialized membrane, as in perforated synapses. 0.5% of the CHA's contain presynaptic specializations in random, ultrathin sections. All except 2 of the synapses we observed were in the expanded, terminal portions of the axons in the middle of the inner nuclear layer, and the axons were mostly presynaptic. 40% of the postsynaptic elements were dendrites of intermediate electron density with very few microtubules, abundant microfilaments, and a few synaptic vesicles. 19% were more electron lucent dendrites with abundant microtubules. 11% of the postsynaptic elements contained abundant, small vesicles and large, dense-cored vesicles. Preliminary observations of labeled tissue indicate that these are tyrosine hydroxylase immunoreactive interplexiform cell processes. Somata in the middle of the inner nuclear layer received the remaining 30% of the synapses from CHA's. We also observed somata and interplexiform cell processes which were presynaptic to the axons, but these were seen far less frequently.

10.11 MONOCLONAL ANTIBODIES AGAINST COMPONENTS OF THE MOUSE RETINA. Grant W. Balkema and Ursula C. Dräger, Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Monoclonal antibodies against mouse retina are useful tools in studying the functional architecture of the retina in normal and mutant mice. We have used two immunization strategies: 1) we immunized with mechanically isolated retinal ganglion cell layer and used conventional fusion techniques. 2) we followed the immunosuppression-in vitro immunization technique of Matthew & Patterson (1983): we immunized either with a retina in which all photoreceptors had degenerated or with a retina from which the ganglion cell layer had been removed, then eliminated all responding B-cells with cyclophosphamide, and finally challenged the surviving B-cells in vitro with whole retina. The conventional technique yielded predominantly antibodies against filamentous components in glial cells. The in vitro fusions gave a much higher number and larger diversity of antibodies, and several of them were directed against the difference between the first and the second antigens. Our antibodies can be roughly classified into eight groups: 1) Several antibodies label the outer segments of the photoreceptors; one of them (2D5) appears to have a preferential affinity for photoreceptors in the periphery of the retina. 2) Antibody 1E9 has a high affinity for ribbon-like structures in the outer plexiform layer, labeling about 5 of them per $1\mu m^2$, and low affinity for cell nuclei in the ganglion cell and inner nuclear layer and for the inner segments of the photoreceptors. On Western blots 1E9 binds with high affinity to a band at 82K, and under low affinity conditions two additional bands appear at 76K, and 74K M_r. 3) Antibody 2B5 labels the ganglion cell layer (but spares the ganglion cell bodies), a laminar pattern in the inner plexiform layer, the outer plexiform layer, scattered round dots (~1μm) in the outer nuclear layer, and the inner segments of the photoreceptors. The labeling pattern in the retina is identical to the histochemical staining for cytochrome oxidase. 4) Antibody 2C6 labels some of the retinal ganglion cells and a few processes that extend into the inner plexiform layer. 5) Antibody 2A6 labels astrocytic processes on the vitreal surface of the retina. 6) Antibody M3G7 labels the outer limiting membrane in the retina. 7) Two antibodies (M3C9 & M3G4) label only the outer plexiform layer. 8) Several antibodies (e.g. 8F2) label the radial Müller glia and one class of horizontal cells.

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10.12 MONOCLONAL ANTIBODIES THAT DETECT CELL TYPE AND LAMINAR DIFFERENCES IN RAT RETINA.
K. Akagawa* & C. J. Barnstable. (SPON: P. R. MacLeish). The Rockefeller University, New York, NY 10021.

To understand the molecular basis of neural structure, function and development it is necessary to identify the molecules involved. We have been generating monoclonal antibodies against adult rat retina using retinal membranes as immunogen. We have previously described antibodies that allow us to label each major cell type of retina both in intact tissue and in monolayer cultures (Barnstable et al., Cold Spring Harbor Symp. Quant. Biol., 48 ,863,1984). Here we describe some new markers that detect novel molecular patterns in the retina.

Antibody 3.5E labelled membranes of bipolar cells. The dendritic processes in the outer plexiform layer, the somata and the axons were all clearly labelled. Labelling of the inner plexiform layer revealed a clear laminar pattern. This antibody differs from our previous bipolar cell markers in that it is not also strongly expressed on photoreceptor inner segments.

Antibody 2.6A labelled both inner and outer plexiform layers. No labelling of cell bodies was detectable. Bundles of axons in the optic nerve fibre layer were unlabelled suggesting that the plexifrom layer specificity was real and not an artefact of densely packed membranes. The labelling given by antibody 3.1A on the other hand was restricted to the outer plexiform layer. A further antibody, 6.1G, gave a highly punctate labelling of both plexiform layers that differed both quantitatively and qualitatively from that given by 2.6A.

Antibody 8.2E labelled the cytoplasm of ganglion cells and some cells in the inner nuclear layer. Since the ganglion cell axons in the optic nerve fibre layer and the horizontal cells were unlabelled this antigen is clearly different from the previously described ganglion cell markers Thy-1 and neurofilaments.

Together with our previously described antibodies these markers allow us to monitor and manipulate the cell development and composition of retina in vivo and in vitro . In addition, molecules restricted to synaptic regions will allow a better understanding of the processes of synapse formation, structure and function.

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10.13 POSTNATAL DEVELOPMENT OF GANGLION CELLS IN RABBIT RETINA. E. V. Famiglietti. Dept. of Anatomy, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

Time of birth for litters of pigmented rabbits was established within 8-16 hrs. Animals were sacrificed at timed intervals, the eyes removed and the isolated, flat-mounted retinas processed by the Golgi-Kopsch method. At 5 days postnatal, ganglion cells are immature with few dendritic branches of undetermined orientation. Morphological types identifiable in the adult by their dendritic branching pattern are not recognizeable at this stage. At 8 days postnatal, wide-field and narrow-field cells are differentiable, and narrow dendritic stratification, including bistratification, is evident. On the other hand, dendritic field diameters are small, and dendritic branching patterns typical of adults are not well established. Some aberrantly stratified dendrites appear to be present. It has been reported that at this stage only 9% of ganglion cells encountered respond to light, and none with differentiated receptive fields (Masland, 1977).

At 10 days of age, just before eye opening, most adult types of ganglion cell may be observed. These differ significantly from adult cells, however, as they are covered with long, slender, and branched spines, and the large-bodied, class I cells have reached only 50 to 60% of their final field diameters. Spines from adjacent dendritic branches are often in contact, bridging between daughter branches in ladder-like fashion, particularly in the case of the highly branched, type 1 bistratified ganglion cells. Most dendritic spines disappear in adult ganglion cells. Some of these may remain in the dendritic trees of type 1 bistratified ganglion cells, however, since slender touching branches or appendages are not uncommon in the adult cells. The latter cells have been identified as ON-OFF directionally selective ganglion cells (Amthor et al., 1983). At this developmental stage, directionally selective cells were found, and 80% of the ganglion cells were visually responsive, but only half had adult receptive field organization (Masland, 1977).

At 10 days of age, the dendrites of retinal ganglion cells receive synaptic input sufficient to yield complex responses indicating direction of motion. Nevertheless, homotypic dendro-dendritic interaction among ganglion cell dendrites, mediated by spinous processes may be equally important at this stage of development, perhaps functioning to regulate the orderly expansion and overlap of the growing dendritic trees of retinal ganglion cells.

EXCITATORY AMINO ACIDS: GLUTAMATE AND GLUTAMATE ANALOGS

11.1 QUISQUALATE-SENSITIVE GLUTAMATE RECEPTOR SUBTYPES STUDIED BY QUANTITATIVE AUTORADIOGRAPHY. S. Halpain, C. Wieczorek*, and T.C. Rainbow. Rockefeller Univ., New York, NY 10021 and Dept. of Pharm., Univ. of Penn. Sch. of Med., Philadelphia, PA 19174

Previously we reported evidence for at least two forms of sodium-independent ^{3}H-glutamate (Glu) binding to rat brain frozen sections prepared for in vitro quantitative autoradiography. The two forms are distinguished by different affinities for quisqualic acid (Quis). In subsequent studies we sought to map the locations of these two possible Glu receptor subtypes throughout the rat brain.

Using densitometric analysis of autoradiograms, we obtained IC_{50} values for each of the two sites in 3 separate anatomical structures. Each of the structures gave similar values for the 2 sites: $IC_{50}1$=300nM, $IC_{50}2$=2mM. Based on this 8000-fold difference in affinities, we calculated that 24uM Quis would occupy 99% of the high affinity site (Site 1) and only 1% of the low affinity site at an ^{3}H-Glu concentration of 300nM. 24uM Quis was therefore included in the next series of experiments to determine the locations of the two sites. High and low affinity Quis sites were distributed non-uniformly. Site 1 was most abundant in the anterior olfactory nucleus (AON) and CA1 and dentate gyrus of dorsal hippocampus, accounting for 50% or more of the total specific binding in these regions. Lesser but still high amounts of Site 1 were found in caudate, cerebellum, and lateral septum. Lowest amounts of Site 1 (<15% of spec. binding) were seen in medial geniculate, central gray, and ventroposterior thalamus.

Recently, a Quis analog, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) has been proposed as a selective agonist at a Quis subtype of glutamate receptor (Nature,284: 64). We therefore used ^{3}H-AMPA to quantitatively localize AMPA binding sites in frozen rat brain sections using procedures identical to those for ^{3}H-Glu. The pharmacological profile of this site agreed well with reports from membrane binding studies. ^{3}H-AMPA binding was more highly localized than ^{3}H-Glu binding. Highest levels were in dorsal CA1 and subiculum of the hippocampal formation and in lateral septum. Next highest levels were in AON, dentate gyrus, and frontal cortex. Lowest amounts of AMPA binding were seen in central gray, medial geniculate, and ventroposterior thalamus.

There appears to be a large degree of overlap in the locations of ^{3}H-AMPA binding sites and the ^{3}H-Glu binding sites which are readily displaceable by 24uM Quis. The "AMPA site" may thus account for much of the high affinity Quis sites labeled by ^{3}H-Glu in frozen sections. (Supported by NS19759, NS20006 to TCR and an Albert Cass Fellowship to SH)

11.2 QUISQUALATE-SENSITIVE L-[^{3}H]GLUTAMATE BINDING TO OLFACTORY BULB DENDRODENDRITIC SYNAPTIC MEMBRANES. M.R. Quinn. Dept. Pathol. Biochem., NYS Office for Mental Retardation and Developmental Disabilities, Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

The secondary dendrites of mitral cells form dendrodendritic synapses with primary dendrites of granule cells in the external plexiform layer of the olfactory bulb. Although a wealth of evidence supports GABA as a neurotransmitter utilized by granule cells, the nature of the mitral cell excitatory neurotransmitter remains controversial. Since glutamate and aspartate have been suggested as likely neurotransmitter candidates of mitral cells, we determined the pharmacologic specificity of Na^+-independent binding of L-[^{3}H]glutamate to membranes prepared from dendrodendritic synaptosomes (DDS) of rat olfactory bulb. DDS were prepared as previously described (Quinn & Cagan, J. Neurochem. 39:1381, 1982) and lysed in 40 vols of deionized water with the use of a Polytron. Membranes of DDS were sedimented at 48,000 g for 10 min and extensively washed in Tris buffer. Binding was measured by incubating membranes with 0.2 μM L-[^{3}H]glutamate and various concentrations of test compounds in 50 mM Tris-HCl (pH 7.1) at 37°C for 20 min. Nonspecific binding was measured in the presence of 0.5 mM unlabeled L-glutamate. Membrane bound ligand was separated from free by rapid filtration through Millipore type AAWP filters. Filters were rinsed with 10 ml of ice-cold buffer and radioactivity retained on the filter was measured by liquid scintillation spectrometry.

Specific binding of L-[^{3}H]glutamate to membranes of DDS reached equilibrium within 20 mins and freeze-thawing the membranes essentially abolished specific binding activity. Specific binding of L-[^{3}H]glutamate to membranes of DDS was saturable and Scatchard analyses revealed the presence of one site with a K_D = 0.56 ± .04 μM and a Bmax = 48 ± 5 pmol/mg protein. Hill plots also indicated the presence of one site and did not reveal cooperativity (n_H = 0.99 ± .03). Several compounds were tested over a range of concentrations for inhibition of L-[^{3}H]glutamate binding to membranes of DDS and the following K_i values (μM) were calculated: quisqualate, 0.57; L-glutamate, 0.62; L-homocysteate, 0.71; ibotenate, 0.83; D-homocysteate, 2.67; L-cysteinsulfinate, 3.54; D,L-2-amino-4-phosphonobutyrate, 4.42; L-aspartate, 9.0; D-glutamate, 11.4; D-aspartate, 15.62; L-glutamate diethylester, 253; quinolinate, N-methyl-D-aspartate, and kainate > 1000. The relative inhibitory activities of the above compounds suggest that a quisqualate-sensitive L-glutamate receptor mediates the excitatory influence of mitral cell dendrites within the olfactory bulb. (Supported in part by NS 18870 from NINCDS).

11.3 N-METHYL-D-ASPARTIC, KAINIC AND QUISQUALIC ACIDS EVOKED CURRENTS IN MAMMALIAN CENTRAL NEURONES. L.M. Nowak and P. Ascher*, Laboratoire de Neurobiologie, Ecole Normale Supérieure, Paris 75230, Cedex 05 France.

N-methyl-D-aspartic acid (NMDA) activates a distinct class of excitatory amino acid receptor in the vertebrate CNS. The distinction between kainic (KA) and quisqualic acid (QUIS) activated receptors is less certain. We have compared KA, QUIS and NMDA evoked responses obtained in patch clamp recordings as a preliminary step in determining the feasibility of separating these receptors by differences in their responses.

Neurones were dissociated from embryos (rat spinal cord, mouse mesencephalon and striatum) and grown in primary culture (3-30days). The whole cell recording mode was used to voltage-clamp small cells (Hamill et al. 1981,Pflügers Arch. 391:85) and study responses to bath applied agonists at room temperature. Extracellular solutions contained in mM: 140 NaCl, 2.8 KCl, 1.0 $CaCl_2$, 10 Hepes-Na^+(pH=7.2) with or without 1mM $MgCl_2$. Patch pipettes contained in mM: 140 CsCl, 4 NaCl, 5 EGTA-K^+/0.5 $CaCl_2$ and 10 Hepes-K^+(pH=7.2).

Not all neurones responded to each of the three agonists (10-50µM NMDA, 10-100µM KA, 0.1-10µM QUIS) since an occasional cell responded well to KA and not to QUIS or NMDA. At -60mV NMDA induced a large increase in noise for a small total inward current response whereas KA and QUIS evoked small increases in noise for comparatively large inward currents suggesting that KA and QUIS channels have a smaller unitary conductance than NMDA channels. Spectra obtained from NMDA and KA noise were fitted with single Lorentzians which corresponded to mean open times of near 5 ms for NMDA and near 2 ms for KA. QUIS noise generally could not be fitted by a single Lorentzian. The current-voltage (I-V) curves produced by all three agonists had reversal potentials of 0mV. QUIS and KA I-V curves both showed outward rectification and were not affected by addition of extracellular Mg^{++}, unlike NMDA curves which showed very marked outward rectification in 1mM Mg^{++}. Reducing Na^+ (to 50mM) and adding choline (104mM) in the bath shifted the reversal potentials to -22mV consistent with these agonists activating non-selective cationic channels. However, choline markedly reduced the NMDA evoked current and augmented the rectification of the QUIS I-V curve without changing the shape of the KA curve, thus having different effects on each of the three responses.

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11.4 SYNTHESIS OF QUINOLINIC ACID BY 3-HYDROXYANTHRANILIC ACID OXYGENASE IN RAT BRAIN TISSUE. A.C. Foster and R. Schwarcz Maryland Psychiatric Research Center, Baltimore, MD 21228.

The excitotoxic compound quinolinic acid (QUIN) is an endogenous metabolite of rat and human brain which is proposed as an etiological factor in human neurodegenerative disorders (Science 219: 316, 1983). In mammalian peripheral organs, QUIN is synthesized from 3-hydroxyanthranilic acid (3HANA) by 3-hydroxyanthranilic acid oxygenase (3HAO), an enzyme involved in the biosynthesis of NAD from tryptophan. We have now investigated 3HAO activity in rat brain tissue.

25 mg brain tissue was incubated at 37°C for 30 min in a solution containing 0.33 mM Fe^{2+}, 0.033 mM L-cysteine, 0.4 mM formate, 67 mM HEPES:NaOH buffer (pH 6.5) and 3 µM (carboxy-^{14}C)3HANA (6.1 mCi/mmol; kindly provided by Drs. E. Shaskan and L. Spitznagle, Univ. Conn.)in a total volume of 0.5 ml. Subsequently, the product (^{14}C-QUIN) was separated from the unreacted substrate on a Dowex 50W (H^+ form) cation exchange column. Blank values were obtained with boiled tissue.

The radioactive enzymatic product formed was identified as ^{14}C-QUIN by 4 criteria: (1) the product had an identical elution pattern to standard QUIN by HPLC analysis; (2) heating both the product and standard ^{3}H-QUIN in glacial acetic acid resulted in the formation of radiolabelled nicotinic acid;(3) purified hog kidney QUIN phosphoribosyltransferase (an enzyme specific for QUIN) converted both the product and standard ^{3}H-QUIN to radiolabelled nicotinic acid mononucleotide and (4) the product and standard ^{3}H-QUIN had identical R_f values by TLC analysis. Kinetic analyses indicated a K_m for the substrate of 2.6 µM and a v_{max} of 32.3±10.9 pmol QUIN/h/mg tissue (N=3). Omission of Fe^{2+} caused 63.7±2.4% inhibition of activity (N=5). The following compounds gave <25% inhibition of activity at 500 µM: QUIN, kainate, N-methyl-D-aspartate, L-glutamate, L-aspartate, kynurenate, picolinate, glutarate and L-tryptophan. A 4-fold variation of enzyme activity was observed between rat CNS regions: olfactory bulb>hypothalamus frontal cortex, hippocampus, striatum>cervical spinal cord, cerebellum. When expressed as a percentage of adult values, forebrain 3HAO activity (per mg wet tissue weight) was 83% at 7 days of age, rising to 143 and 168% at 12 and 15 days, respectively, and returning to 103 and 106% at 22 and 27 days of age.

Since our data show that rat brain tissue can synthesize QUIN by the action of 3HAO, it will be interesting to examine a possible involvement of this enzyme in excitotoxic phenomena.

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11.5 QUINOLINIC ACID PHOSPHORIBOSYLTRANSFERASE IN HUMAN AND RAT BRAIN: ACTIVITY IN HUNTINGTON'S DISEASE AND LESIONED RAT STRIATUM. R. Schwarcz, A.C. Foster, E.D. Bird and W.O. Whetsell, Jr. Maryland Psych. Res. Ctr., Baltimore, MD 21228, Brain Tissue Resource Ctr., McLean Hosp., Belmont, MA 02178 and Dept. Pathology, Vanderbilt Univ., Nashville, TN 37232.

Quinolinic acid (QUIN), an excitotoxic compound present in the mammalian brain, has been hypothetically linked to neurodegenerative disorders such as Huntington's disease (HD). Its specific catabolic enzyme, QUIN phosphoribosyltransferase (QPRT), has been characterized in rat brain (Abs. Soc. Neurosci. 9:327.3, 1983). To obtain information about the biochemistry of QUIN in neurodegenerative states, we have now measured QPRT activity in post-mortem samples of human brain from control and HD patients and in the rat striatum following excitotoxic lesions.

Human brains were obtained 1.5-10 hrs post-mortem and stored at -80°C until analysis; HD was confirmed histologically. Injections of QUIN (300 nmol) or kainic acid (10 nmol) in 1 µl were made into the left striatum of anesthetized rats.

In control human brains, QPRT activity was present in the caudate and substantia nigra>thalamus, hypothalamus, frontal cortex, hippocampus>cerebellum and putamen (N=5-9 each). The identity of the enzymatic product was confirmed by HPLC analysis. In control caudate, K_m values for QUIN and its co-substrate PRPP were 1.6 and 69.5 µM and v_{max} values 16.18 and 0.35 fmol/h/mg protein, respectively. QPRT activity in HD caudate tended to be higher than controls, the respective values (mean±SEM, N=9 each) being 365.7±52.5 and 242.0±50.8 fmol/h/mg protein ($0.1>p>0.05$, t-test). Kinetic analyses in HD caudate indicated similar K_m values for QUIN and PRPP but increased v_{max} values when compared to control. No differences in QPRT were apparent in putamen.

QPRT was elevated in QUIN-injected rat striata. Enzyme activities (ipsilateral as a percentage of contralateral striata) at various timepoints after injection were: 2 days: 163.6±19.5%; 14 days: 344.4±52.1%;7 months: 198.9±29.8% (N= 7 for each group). Kinetic analyses in the 7 month QUIN-lesioned group indicated an increase of v_{max} only. Similar increases in QPRT were observed 12 days and 1 month following striatal kainic acid lesions.

The results indicate that QPRT can increase in response to specific neurodegenerative events. However, the elevation of QPRT activity is not an exclusively QUIN-related process and could be associated with (glial?) tissue reactions to a loss of nerve cells.

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11.6 TISSUE LEVEL DETERMINATION AND BRAIN DIALYSIS SUGGEST SELECTIVE BUT DISTINCT RÔLES FOR NOREPINEPHRINE AND TAURINE IN THE ACUTE PHASES AFTER INTRAHIPPOCAMPAL QUINOLINATE INJECTION. Annamaria Vezzani and Robert Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228.

There exists a vast body of literature on the possible roles of norepinephrine (NE) and taurine (TAU) as modulators of seizure phenomena. The present study was designed to examine if the mechanisms which govern the actions of NE and TAU (and other neuroactive amino acids) in the brain are impaired in response to an intrahippocampal injection of quinolinic acid (QUIN). We had recently hypothesized that the actions of QUIN, an excitotoxic, convulsant brain metabolite, may be causally related to the precipitation of epileptic phenomena (Exp. Neurol. 84:1, 1984).

In a first set of experiments, hippocampal levels of NE, aspartate, glutamate, glutamine, glycine, TAU and GABA were measured bilaterally from 10 min up to 6 hr after the unilateral application of 120 nmol QUIN to unanesthetized rats. Only NE levels changed significantly as compared to controls. Starting 10 min after QUIN, NE levels decreased bilaterally, reaching a nadir of -70% at 120 min and returning to control within 6 hr. In order to gain insight into dynamic processes surrounding QUIN-induced seizures, amino acids (but not NE) were measured in perfusates obtained every 10 min through dialysis fibers bilaterally implanted into the hippocampi one day earlier. The fibers (adjusted to our needs with the kind assistance of A. Eliasson, U. Ungerstedt and E. French) were glued to bipolar electrodes and, unilaterally, to a guide cannula for intracerebral QUIN injection. Thus, the setup permitted continuous sample collection with simultaneous EEG recordings as well as QUIN application in unanesthetized rats. Of all amino acids measured, only TAU changed after a convulsant dose of 120 nmol QUIN (267±37% of baseline values during the first 2 hr; N=3). TAU increased on the QUIN-injected side only. There was no correlation between seizure activity and the TAU response.

The data indicate a close link between QUIN-related seizure mechanisms and noradrenergic function. Increases in TAU in the perfusates, apparently triggered by the physical existence of QUIN in the hippocampus, may reflect a selective tissue response to an overabundance of extracellular excitotoxins. Since tissue level determinations failed to reveal the dramatic increase in extracellular TAU, brain dialysis is clearly the method of choice for the further examination of the link between QUIN and TAU.

Supported by USPHS grant NS-16102 (to R.S.).

11.7 EXCITATORY AMINO ACID NEUROTOXICITY IS PRODUCED BY PASSIVE CHLORIDE INFLUX. S. M. Rothman. Departments of Pediatrics, Neurology and Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

The neurotoxic properties of glutamate (GLU) and other dicarboxylic amino acids have been repeatedly documented since 1969. Over this period evidence has accumulated that GLU may be a major excitatory transmitter in the central nervous system and may also be involved in the pathogenesis of a variety of neurological diseases. In the CNS, GLU opens channels permeable to at least sodium, which explains its depolarizing action. The factors responsible for toxicity are poorly understood. Possibilities suggested include: (1) increased frequency of action potentials and synaptic potentials; (2) sustained depolarization; and (3) excessive calcium (Ca) influx. Experiments with dissociated rodent hippocampus in tissue culture have provided new insights into the pathophysiology of amino acid neurotoxicity.

In one set of experiments, cultures were exposed to a balanced salt solution (BSS) containing 3 μM tetrodotoxin (TTX), 5 mM $MgCl_2$, no added Ca, and 1 mM GLU. The TTX and $MgCl_2$ were added to block action potentials and synaptic potentials. All neurons still died within 30 min; the culture appearance was indistinguishable from cultures kept in control BSS with GLU. In BSS with Ca, the Ca ionophore A23187 (30 μM) also failed to produce detectable changes after 30 min. These experiments indicate that action potentials, synaptic potentials, and calcium influx play no role in GLU-induced neuronal death.

When sodium was replaced by the impermeable cation benzoylcholine, neurons were protected from GLU. In addition, replacement of chloride by isethionate or sulfate, which are impermeable anions, was also protective. These observations suggested that GLU toxicity was mediated by passive chloride influx resulting from depolarization. This hypothesis was supported by other ion substitutions: depolarization with 90 mM potassium killed neurons when chloride, but not sulfate, was the balancing anion. Chloride- or sodium-free BSS also protected neurons from kainic acid and N-methyl-D-aspartate.

These results suggest a straightforward pathophysiology for excitatory amino acid neurotoxicity: depolarization leads to chloride influx down an electrochemical gradient; this causes increased cation influx, osmotic overload, and cell lysis.

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11.8 THE IONIC BASIS OF EXCITOTOXIN-INDUCED NEURONAL NECROSIS. J.W. Olney, M.T. Price, L. Samson* and J. Labruyere*, Washington Univ Sch Med, Dept Psychiatry, St. Louis, MO

It is thought that the neurotoxic activity of glutamate (Glu), aspartate (Asp) and related molecules (excitotoxins) is mediated through Glu/Asp excitatory receptors and entails sustained depolarization and altered permeability of postsynaptic dendrosomal membranes. While increased membrane permeability is presumably an essential precondition, the specific transmembrane ionic changes that play crucial roles in the ensuing neuronal necrosis have not been clarified. Contrary to speculation that excessive Ca^{++} influx into the postsynaptic neuron may be critical, Rothman (see abstract, this meeting) recently found that the toxic action of Glu on cultured rat hippocampal neurons in vitro is prevented by removal of Na^+ or Cl^- from the medium but not by removal of Ca^{++}. Using the chick embryo retina as an alternate in vitro system for studying Glu neurotoxicity, we have confirmed Rothman's findings.

Fifteen day chick embryo retinas were incubated for 30 min with Glu (0.6-1.2mM) in Hanks balanced salt solution (BSS) variously modified to alter the ionic makeup. Glu, in normal BSS, produced a fully developed lesion in 30 min. Substituting benzoylcholine (an impermeable cation) for Na^+, or $SO_4^=$ (a relatively impermeable anion) for Cl^-, prevented Glu toxicity, but eliminating Ca^{++} (plus adding EDTA) failed to alter the toxic reaction. Replacing the entire Na^+ content with K^+ (balanced by Cl^- as the major anion) but without including Glu, resulted in an acute cytotoxic reaction similar but not identical to the Glu reaction. This KCl-mediated toxic reaction failed to occur in high K^+ but low Cl^- medium. Thus, both K^+ and Glu have neurotoxic potential that is presumably depolarization-mediated and dependent upon excessive Cl^- influx. We also report that the potent excitotoxins kainate and N-methylaspartate destroy neurons in the inner layers of the in vitro chick embryo retina with potencies proportional to their excitatory potencies, that this neurodestructive action is Na^+ and Cl^- but not Ca^{++} dependent and that D-α-aminoadipate, D-2-amino-5-phosphonopentanoate and kynurenate, with varying degrees of receptor specificity, effectively block the neurotoxicity of excitotoxins in this retina preparation.

Our findings support Rothman's interpretation that the steady depolarization induced by excitotoxins entails, in addition to initial cation conductance changes, an unregulated passive influx of Cl^-, balanced ionically by further cation influx and osmotically by cytolytic increases in cell water. Excessive Ca^{++} influx does not appear to be a critical ingredient of this neurotoxic formula. Supported by RSA MH38894 to JWO.

11.9 KYNURENATE PROTECTS AGAINST EXCITOTOXIN-INDUCED NEURONAL NECROSIS IN CHICK RETINA. L. Samson*, J.W. Olney, M.T. Price and J. Labruyere* (SPON: E. Robins), Washington Univ Schl Med, Dept Psychiatry, St. Louis, MO.

The chick embryo retina displays a distinctive pattern of acute neuronal degeneration when incubated for 30 min in balanced salt solution containing low concentrations of the excitatory amino acids (EAA), glutamate (Glu), N-methyl-DL-aspartate (NMA) or kainic acid (KA). Removal of Na^+ or Cl^- from the incubation medium blocks the toxic action of these EAA but removal of Ca^{++} does not (Olney et al., this meeting). In the present experiments, we tested certain EAA receptor antagonists for their ability to protect the chick embryo retina against Glu, NMA or KA neurotoxicity.

Antagonists were tested in a range of concentrations against a fixed agonist concentration determined to be slightly above that required to consistently cause a fully developed retinal lesion (Glu 1mM, NMA 0.2mM, KA 0.025mM). Glutamic acid diethylester at concentrations up to 3mM was totally ineffective against any agonist. D-α-aminoadipate at 3mM slightly antagonized Glu and KA and provided nearly complete protection against NMA. D-2-amino-5-phosphonopentanoate at doses up to 1mM partially antagonized Glu and KA and at a lower concentration (0.3mM) completely blocked NMA toxicity. Kynurenate (Kyn) at 3mM protected completely against NMA and KA and partially against Glu. An hierarchy of cellular sensitivities to Kyn blocking was demonstrated; eg, when Kyn was paired with KA, gradually lowering the concentration of Kyn revealed loss of blocking first against one cell type, then another, etc. until all blocking was lost at 0.2mM Kyn (vs 0.025mM KA). Xanthurenate (Xan), a close structural analog of Kyn, at 3mM failed to block the toxicity of any agonist. Our finding that Kyn, which may be a natural CNS constituent, effectively protects against either NMA or KA toxicity and that Xan is without blocking action, confirms similar findings by Foster and Schwarcz (Neurosci Abst 9, 1983) in the in vivo striatum and hippocampus. Stone et al. (Brain Res, 1982) and others have shown Kyn effective in blocking the depolarizing actions of exogenous EAA or synaptic transmission at several CNS sites where EAA (Glu or aspartate) may be the natural transmitter. Thus, Kyn has several interesting properties: it is inexpensive, readily available, may be endogenously present in the CNS and effectively blocks both the excitatory and toxic activities of EAA at several EAA receptor subtypes. (Supported by RSA MH38894 to JWO.)

11.10 DOES CALCIUM MEDIATE EXCITOTOXIN-INDUCED NEURONAL NECROSIS? M.T. Price, J. Labruyere* and J.W. Olney, Washington University Sch Med, Dept Psychiatry, St. Louis, MO.

It is thought that the neurotoxic activity of glutamate (Glu), aspartate (Asp) and related molecules (excitotoxins) is mediated through Glu/Asp excitatory receptors and entails sustained depolarization and altered permeability of postsynaptic dendrosomal membranes. While increased membrane permeability is presumably an essential precondition, the specific transmembrane ionic changes that play critical roles in the ensuing neuronal necrosis have not been clarified. Current speculation has focused on excessive Ca++ influx as the mechanism responsible for Glu-induced cell death. The present studies were undertaken to determine whether the well established neurodegenerative reaction induced in the hypothalamus by systemic excitotoxin administration is associated with selective accumulation of Ca++ in degenerating neural elements and whether the Ca++ channel blocker nimodipine (believed to act postsynaptically to block voltage dependent Ca++ influx) can prevent excitotoxin-induced neuronal degeneration.

The potent excitotoxic analog of Asp, N-methylaspartate (NMA), was administered to 30 day old mice (100 mg/kg sc) and the arcuate hypothalamic (AH) nucleus examined 2, 6 and 24 hr later by the oxalate-pyroantimonate method for ultrastructural localization of Ca++. Although results were somewhat variable with respect to the intensity of Ca++ like precipitation, there was a consistent pattern of increased precipitation localized selectively in degenerating dendritic structures within the lesioned area. Since this merely establishes an association, not a causal relationship, between Ca++ influx and NMA-induced degeneration of AH neural elements, we pursued the issue further as described below.

Mice (30 days old) were injected with NMA (100 mg/kg sc) or nimodipine (3-10 mg/kg sc) or the same doses of both agents simultaneously and sacrificed after a 4 hr observation period for histopathological evaluation of the AH. Mice receiving nimodipine or NMA alone were asymptomatic following treatment but those receiving both agents displayed hyperexcitability and compulsive tail chewing. Nimodipine by itself induced no histopathological changes in brain. NMA induced a typical excitotoxin-type neurodegenerative reaction in AH. Nimodipine plus NMA resulted in a substantial increase in the number of AH neurons destroyed and in the tendency of the lesion to spread beyond the confines of the AH region. Thus, instead of preventing the neurotoxic action of NMA on AH neurons nimodipine, by a mechanism yet to be determined, augmented this action. For more definitive evidence contradicting the hypothesis that excitotoxin-induced neuronal necrosis is mediated by Ca++ influx, see Rothman and Olney et al., this meeting. (Supported by RSA MH38894 to JWO.)

11.11 ATTEMPTS TO RAPIDLY CONDITION INCREASED ACTIVITY TO CLICK IN SINGLE CORTICAL NEURONS OF AWAKE CATS USING GLABELLA TAP AND IONTOPHORETICALLY APPLIED GLUTAMATE. C.D. Woody, Y. Oomura, E. Gruen*, J. Miyake*, and V. Nenov*. Depts. of Anatomy and Psychiatry, UCLA Med. Center, Los Angeles, CA 90024

Earlier studies (J. Neurophysiol., 49:767-779, 780-791, 1983) found that rates of eyeblink conditioning could be accelerated by adding electrical stimulation of hypothalamic regions (HS) to presentations of click-CS and glabella tap-US and that short-latency activation of cortical neurons was a consequence of hypothalamic stimulation that could produce this effect. Further studies (Cooper and Woody, Soc. Neurosci. Abstr., 1983) found that this short-latency cortical unit activation could be suppressed by glutamic acid diethyl ester (GDEE), as could activation of the same units by glutamate.

Present studies averaged click-evoked activity in single units (n = 104) of the motor cortex before and after ten serial presentations of click plus glutamate (c + g), click plus glabella tap plus glutamate (c + t + g), and glutamate plus click plus glabella tap (g + c + t) and, in other cells (n = 20), after click plus glabella tap plus chloride (c + t + Cl^-). Unit activity was recorded through the same electrodes used to apply 0.5 M glutamate or 1.5 M Cl^- extracellularly (90 nA, 300 ms). Mean peak responses to click $\geq$ 3 sd above mean baseline levels of activity were found only during the initial ten click presentations and after the ten presentations of c + t + g. Some units responsive to click could be found within each group of cells tested. Among the responsive cells, responses to the ten "test" clicks were largest after presentation of c + t + g, with or without subtraction of mutual baseline activity. All but one of the cells responding after c + t + g showed increased activity in response to glutamate. Our results show that application of glutamate after click-CS and tap-US can produce effects on cellular activity resembling those found after adding HS to the same CS and US. The evidence favors the hypothesis that glutamate or a glutamate-like chemical is released at these cortical neurons by HS and that the resulting increase in activity contributes in some way to the rapidity of conditioning. (Supported by AFOSR F49620-83-C-0077)

11.12 AN IN VITRO PREPARATION FOR THE STUDY OF THE PHARMACOLOGY AND ELECTROPHYSIOLOGY OF THE VESTIBULAR NUCLEI. M. R. Lewis*, J. P. Gallagher and P. Shinnick-Gallagher. Dept. Pharmacology & Toxicology, Univ. Texas Med. Br., Galveston TX 77550.

The pharmacology of the vestibular nuclei has remained poorly understood due to the equivocal results obtained from in vivo studies. We have therefore developed a brain slice preparation containing the medial (MVN) and lateral (LVN) vestibular nuclei as well as a portion of the VIIIth nerve and Scarpa's ganglion. This preparation allows intracellular recording from the MVN while stimulating the vestibular nerve fibers or tract to evoke synaptic potentials. The slice is a sagittal section of approximately 400μ taken from the rostral medulla of a rat, and placed in warmed, oxygenated artificial cerebrospinal fluid. Intracellular recordings show MVN neurons have average resting potentials of -60mV, with approximately 80% demonstrating spontaneous action potentials of 70-80mV and firing at an average frequency of 20Hz. In certain cells, stimulation of the VIIIth nerve fiber tract within the slice generates graded excitatory postsynaptic potentials (EPSPs) or orthodromic spikes of 4msec latency with a conduction velocity of 1-20m/sec. These results suggest that these MVN neurons are second-order.

Second-order neurons in the MVN can be classified on the basis of their active or passive properties. Two types of spikes can be discerned: one type displaying a slow long lasting hyperpolarization following a fast afterhyperpolarizing potential, and another type of spike without the slow hyperpolarizing potential. Two types of responses to hyperpolarizing current injection are observed: 1) a non-rectifying electrotonic potential, and 2) a highly rectifying electrotonic potential.

During recording of VIIIth nerve tract evoked EPSPs, superfusion of atropine (5μM) and hexamethonium (500μM) had no effect on the EPSPs indicating the second-order synapse is non-cholinergic. The H_1 antagonist diphenhydramine at 5μM also had no effect. However superfusion of 0.5mM α-aminoadipate, an excitatory amino acid antagonist acting preferentially at N-methylaspartate receptors, caused marked depression of the evoked EPSPs while not affecting cell conductance or resting membrane potential . This indicates that neurotransmission at this synapse may be mediated by an excitatory amino acid. (Supported by NASA NAG-2-260)

11.13 THE EFFECT OF GLUTAMATE ON THE SEMICIRCULAR CANAL OF THE FROG. P.S. Guth, P. Valli, G. Zucca, L. Botta, C. Casella. Dept. of Pharmacology, Tulane Univ. Sch. Med., New Orleans, LA 70112 and Ist. Fisiol. Gen., Univ. Pavia, 27100 Pavia, Italy.

In the frog semicircular canal L-glutamate (Glu) has at least two sites of action: the hair cell (presynaptic) and the primary afferent nerve endings (postsynaptic). Glutamate's action on the hair cell results in the release of the natural transmitter. This presynaptic action: (1) requires Ca^{++} in the perilymph, (2) requires K^+ in the endolymph, (3) is antagonized by the excitatory amino acid antagonist D-alpha aminoadipate (DαAA), and (4) appears responsible for the large increase in frequency of firing of the nerve fibers. On the afferent nerve fibers Glu produces a long-lasting depolarization that: (1) does not, by itself, by the mode of application used, elicit action potentials; (2) is only weakly antagonized by DαAA; (3) causes refractoriness to the effects of the natural transmitter; (4) does not exhibit desensitization; (5) is not antagonized by Mg^{++}; and (6) does not seem to require K^+ in the endolymph or Ca^{++} in the perilymph. We have therefore reached the tentative conclusion that Glu is probably not the transmitter released by the hair cells of the frog semicircular canal because: (1) transmitters commonly cause a reduction of their own release rather than an enhancement, as seen in the present work; (2) Glu is incapable of inducing action potentials when the release of the natural transmitter is reduced or prevented; (3) the long-lasting depolarization (postsynaptic) causes a long-lasting refractoriness to the effects of the natural transmitter, a condition not suited to the rapid responses necessary in equilibrial organs; and (4) the depolarization of the nerve terminals caused by Glu does not exhibit desensitization or antagonism by Mg^{++}, as is seen at some glutamatergic synapses. Also to be described is a new method of applying substances to the isolated labyrinth or isolated semicircular canal that has distinct advantages over previous methods used. (Supported by a Fogarty Senior International Fellowship from NIH and a grant from the Consiglia Nazionale di Ricerche of Italy).

12.1 AUTORADIOGRAPHY USING TRITIATED AGMATINE AS AN INDICATOR OF PHYSIOLOGIC ACTIVITY IN HERMISSENDA VISUAL NEURONS. A.M. Kuzirian*, L.Hill*, J.T.Neary and D.L.Alkon (SPON: J.Disterhoft). Lab. of Biophysics, NINCDS-NIH, Marine Biological Laboratory, Woods Hole, MA 02543.

Yoshikami (Science 212:929,1981) demonstrated the potential use of a tritium labeled amine, agmatine (4-[1-^{3}H] (aminobutyl) guanidine) to identify and assess sensitivity of specific neurons to acetylcholine (ACh) activation of ionic conductance channels. Such small cationic amines have been found to permeate ACh-activated ionic channels in sympathetic neurons and endplate channels in vertebrate skeletal muscle (Dwyer et al., J. Gen. Physiol. 75:469,1980). Elements of the photic pathway in the nudibranch Hermissenda crassicornis have also been demonstrated to be cholinergic (Heldman et al., J. Neurophysiol. 42:153,1979) and synaptic interactions have been studied electrophysiologically (Alkon, Biol. Bull. 159:505,1980). Experiments were conducted on isolated nervous systems to test the usefulness of ^{3}H-agmatine to identify autoradiographically post-synaptic cells and as a possible indicator of physiological activity within the photic pathway under conditions of darkness and intermittent flashes of light. Under conditions of illumination, type B photoreceptors (central or medial) showed distinct agmatine uptake above background as did putative interneurons located within the cerebropleural ganglion (CPG) in proximity to the region of demonstrated cholinesterase activity by optic nerve fibers. Nervous systems kept in total darkness showed no significant uptake by the type B photoreceptors and less uptake by cells along the photic pathway within the CPG. The light elicited uptake of the radiolabel is consistent with the known post-synaptic cholinergic function of the photoreceptors. Specific optic ganglion cells in both light and dark conditions also accumulated the radiolabel as did sensory hair cells in the statocyst and their axons within the static nerve. Several identifiable neurons (75-150 μm diameter) in the CPG and pedal ganglion (PG) showed high levels of agmatine but only a few consistently showed changed uptake patterns between light and dark conditions. These results indicate that tritiated agmatine autoradiography provides a histologic means of monitoring physiologic activity within known elements of the Hermissenda visual system.

12.2 CABLE PROPERTIES OF APLYSIA R2 NEURON. D. Junge. Sch. of Dentistry and Dept. of Physiology, Univ. of Calif., Los Angeles, CA 90024.

When a current is injected into the cell body of an R2 neuron, how much of it flows into the axon? I have attempted to answer this question by measuring somatic and axonal parameters directly, and combining them in an overall model of the cell. For this study I used adult animals of 15-20 cm in length. First, the responses of somas with more than 4 cm of attached axon were studied. Potential changes and time derivatives of potential were recorded in response to small inward steps of current. The values of input resistance and time-constant found were 0.97 ± 0.39 (S.D.) MΩ and 0.131 ± 0.036 s. The somatic charging curves were only approximately fitted by single exponential functions. Charging curves were better fitted by the Rall theory, but equally good fits were obtained over a sixfold variation of ρ, the axon/soma conductance ratio, by small adjustments of the membrane time constant. In order to better determine the axon/soma coupling the axonal parameters were measured directly with two electrodes, one of which was connected to a stimulus-subtracting circuit. The values obtained by this method were R_{in} = 1.82 ± 1.09 MΩ, λ = 0.208 ± 0.071 cm, and τ = 0.31 ± 0.094 s. The length constant was smaller than the amount previously measured with one electrode in the soma and one in the axon (Tauc, L., 1962, J. Gen. Physiol. 45, 1077; Junge, D. and Miller, J., 1974, Nature 252, 155.) This could reflect the different properties of the initial segment, or trophospongium, which is interdigitated by Schwann cells (Coggeshall, R.E., 1967, J. Neurophysiol. 30, 1263). If the axon was considered as homogeneous throughout, then the end-resistance would be twice R_{in}, or 3.64 MΩ. Since the conductance of the soma-plus-axon was 1.03 μS and the axon end-conductance was 0.275 μS, the soma conductance should have been 0.755 μS, which indicated that ρ was about 0.364. A numerical model based on measured axon parameters was able to give good fits to the axon charging curves. The charging curves were best fitted when the length-specific internal resistance of the initial segment was less than that in the remainder of the axon. This finding could account in part for the larger length constant measured from soma-to-axon than with both electrodes in the axon. In conclusion, the answer to the original question appears to be "about 27%," with the remaining 73% going through the somatic membrane, the ratio of these fractions being ρ.

12.3 A NOVEL CYTOSKELETAL PROTEIN OCCURRING IN SUBSETS OF BRAIN NEURONS. B. Riederer* and A. Matus. Friedrich Miescher Inst., P.O.Box 2543, 4002 Basel, Switzerland.

A library of monoclonal antibodies prepared against rat brain postsynaptic densities (PSDs) contained two independent primary clones which secreted antibodies reacting with a Mr 160,000 PSD polypeptide. Both antibodies stained only neurons and in most brain areas labeling was strikingly confined to subsets of neurons. In the hippocampus CA3 pyramidal cells were strongly stained whereas CA1 cells and granule cells of the dentate gyrus were entirely negative. Subsets of pyramidal cells were also stained in the neocortex, and there was selective labeling of neurons in the basal ganglia, brain stem and cerebellum.

In all labeled cells the staining pattern was the same, with the antigen present throughout cell body, dendrites and axon. Within the cytoplasm the stained material was distributed in a fibrous pattern suggestive of a cytoskeletal affiliation. To test this possibility we prepared brain microtubules, neurofilaments and actomyosin and examined them for their content of the 160,000 peptide by western blotting. All were negative. Western blots of a series of brain fractions showed that the antigenic protein was present in brain homogenate, the 1000xg (P1) pellet and in the PSD fraction. These results suggest that this protein is associated with filamentous material in the cytoplasm of specific types of neurons. Although it does not appear to be an integral component of the known neuronal cytoskeletal structures, it may be associated with one of them in a manner which does not survive cell fractionation. By contrast its presence in isolated PSDs may indicate that it is an integral PSD component.

12.4 FLOW CYTOMETRIC ISOLATION AND CULTURE OF A NEURONAL SUB-POPULATION FROM THE EMBRYONIC MOUSE SPINAL CORD (SC). G. Kapatos, J. Mazzetta*, J.L. Barker, A.B. MacDermott and M.T. Caserta. LNP, NINCDS, NIH. Bethesda, Md. 20205.

The development of a research strategy for the isolation and culture of specific populations of neurons from the mammalian central nervous system (CNS) remains a major goal of contemporary neurobiology. We have previously reported using in vitro sensitization techniques to generate a panel of surface-reactive monoclonal antibodies (mAbs). One mAb, an IgM designated G1, recognizes a surface antigen present on approximately 30% of the neurons in 1-4 week old cultures derived from embryonic mouse SC. Western blot analysis of membrane proteins prepared from these cultures show that this mAb binds primarily to a 41KD protein. Probing identical blots with an IgM class mAb directed against actin indicates that this 41 KD protein is not actin. Ligand competition studies performed on blots suggest that the epitope recognized by the G1 mAb is composed in part of a carbohydrate moiety containing N-acetyl-glucosamine. Flow cytometric analysis shows that 10-20% of cells dissociated from the embryonic mouse SC are G1+ and that while cell-surface proteolysis with trypsin (0.1% trypsin for 30 min. at 37° C) eliminates G1 binding, removal of sialic acid residues with neuraminidase (50U/ml for 30 min. at 37° C) increases the number of G1+ cells. These data are in agreement with the biochemical characterization of G1 antigen as a glycoprotein. Re-analysis of sorted G1+ cells stained with propidium iodide indicates a sorting efficiency of at least 90%, and variable cell viability. G1+ cells isolated by flow-cytometry have been maintained for weeks in culture on feeder layers of cortical astrocytes. Cultures of G1+ cells reacted again with the G1 mAb show that 90-95% of these cells are G1+. These cells exhibit neuronal morphology, synthesize neuron-specific enolase and glutamic acid decarboxylase (see Caserta et.al.,this volume), and display excitable membrane properties. The latter can include firing of action potentials, repetitive firing of action potentials during sustained depolarization and spontaneous synaptic activity. We conclude that the G1 antigen is a glycoprotein on the plasma membrane of a specific subset of neurons derived from the embryonic mouse SC. More generally, these results suggest that isolation of neurons from the embryonic CNS using immunofluorescent surface markers and flow cytometry may well be applicable to many, if not all, subtypes of embryonic CNS cells.

12.5 A PUTATIVE CELL-SURFACE MARKER IDENTIFIES A SUBPOPULATION OF GABAERGIC NEURONS IN MOUSE SPINAL CORD. M.T. Caserta, J. Mazzetta*, J.L. Barker and G. Kapatos. Lab. of Neurophysiology, NINCDS, NIH, Bethesda, MD 20205.

We have previously shown (Kapatos et al., Soc. Neurosci, Abstr. 9(1): 343, 1983) that a monoclonal antibody (G1) directed against the surface of GH3 cells, a pituitary tumor cell line, identifies a population of neurons in the embryonic mouse spinal cord. These neurons can be isolated from the spinal cord and grown in tissue culture for many weeks (Kapatos et al., this volume). We report here that the majority of these neurons are glutamic acid decarboxylase positive (GAD+) and are presumably GABAergic.

When embryonic mouse spinal cord cells were reacted with the G1 antibody, a fluorescent secondary antibody and sorted by flow cytometry, a population of G1-positive (G1+) and G1-negative (G1-) cells were collected, plated on astrocytic feeder layers and grown in culture for several weeks. These G1+ cultures were stained with an antibody to GAD which has previously been shown to label GABAergic neurons in culture (Caserta and Barker, Soc. Neurosci. Abstr. 9(1): 7, 1983). Cell counts revealed that 70-90% of the neurons in the G1+ cultures were GAD+. G1- cultures also contained GAD+ neurons, but in lower number (20-30%). To control for the effect of plating density, feeder layer induction and sorting artifact, mouse spinal cord cells were labelled with a monoclonal antibody that identifies the surface of all neurons (A2B5), sorted and plated like the G1 cultures. These were then stained with the GAD antibody and GAD+ cells counted. The results indicate that neither plating density nor feeder layer nor sorting affect the expression of GAD positivity in culture. In these cultures 20-30% of the neurons were GAD+, which is the same percentage found in routine mouse spinal cord cultures. Double labelling of routine cultures with the G1 antibody and the GAD antibody revealed that 70-80% of the G1 labelled neurons were GAD+. There were numerous GAD+ neurons that were not labelled with G1 (approx. 50%).

Therefore our monoclonal antibody G1 labels a subpopulation of GABAergic neurons and might be considered a putative cell-surface differentiation marker for these cells. Recent experiments have demonstrated that some of these G1+ neurons also contain either somatostatin, met-enkephalin or substance P. Further studies are now in progress to determine whether the G1 antibody marks a population of neurons in which GABA is co-localized with another neurotransmitter.

12.6 CHARACTERIZATION AND FLUORESCENCE ACTIVATED CELL SORTING OF HIPPOCAMPAL NEURONS WITH A MONOCLONAL ANTIBODY GENERATED AGAINST DEVELOPING RAT DENTATE GYRUS. Anne E. Schaffner and Joseph R. Moskal*, Lab.of Neurophysiology, NINCDS, NIH and Lab. of Cell Biology, NIMH, Bethesda, MD 20205.

The hippocampus, with its well-defined internal structure but complex connections and ascribed functions is a particularly interesting brain structure. We have previously described several monoclonal antibodies which bind to cell-surface, trypsin-sensitive antigens in the hippocampal formation. Employing histochemical, fluorescence-activated cell sorting (FACS) and radioimmunoassay techniques we have examined the developmental appearance of the antigen recognized by the antibody, G6E3. In the adult rat, antibody binding appears to be restricted predominantly to pyramidal and dentate gyrus cells and cerebellar Purkinje cells. At embryonic day 19 antibody binding has been observed in olfactory bulb, cortex and spinal cord as well. Furthermore, the antigen appears as early as embryonic day 13 in brain and spinal cord. Antibody binding is also present on approximately 50% of cell bodies and their proximal processes in cell cultures derived from 19-day rat embryonic hippocampi. Two week old cultures were stained for G6E3 reactivity by indirect immunofluorescence followed by PAP histochemistry for neuron specific enolase (NSE) reactivity. G6E3 fluorescence was coincident with NSE+ cells. When cultures were stained for both G6E3 and glutamic acid decarboxylase (GAD) reactivity over 95% of the G6E3+ cells were GAD negative. However their cell bodies and processes were covered with GAD+ boutons. It therefore appears that the antigen is present on hippocampal neurons that receive GABAergic innervation. G6E3+ cells from 19 day embryonic or 2 day postnatal rat hippocampi were sorted on a BD FACS 440 and plated on a feeder layer of nonneuronal cells in the presence of hippocampal conditioned medium. After 1 week all sorted neurons restained positively with G6E3. Optimal conditions for maximal survival and long term maintenance are currently under investigation.It should now be possible to directly correlate the presence of the antigen with specific electrophysiological and pharmacological properties as well as study the function of the antigen itself. By exploiting the binding properties of G6E3, the different birthdates of pyramidal, granule and Purkinje cells and their anatomical distributions we hope to be able to isolate and maintain in culture pure populations of these cells.

12.7 RADIOAUTOGRAPHIC IDENTIFICATION OF ^{125}I-NERVE GROWTH FACTOR BINDING TO CELLS FROM NEUROFIBROMAS. Kenneth H. Sonnenfeld*, Paulette Bernd, and Allan E. Rubenstein. Dept. of Neurology, Mt. Sinai Sch. Med., New York, N.Y. 10029

Benign dermal neurofibromas are tumors often associated with neurofibromatosis (NF). The embryonic origin of the cells contributing to the tumor mass is thought to be the neural crest, a structure that gives rise to elements of the nervous system such as neurons of the sympathetic and dorsal root (DRG) ganglia, Schwann cells and perineural fibroblasts. Nerve growth factor (NGF), a polypeptide required for the normal development and function of sensory and sympathetic neurons may be involved in the pathogenesis of NF. As a means of identifying cells in neurofibromas that might be regulated by NGF so as to contribute to cell proliferation and tumor growth, cell cultures of dissociated neurofibromas were established and tested for the presence of cells that bind ^{125}I-NGF. Neurofibromas were dissociated during digests with either collagenase-trypsin or collagenase-dispase. Suspensions of single cells were plated into 24 well Limbro plates containing glass coverslips and incubated for five days in RPMI 1640 medium containing 15% fetal calf serum (FCS) and antibiotics in a 37^{o}C humidified atmosphere of 95% air-5% CO_2. After 5 days, the glass coverslips and attached cells were washed for 1 hour in fresh RPMI-FCS and incubated for 1 hr at 37^{o}C, 5% CO_2 in medium containing ^{125}I-NGF (5 ng/ml). Coverslips were then processed for radioautographic study.

Following 6 washes in PBS and fixation in 2.5% glutaraldehyde coverslips were mounted on glass slides and coated with photographic emulsion (Ilford L4). Development after 1 wk of exposure revealed a subpopulation of cells with a dense accumulation of grains indicating binding of ^{125}I-NGF. Binding to cells incubated in parallel with unlabeled NGF, 10 ug/ml, was not observed. Histochemical and immunocytochemical studies are in progress to further characterize this cell population. These studies indicate that neurofibromas contain cells having NGF receptors. The functional significance of these receptors to this cell population and to the overall growth of the tumor is unknown. Investigations concerning NGF effects on cell proliferation, survival and chemotaxis are underway. Such studies should help to determine whether cells with NGF receptors are primary components of the tumor or reactive, migrating toward a high source of NGF-like activity. This study may also suggest that additonal target cells for NGF besides those previously described exist in the periphery.

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12.8 MONOCLONAL ANTIBODIES TO TURTLE VISUAL CORTEX. J.M. Shen*, A.R. Kriegstein, J. Boughton*, and N. Eshhar*. Dept. Neurol, Stanford Univ. Sch. of Med., Stanford, CA 94305.

The general cortex of the turtle has attracted the attention of neurobiologists because of its relationship to mammalian neocortex. The turtle cortex is a relatively simplified structure containing only three cortical layers and two basic cell types, pyramidal cells and stellate interneurons, subclasses of which have not yet been defined. We are preparing monoclonal antibodies to turtle cortical tissue (P. scripta) in order to help answer several specific questions: 1) Are there distinct subclasses of pyramidal and stellate cells with similar physiological, chemical and structural properties that can be identified by monoclonal antibodies? 2) When are neuron-specific antigens expressed during development? 3) Are there homologies that exist between turtle cortical neurons and their counterparts in the neocortex of higher animals?

Spleen cells of mice immunized with adult turtle cortex were fused with an X63-Ag8-653 myeloma cell line, and antibody-secreting hybridomas were identified by radioimmunoassay (RIA) and visualized by indirect immunofluorescence on unfixed frozen cortical sections and/or immunoperoxidase staining on fixed tissue. Monoclonals were obtained using the technique of limiting dilutions and then injected into pristane-primed mice for ascites. Based on these results, we have established several stable, independent hybridoma colonies which generate antibodies that are directed against anatomically localizable antigenic determinants.

Several antibodies bind to neuronal membrane antigens. Among these are TC1 which stains a subset of pyramidal cell bodies and their proximal dendrites, and TC2 which outlines cell bodies in some areas of the pyramidal layer. Neither one selectively labels neurons in the molecular and subcellular zones. Two antibodies label processes that run perpendicular to the surface of the cortex: TC5 stains cell bodies and radial fibers of glia-ependymal cells, and TC6 labels fine processes found throughout the cortex. RIA shows that the antibodies have different levels of cross-reactivity with turtle spleen cells and/or newborn rat cortex. Histological studies on mammalian brain tissue are currently underway to localize cross-reacting determinants.

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12.9 CALCIUM-BINDING PROTEIN IMMUNOCYTOCHEMISTRY OF PURKINJE NEURONS IN CULTURE. W.J. Hendelman, S.S. Jande* and D.E.M. Lawson*. Dept. of Anatomy, Univ. of Ottawa, Ottawa, Canada, K1H 8M5; Dunn Nutritional Laboratory, Univ. of Cambridge, Cambridge, England, CB4 1XJ.

Vitamin D-dependent calcium-binding protein (D-CaBP), a protein present in intestine, kidney and other tissue, is also known to be present in the brain. In the intact animal, D-CaBP has been demonstrated using immunocytochemistry in a wide variety of neurons and particularly in the Purkinje neuron. Explant cerebellar cultures from the newborn mouse, grown in the Maximow chamber, develop in vitro in about 3 weeks. These organized cultures consist of a cortical region (with Purkinje neurons) and a deep nuclear region. Cultures were maintained with a medium consisting of 25% human serum, 25% chick embryo extract and 50% Eagle's medium, supplemented with glucose to a final concentration of 11 gm/L. Mature cultures were fixed with Carnoy's, embedded in paraffin, detached from their coverslip and sectioned at 4µm. After the immunocytochemical reaction for D-CaBP, sections were reacted using the peroxidase-antiperoxidase technique. The Purkinje neuron was stained in its entirety - soma, dendrites, axon and terminals in the deep nuclear region. Deep nuclear neurons, which do not react in vivo, did not stain in these cultures. Some cultures were grown in serum-free defined medium and had fewer Purkinje neurons at maturity; the results were qualitatively the same as in the serum plus embryo-extract medium. Preliminary results indicate that the Purkinje neurons of the mouse in the late fetal stage already stain for D-CaBP. Although the role of this protein in the function of the neuron is unknown, this property of the Purkinje neuron is apparently maintained in these cultures. This immunocytochemical marker may prove useful to identify neurons of a specific type in culture.

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12.10 **MOLECULAR STUDIES OF NEURAL ORGANIZATION,** 1. O.Sundin, 2. S. Hockfield, 3. Ronald McKay MRC Lab. Mol Biol, Cambridge, England; Cold Spring Harbor Lab, NY; Biology & Whitaker College MIT

Recently the tools of molecular biology have become sufficiently simple to allow the experimental investigation of patterns of gene expression in complex tissues. We have used hybridoma technology to study both the molecular complexity and the cellular organization of the nervous system. More recently we have applied recombinant DNA techniques, particularly cDNA cloning, plus minus screening and *in situ* hybridization to these same problems.

Using hybridoma technology we showed that the nervous system of the leech and of the cat was composed of antigenically diverse cell types. With both invertebrate and vertebrate systems it became clear that specific monoclonal antibodies allowed a high resolution analysis of *in vivo* cellular organization (Hockfield and McKay, 1983 PNAS 80 5758; 1983 J. Neurosci 3 369; Hockfield et al.,1983 CSH Symp, 48 877; Hendry et al., 1984,Nature, 307 267; McKay et al. 1983,Science 222 788; 1983 CSH Symp 48 599).

We are now using recombinant DNA techniques to obtain systematic data on the molecular complexity of the vertebrate brain. cDNA libraries in various plasmid vectors have been made with oligodT selected sequences from the embryonic neural tube and different regions of the adult brain of the rat. We are now using plus minus screening and *in situ* hybridization to obtain probes which mark cell types during particular stages of differentiation. Our results with these cDNA libraries show that diferential gene expression results in many distinct cell types in the nervous system. The technical advantages of using cDNA libraries suggest that we may be able to obtain a more complete description of the number and organization of different cell types in the vertebrate brain.

12.11 SCHWANN CELL SURFACE MARKER DEFINED BY A MONOCLONAL ANTIBODY (224-58) WITH CROSS SPECIES REACTIVITY. B. Zalc‡A.Guerci‡ C. Gouget-Zalc‡M. Monge‡A. Baron-Van Evercooren‡ S. Dancea‡ J.M. Boutry*(SPON: J.P. CHANGEUX) Lab of Neurochimie, INSERM U-134, Hôp. de la Salpêtrière, 75651 Paris, France.

Mice (C57Bl/6) were immunized with human brain myelin. Spleen cells from the sensitized animals were fused with a mouse non secreting cell line (Sp2/0-Ag14). Supernatants from the hybridomas were screened by Elisa on human brain myelin. Positive secreting hybrids were cloned. One of them (224-58)secreted an IgM which reacted with sulfogalactosylceramide (SGC) by Elisa, complement fixation and immunoautoradiographic detection on thin layer chromatography.

Chemical and enzymatic treatments of SGC and total lipid extracts, combined with immunological screening against various glycolipids closely related to SGC, have allowed us to analyze precisely the molecular structure of the epitope recognized by the monoclonal (mAb) 224-58.This epitope is formed by the sulfate group in the C_3 position of galactose, the β1-1 osidic bond and the carbonylic function of the fatty acid.

Interestingly enough, the structures labelled by the mAb 224-58 on tissue sections treated for indirect immunofluorescence had a completely different topographical distribution from the ones previously described using a polyclonal affinity purified specific antiSGC antibodies preparation (Zalc et al. Brain Res.,211:341,1981).Indeed, on tissue sections, only peripheral nerve preparations were 224-58 positive, while brain, kidney, spleen or liver were always 224-58 negative.The mAb 224-58 reacted as well on mouse, rat or human sections of peripheral nerve. On these latter sections, only the Schwann cells membrane was 224-58 positive. The surface labelling of Schwann cells was confirmed by immunolocalisation on sciatic nerve teased fibers or dissociated cells preparations and also on Schwann cells primary cultures. Double labelling experiments with rabbit anti-Thy1-1 or anti laminin antiserum have also confirmed the specificity of 224-58 for Schwann cells. The discrepancy between the staining observed with the polyclonal antiSGC antibodies and the mAb 224-58 will be discussed.

12.12 GLYCOPROTEINS IMMUNOCYTOLOGICALLY DISTRIBUTED ON NEURONS AND GLIAL CELLS IN THE LEECH CENTRAL NERVOUS SYSTEM, ARE THEY MOLECULARLY RELATED? T. Flanagan, M. Flaster, J. MacInnes* and B. Zipser. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

Three MAbs raised against leech nerve cord homogenates immunocytologically stain the surfaces of neurons and glial cells. Two of these MAbs stain axons which project in bundles, and the 3rd MAb labels antigens on axons and on glial cells. In Western blot studies, these MAbs recognize multiple antigens; they each bind a 130 kd antigen and 1 to 4 additional lower molecular weight antigens. The 130 kd antigen is clearly a glycoprotein. It has been affinity isolated from lectin columns, but does not bind to recine columns. The 130 kd glycoproteins are not members of the major sets of concanavalin A-binding glycoproteins detected in extracts of the leech nervous system. Cross immunoprecipitation studies indicates that each MAb recognizes a distinct 130 kd glycoprotein, but persistence of a low level of crossreactivity between 2 of these MAbs suggests some molecular homology between their respective antigens. The strength of this homology is additionally examined with isoelectric focusing. Partial homology among sets of glycoproteins may indicate that they are derived from a common ancestral molecule and hence related. These glycoproteins are not members of the major glycoprotein sets within the leech CNS. and, therefore, they may be a class of molecules associated with specific interactions between select cell types.

13.1 SPINAL PROJECTIONS OF THE GIGANTOCELLULAR RETICULAR FORMATION IN THE RAT. EVIDENCE FOR DIFFERENTIAL PROJECTIONS TO LAMINAE I, II AND IX. G.F. Martin and R. Waltzer, Department of Anatomy, The Ohio State University, Columbus, Ohio 43210. R.P. Vertes, Mercer University, School of Medicine, Macon, Georgia 31207.

We have used axonal tracing techniques to study the organization of bulbar reticulospinal connections in the rat. Injections of ^{3}H-leucine were made into each of the reticular nuclei of the medulla shown by retrograde transport studies to innervate the spinal cord. All animals were anesthetized for the injections. Twelve to fourteen days after surgery the animals were sacrificed and perfused so that their brain and spinal cord could be removed for autoradiographic processing. Exposure times of 4 to 12 weeks were employed. Of particular note was the evidence for bilateral projections from the gigantocellular reticular nucleus (the Gi of Paxinos and Watson, 1982) to laminae I, II and IX. Laminae I and II labelling was found when the injections included that part of the rostroventral Gi located medial to the facial nucleus, whereas lamina IX labelling was present when the injections included caudal areas of Gi. Projections from Gi to laminae IV-VIII and lamina X as well as to autonomic nuclei have also been documented. Our results suggest that bulbar reticulospinal projections of the rat are comparable to those reported previously for the opossum (Martin et al., J. Comp. Neurol., 196:663-682, 1981) and cat (Basbaum et al., J. Comp. Neurol., 178:209-224, 1978; Holstege, G. and Kuypers, H.G.J.M., Prog. Brain Res., 57:145-175, 1982). The fact that laminae I and II labelling was not produced by Gi injections in recent studies of the rat (Zemlan et al., Brain Res., 292:207-220, 1984) can probably be explained by the caudal placement of their injections. Our data suggest that the Gi of the rat, as defined in the atlas of Paxinos and Watson (1982), is not homogeneous, but can be divided into at least three parts: a rostroventral part which projects to laminae.I and II, a rostrodorsal part which does not project to laminae I and II and a more caudal portion which innervates lamina IX. (Supported by BNS-8309245 and NS-10165-10).

13.2 PRIMARY AND SECONDARY UPPER CERVICAL AFFERENT PROJECTIONS TO PRE-COLLICULAR AND PRE-CEREBELLAR RELAY NUCLEI IN THE DORSAL MEDULLA OF THE CAT. M.T. Stechison* and J.A. Saint-Cyr. Playfair Neuroscience Unit, and Dept. of Surgery, Div. of Neurosurgery and Dept. of Anatomy, Univ. of Toronto, Toronto, Canada M5T 2S8.

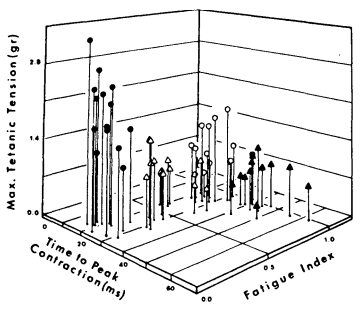
Projections from upper cervical dorsal root ganglia and the cervical spinal cord (C1,2,3) were studied in order to find links with nuclei known to project to the superior colliculus and cerebellum. Projections arising from the lower cervical (C4,5,6) and lumbar cord (L5,6) were examined for comparison. Injections of WGA-HRP (2%; 0.5-1.0ul) were made in 11 cats under pentobarbital anesthesia. Tissue was processed using tetramethyl benzidine and glucose oxidase methods. Few medullary structures project directly to the superior colliculus i.e. nuclei intercalatus (INT) and prepositus hypoglossi (PH) (medial (MVN) and descending (DVN) vestibular nuclei, weakly) cf. Stechison et al., Neurosci. Abstr. 1983, 9: 1088. Labelled terminals were demonstrated only in INT after injections either in C1,2,3 dorsal root ganglia, or in corresponding levels of cervical cord. The C1 ganglion projected only to the ventrolateral aspect of the caudal one-half of contralateral INT, while C2 and C3 ganglia projected to the caudal halves of INT bilaterally. Precerebellar structures of the dorsal medulla demonst rated to receive primary cervical afferents from C1, 2, 3 in the present study include INT ipsilaterally and its ventrolateral aspect contralaterally, and the lateral portions of the cuneate and external cuneate nuclei ipsilaterally. A similar pattern of terminations in INT followed a C1, 2, 3 cord injection. Anterograde labelling was also present in the caudal cuneate and external cuneate nuclei, caudal group x, and weak terminations in the MVN, DVN, and lateral vestibular nucleus (LVN). Despite retrogradely labelled cells in MVN, DVN, LVN, and the ventrolateral border of INT bilaterally following C4, 5, 6 cord injections, there was no evidence of anterograde transport except in the very medial portions of the cuneate and external cuneate nuclei. Lumbar cord injections (L5, and L6) resulted in anterograde and retrograde labelling in MVN and DVN bilaterally as well as strong ipsilateral retrograde labelling in LVN. This study demonstrates potential relays for the transmission of neck afferent information to the superior colliculus and cerebellum. While most pathways do not overlap, INT may act as a relay to both structures. Support by MRC Grant MT 7209 to J.S.C.

13.3 FACTORS DETERMINING RHEOBASE VARIATION AMONG CAT MOTONEURONES. M.J. Pinter and B. Gustafsson*. Dept. of Physiol., Univ. of Göteborg, Box 33031, S-400 33 Göteborg, Sweden.

This study represents an attempt to specify the cellular properties that are most important in determining the functional variation of intrinsic motoneurone (MN) excitability. The variation of rheobase currents obtained from 153 cat hindlimb MNs (pentobarbital anesthesia) was compared with the variation of other cell parameters. In general agreement with previously reported results, rheobase was well correlated with input conductance (r^2=0.81; p<.001). However, the range of rheobase exceeded that of input conductance by a factor of 2. Similarly significant correlations existed in MN subgroups classified according to rheobase magnitude or afterhyperpolarization duration. It may thus be expected that those factors determining the variation of input conductance (or co-variant parameters) also function importantly in determining rheobase. Two properties are of particular importance; specific membrane conductivity and cell surface area. An index of specific conductivity was obtained from the inverse of the membrane time constant determined using brief, hyperpolarizing pulses. These values were well correlated with rheobase (r^2=0.77; p<.001). This correlation was also evident within MN subgroups. Assuming that MNs can be represented as equivalent cylinders and that specific membrane capacitance is identical for all MNs, a cell surface area estimate can be obtained by calculating the total capacitance of individual MN equivalent cylinders. The values were distributed in an approximately normal manner and had an overall range virtually identical with surface area estimates obtained from HRP-labelled MNs. These surface area estimates were only weakly correlated with rheobase (r^2=0.22; p<.001). The results indicate that the functional variation of intrinsic excitability both across the MN pool and within subgroups is governed chiefly by the variation in specific membrane conductivity and other membrane properties that co-vary with conductivity. Cell size itself and factors that co-vary with it appear to play a more limited role.

13.4 THE MOTOR UNIT POPULATION OF THE CAT TENUISSIMUS MUSCLE A. Lev-Tov, C. A. Pratt and R. E. Burke Lab. of Neural Control, NINCDS, NIH, Bethesda, MD and Dept. of Anatomy, Hebrew University, Jerusalem, Israel.

The cat tenuissimus (TEN) is a long, very thin muscle which originates from the 2nd caudal vertebra and inserts onto fascia below the knee. It presumably can act as a weak (tetanic force : 25 g) hip extensor and knee flexor, but its main role may well be as a positional transducer. Retrograde HRP labeling disclosed a maximum of 28 TEN motoneurons scattered along the L7 ventral horn. This scattering permitted study of relatively large fractions (6 - 15 units per cat) of the TEN motor unit population by stimulating single TEN axons in dissected ventral root filaments, with the cut distal muscle insertion connected to a sensitive strain gauge (2g passive force; approx. peak of the very flat length-tension curve). Fatigue index and "sag" property criteria applied to other cat hindlimb muscles (Burke et al., J. Physiol. 234:723, 1973) were used to classify TEN motor units into 4 types (see Fig.: FF (●), FI (△), FR (○), and S (▲). Unlike motor unit populations in larger cat hindlimb muscles, TEN exhibited a relatively high proportion of FI units (26%; fast twitch, intermediate [.25 - .75] fatigue resistance) and a relatively narrow range of tetanic tensions (1.2 to 3.3g, or 16-fold). Functional interpretation of these findings must await studies of TEN kinesiology in situ.



(Dr. Lev-Tov was supported by the Israel Academy of Sciences and Humanities.)

13.5 ALTERATIONS OF INPUT RESISTANCE OF PONTINE RETICULAR NEURONS DURING THE SLEEP-WAKE CYCLE. R.W. McCarley and K. Ito. Lab. of Neurophysiology, Harvard Med. Sch., Boston, MA 02115.

Intracellular recordings in naturally sleeping, undrugged cats indicate that the population of medial pontine reticular formation (mPRF) neurons shows a membrane depolarization specific for desynchronized sleep (D). Since this depolarization appears to be critical for the generation of increased spike potentials during D, it is important to determine the mechanism of its production. Our intracellular connectivity studies suggest that a positive feedback existing between mPRF neurons (dense monosynaptic excitatory interconnections) might be one mechanism; this would be compatible with a decreased neuronal input resistance (Rn) during D. Another (nonexclusive) hypothesis about the mechanism is the withdrawal, during D, of a classical inhibitory influence present during W; this would predict a higher Rn in D. To measure Rn during naturally occurring sleep-wake cycles in the cat we utilized a high input impedance preamplifier with bridge circuitry and injected hyperpolarizing current (1-10 nA) through the recording micropipette and determined Rn by the voltage change after initial capacitative effects had disappeared. We recorded intracellularly and monitored membrane polarization levels and responses to current injection in each behavioral state and also extracellular reference responses. We have thus far measured state-dependent Rn alterations in 5 mPRF neurons and have consistently found the following rank ordering of Rn: Waking (W)=early slow wave sleep (S)>the transition period (T, PGO waves but no other signs of D; T had a median of 25% Rn decrease relative to W) >D (median of 35% decrease relative to W). Absolute values of W Rn ranged from .6 - 1.9 Mohm. There was a high correlation between the time course of D-related membrane depolarization and Rn change over the sleep cycle: Rn was relatively constant during W and early S, with decreases in Rn beginning with the onset of membrane depolarization prior to the occurrence of PGO waves and with a further decrease in Rn during T. During D there was a tonically low level of Rn (and a tonic membrane depolarization), with a resumption of a higher level of Rn at the end of D and onset of W. We have also measured Rn in 5 neurons in pentobarbital anaesthetized cats to form a baseline for the chronic measurements; Rn levels approximated those measured in spinal motoneuron pools: mean Rn was 1.2 Mohms. Our data is thus consistent with the production of the D-related depolarization by a positive (excitatory) feedback producing an increased opening of membrane gates and hence a decreased Rn.

13.6 HISTOCHEMICAL LOCALIZATION OF CYTOCHROME OXIDASE IN THE SPINAL CORD, DORSAL ROOT GANGLION AND SKELETAL MUSCLE OF NORMAL RAT, CAT AND MONKEY. M. Wong-Riley and G. Kageyama. Dept. of Anat., Med. Coll. Wis., Milwaukee, Wis. 53226.

The close correlation between cytochrome oxidase (C.O.) levels and neuronal activity in the brain has been demonstrated by our laboratory and others. In order to determine whether differential patterns of C.O. existed in the spinal cord, dorsal root ganglia and skeletal muscles, we examined these regions in normal rats, cats and monkeys. A similar pattern prevailed in all three species. In the spinal cord, the gray matter was moderate to highly reactive for C.O., while the white matter was consistently low. Substantia gelatinosa was usually less reactive than the rest of the dorsal and ventral horns. Darkly reactive neurons were observed in the dorsal nucleus of Clarke, the lateral cervical nucleus of the cat, the intermediate cell columns of the thoracic and upper lumbar levels (possibly preganglionic sympathetic neurons), cells of Waldeyer in the marginal layer, and small fusiform neurons bordering the ventral gray. In the ventral horn, reactive and nonreactive neurons fell within all three size categories (large, medium and small). In general, darkly reactive neuronal somata were observed more frequently in monkeys and cats than in rodents. Dorsal root ganglia neurons ranged in size from large to small, and in each size category, dark, moderate and lightly reactive cells were found. There appeared to be a greater concentration of reactive neurons along the external border of the ganglia than within the center. Glial cells were not reactive for C.O.. We have also examined skeletal muscles of the cat. The extensor digitorum longus had a mixed muscle population. The mean size of the dark (presumably slow oxidative, SO), moderate (presumably fast oxidative glycolytic, FOG) and lightly (presumably fast glycolytic, FG) reactive fibers tended to be small, medium and large respectively. The soleus muscle, on the other hand, had a rather homogeneous distribution of moderately reactive SO fibers.

Our results indicate that motor neurons of the CNS and sensory neurons of both the CNS and PNS exhibit distinct levels of C.O. staining that may prove to be a useful marker for their individual metabolic and synaptic demands. The staining in the skeletal muscles is consistent with established data based on other metabolic enzymes, and correlates with known patterns of contractile activity. (Supported by NIH NS18122).

13.7 BRAINSTEM AREAS AND DESCENDING PATHWAYS FOR THE INITIATION OF FLYING AND WALKING IN BIRDS. J.D. Steeves and G.N. Weinstein*. Dept. of Zoology, UBC, Vancouver, British Columbia, V6T 2A9

The initiation and control of spinal locomotor mechanisms by supraspinal brainstem centers has been studied in many vertebrate species. However, there is very little information regarding brainstem pathways involved in avian locomotion. To undertake these studies, birds (geese or ducks) were placed in a stereotaxic frame and decerebrated under halothane anesthesia. After anesthesia was discontinued, discrete brainstem regions were stimulated with monopolar stimulating electrodes. Preliminary experiments indicated that locomotion could be evoked from several areas within the avian brainstem. Stimulus thresholds ranged from 25 - 100 uA at 30 - 60 Hz (stimulus pulse duration = 0.5 ms). Varying the stimulus strength altered the speed of hindlimb stepping and forelimb wing flapping. Transecting the dorsal half of the thoracic spinal cord did not abolish brainstem-stimulated walking. Thus, the neuronal pathways, necessary for locomotion, are probably confined to the ventral and ventrolateral funiculi of the spinal cord. This is also in agreement with our observation that transection of the dorsal thoracic cord in intact birds does not inhibit locomotion.

Histological examination of the stimulation sites indicated that the predominant locomotor areas were restricted to the ventromedial regions of the pontine and medullary reticular formation. To ascertain whether neurons in these regions of the avian brainstem project to the spinal cord, the low thoracic spinal cord was injected with a fluorescent retrograde tracer, true blue. In an attempt to restrict retrograde transport to neurons descending via the ventral and ventrolateral funiculi, the dorsal half of the spinal cord was transected rostral to the injection site. Comparison of the retrogradely labelled cell bodies with brainstem stimulation sites that evoked locomotion indicated a remarkable degree of overlap. (supported by NSERC)

13.8 SPINAL CORD STIMULATION IN SPINAL CORD INJURY. J.B. Myklebust, B.M. Myklebust, G. Barolat-Romana* and W. Wenninger.* Medical College of Wisconsin, Milwaukee, WI 53226, Rush Medical College, Chicago, IL 60612, and VA Medical Center, Wood, WI 53193.

Muscle spasm is one of the most functionally disabling aspects of the spasticity secondary to spinal cord injury. Spinal cord stimulation has been used to treat the spasticity due to cerebral palsy, multiple sclerosis, and head and spinal trauma.[1] It has been suggested that the most efficacious electrode placement in spinal cord injury is below the lesion. To evaluate spinal stimulation as a treatment for spasticity, studies have been conducted with spinal cord injured patients.

Monopolar stimulation electrodes have been implanted into the thoracic epidural space in patients with cervical spinal cord injury at the Wood VA Spinal Cord Injury Center. Surface EMG recordings were made from hamstrings and quadriceps muscles with patellar tendon taps, isometric knee flexion and extension and with isokinetic contractions of the knee (4 - 200 deg/sec). Additional recordings were made during muscle spasms occurring spontaneously or evoked by tactile stimulation.

Preliminary results indicate that spinal stimulation has an inhibitory effect on muscle spasm. In some cases this effect occurs within minutes of onset of stimulation. The effects have been observed to last for several hours following cessation of stimulation. Data will be presented to demonstrate the effects of spinal cord stimulation on reflexive, isometric and isokinetic muscle activity and muscle spasm.

[1]Sherwood, A.M. Electrical stimulation of the spinal cord in movement disorders. Chapter in Neural Stimulation, J.B. Myklebust, A. Sances, Jr., S.J. Larson, and J.F. Cusick, Eds., CRC Press Uniscience Series, 1984.

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13.9 SPINAL CORD CATECHOLAMINE CHANGES AFTER EXPERIMENTAL CALCIUM-INDUCED MYELOPATHY. J.C. de la Torre, M.T. Richard* and L.P. Ivan*. Division of Neurosurgery, Ottawa General Hospital, Ottawa, Ontario. K1H 8L6

Chemical myelopathy has been previously induced in rats by dripping the exposed spinal cord with a syringe containing 1 ml of 10% calcium chloride for 10 minutes. The resulting morphologic damage consisted of disintegration of the axoplasm combined with intramyelinic edema which was most marked 24 hours following exposure to the $CaCl_2$ (Balentine & Hilton, J. Neuropath. Exp. Neurol., 39:339, 1980). Besides the morphologic changes following $CaCl_2$, rats developed paralysis of the rear limbs.

We investigated the effects of $CaCl_2$ on rat spinal cord catecholamines using a modified animal model from the above. Adult male Long-Evans hooded rats underwent laminectomy at T_{11-12} and were fitted with a perispinal catheter system connected to an osmotic minipump which was placed under the skin as previously described by us (de la Torre & Carvajal, Lab. An. Sci., 31:701, 1981). This technique allows the chronic administration of chemicals or solutions to drip on the cord surface at a constant rate of 1 µL/hr. in the conscious animal. Rats were sutured with skin clips and allowed to recover from surgery to assure that they were neurologically intact following the laminectomy. Ten days after recovery, rats were quickly anesthetized with ether, the skin clips were opened and the osmotic minipump was filled with either 10% $NaCl_2$ (control) or 10% $CaCl_2$. The perispinal catheter was then filled with the same solution as the minipump and the rats were allowed to recover. Rats were sacrificed after 2,4,8 and 16 hours following this treatment and the spinal cords were removed for histofluorescent examination of catecholamines using the SPG method and for light microscopic analysis.

Results show a correlation between changes seen in catecholamine-containing varicosities of spinal cord gray matter at the site of the $CaCl_2$ exposure and the hind-limb paralysis that develops in these rats.

These preliminary results support the hypothesis that calcium may have a role in the pathophysiology leading to spinal cord paralysis.

Supported by the American Paralysis Association.

13.10 STUDIES ON THE OPTIMAL CONDITIONS FOR LONG-TERM SURVIVAL OF MAMMALIAN BRAIN IN VITRO. M. Mühlethaler* and R. Llinás (SPON: V. de Crescito). Dept. Physiol. Biophys., New York Univ. Med. Ctr., 550 First Ave., New York 10016.

An in vitro technique of isolated and arterially perfused mammalian brain tissue has been recently introduced and applied to the brainstem (Yarom & Llinás, Soc. Neurosci. Abst. 5:109, 1979), the brainstem cerebellum "en bloc" (Llinás, Yarom & Sugimori, Fed. Proc. 40:2240, 1981), or the whole brain of guinea pigs (Walton & Llinás, Soc. Neurosci. Abst. 8:935, 1982). This simple and rapid technique allows good survival of the tissue for several hours as ascertained by extracellular and intracellular recordings. Nevertheless, following long-term perfusion (6 hr) these preparations show some degree of extracellular edema and a decrease in synaptic activity despite the fact that intracellular recordings of excellent quality can still be obtained. We have therefore studied the various factors involved in long-term preservation. Using the isolated cerebellum-brainstem preparation, the resistance of the capillary bed, tissue wet weight, permeability of the blood brain barrier and compound potentials evoked in the cerebellar cortex have been monitored. In particular, surface, juxtafastigial or olive stimulation were used since they reflect the overall status of the perfused brain better than do intracellular recordings. The following factors are significant in optimal survival. (1) Perfusing media. In addition to pH, temperature, O_2, and osmolarity of the Ringer's solution, viscosity and oncotic pressure were considered, and the latter was found to be important. Mannitol, albumin, hydroxyethyl startch, dextran, polyvinylpyrrolidone and combinations thereof were tested at various concentrations. (2) Perfusion technique. Constant pressure and constant flow perfusion were compared. Oxygenation of the medium by simply bubbling or by membrane oxygenator in addition to H_2O_2 were compared as was pulsatile vs non-pulsatile flow. (3) Pretreatment. We tested the effects of heparin, steroids, vasodilators, metabolic substrates, various doses of barbiturates, and intracardiac perfusion of cold Ringer's solution prior to brain removal. Data will be presented concerning the optimal conditions of survival of this preparation and its use in the analysis of the electrophysiological properties of neurons of the cerebellar nuclei. Supported by Swiss Fellowship 83119083 and by NIH grant NS13742.

13.11 CHARACTERIZATION OF MULTIUNIT RHYTHMIC BURSTING DEVELOPING SPONTANEOUSLY IN MONOLAYER NETWORKS CULTURED ON MULTIMICROELECTRODE PLATES. M.H. Droge and G.W. Gross, Dept. of Biology, Texas Woman's University, Denton, Texas 76204.

We have previously reported the development and successful testing of photoetched multimicroelectrode plates (MMEPs) that allow the simultaneous monitoring of spike activity from 36 active units in culture (Gross and Lucas, J.Electrophysiol. Tech. 9:55, 1982). These MMEPs are now being systematically applied to characterize activity developing in low density cultures of dissociated mouse spinal tissue. As our first consistent observation, we report the spontaneous emergence of complex, multiunit activity at several recording sites. More than 80% of this activity involves single or multiunit bursting. Almost all of the bursting is rhythmic with a broad range of cycle periods (20ms - 2 min) observed in different cultures. In all experiments involving simultaneous monitoring, the rhythmic bursting was coordinated among 2 to 14 recording sites (average: 5) separated by as much as 800 µm. This activity is extremely sensitive to small, sudden changes in osmolarity and pH as well as to movement of the medium. Under optimal conditions it has been shown to be stable for 3 days. Maximum recording periods (spot checks) have exceeded 70 days, but network instability caused major changes in the observed activity.

The development of vigorous, spontaneous rhythmic activity from completely dissociated embryonic neurons can be explained only by assuming that specific synaptic interconnections are formed in culture or that random circuits are capable of rhythmogenesis. We consider it likely that central pattern generator (CPG) circuits are partially reestablished in culture and that the activity seen may be similar to CPG rhythmogenesis monitored in "fictive", vertebrate locomotor preparations. We are initiating statistical analyses of cycle periods, burst duration, burst composition, dependence of burst duration on cycle period, and a correlation of the coordinated activity with the morphology of the monolayer network. Laser cell surgery is being applied to circuit simplification during recording. Supported by NIH grant NS15167.

13.12 MODIFICATION OF TOOTH PULP STIMULATION-INDUCED RHYTHMIC JAW MOVEMENTS BY FENTANYL AND CHLORPROMAZINE IN THE RABBIT. D.E. Myers*, R.L. Wynn*, and N.R. Myslinski. Depts. of Physiology and Pharmacology, Univ. of Maryland, Sch. of Dentistry, Baltimore, MD 21201

Evidence exists that the rhythmic activity of jaw and tongue muscles during mastication is controlled by a brain stem pattern generator. It has been demonstrated that this rhythmic pattern can be modified by serotonin but not by glycine. In the present study we attempted to examine the effects of fentanyl and chlorpromazine on the rhythmic masticatory-like pattern produced by tooth pulp stimulation in the rabbit.

Eight rabbits had their tooth pulps stimulated through prepared cavities by a digital ramp stimulator. Threshold voltages (TV) to elicit rhythmic jaw movements (lick/chew) were recorded. Following this, fentanyl in 5 doses ranging from .025 to .20 mg/kg was administered I.V. Five minutes later the tooth pulp stimulation was repeated. TVs for lick/chew and other jaw movements were recorded. In an additional 12 rabbits naloxone or saline was administered I.V. before or after fentanyl administration and again TV for lick/chew and other jaw movements was recorded. In an additional 8 rabbits chlorpromazine (10 mg/kg I.V.) was administered. TVs for lick/chew and other jaw movements were recorded before and after drug administration.

Both fentanyl and chlorpromazine raised the TV for lick/chew at all doses tested. The ED_{50} for blocking lick/chew entirely was .10 mg/kg for fentanyl. The dose of chlorpromazine studied, 10 mg/kg, represented an ED_{60} for blocking lick/chew entirely. In animals when the lick/chew response was blocked by either fentanyl or chlorpromazine, a reproducible stereotyped oral behavior occurred in response to tooth pulp stimulation. This response was a slow, non-rhythmic lateral jaw movement, resulting in tooth grinding which bears great similiarity to the human oral parafunction known as bruxism.

14.1 HEMODYNAMIC EFFECTS EVOKED BY ELECTRICAL STIMULATION OF THE ARCUATE NUCLEUS (AN). T.P. O'Neill* and M.J. Brody. Department of Pharmacology and the Cardiovascular Center. University of Iowa, Iowa City, IA 52242

The application of immunohistochemical techniques to the study of central opiocortin (OC) systems provides strong evidence that OC-containing fibers from AN project to a number of cardiovascular regulatory centers, including the paraventricular and parabrachial nuclei, the solitary nucleus, and the spinal cord. The purposes of this study were to determine 1) the cardiovascular effects of electrical stimulation of AN (200 μA, 0.5 msec, 20, 40,60 Hz for 20-30 sec), and 2) the neural and humoral mechanisms of the responses.

Experiments were performed on urethane-anesthetized male Sprague-Dawley rats instrumented with miniaturized pulse-Doppler flow probes to measure changes in renal (RR), mesenteric (MR), and hindquarter (HQR) vascular resistance. Arterial pressure (AP) and heart rate (HR) were monitored via a femoral arterial catheter. Drugs were administered via a femoral vein. In addition, one group of rats underwent acute sinoaortic deafferentation (SAD) by the method of Krieger.

In non-SAD rats, stimulation of AN did not significantly change any hemodynamic variable. However, in SAD animals, AN stimulation produced frequency-dependent increases in BP, HR, MR, RR, and HQR. These increases had long onset latencies (10-20 sec) and durations (2-5 min). These responses were reduced by α-adrenergic receptor blockade (phentolamine, 1 mg/kg i.v.), but were markedly attenuated by both ganglionic blockade with an equidepressor dose of chlorisondamine (1 mg/kg i.v.) and vasopressin blockade (PMP^1-o-methyl-Tyr^2-Arg^8-VP, 20 μg/kg i.v.). The discrepancy between the effects of α-adrenergic and ganglionic blockade suggests an action of chlorisondamine at a site other than sympathetic ganglia.

To control for possible spread of stimulating current to the adjacent median eminence, we directly stimulated the median eminence in SAD rats. Although the responses evoked by median eminence stimulation were qualitatively similar, these responses were significantly smaller than those evoked by AN stimulation.

In summary AN stimulation produces an increase in AP and vascular resistances by a mechanism that requires the participation of both the sympathetic nervous system and VP. Futhermore, the expression of this response requires suppression of the baroreflex. (Supported by HLB-14388, HLB-07121).

14.2 RECIPROCAL CONNECTIONS BETWEEN CARDIOVASCULAR SITES IN THE NUCLEUS OF THE SOLITARY TRACT AND PARAVENTRICULAR NUCLEUS IN THE CAT. J. Ciriello. Department of Physiology, University of Western Ontario, London, Canada N6A 5C1.

It has recently been shown that neurons in the nucleus of the solitary tract (NTS) relay cardiovascular afferent information directly to the region of the paraventricular nucleus of the hypothalamus (PVH) and that stimulation of the PVH alters the reflex cardiovascular responses to activation of carotid sinus afferent fibers. These findings suggest that reciprocal connections involved in cardiovascular regulation exist between the NTS and PVH. To investigate the reciprocal connections between cardiovascular sites in these two nuclei small deposits of HRP were placed in portions of either structure which have previously been shown to receive cardiovascular afferent inputs and to elicit cardiovascular responses when stimulated. After a survival period of 1-5 days, 40 μm transverse frozen sections of the brain were processed according to the HRP-tetramethyl benzidine method. HRP deposits localized to the medial NTS and dorsal aspect of the dorsal motor nucleus of the vagus (DMV) resulted in bilateral retrograde and anterograde HRP labelling in the PVH, with an ipsilateral predominance. Retrogradely labelled neurons were found in the anteromedial aspects of the ventral portion of the dorsal component of the PVH. On the other hand, anterograde labelling was localized primarily to the dorsomedial aspect of the parvocellular component of the PVH. Additional scattered anterograde labelling was observed in the posterior portion of the dorsal component of the PVH. HRP deposits localized to the PVH resulted in bilateral retrograde and anterograde labelling in the NTS region, with an ipsilateral predominance. Retrogradely labelled neurons were observed from approximately 2.5 mm rostral to 1 mm caudal to the obex primarily in the medial subnucleus of the NTS. Labelled neurons were also found in the dorsolateral, ventrolateral, intermediate and commissural subnuclei of the NTS and lateral aspects of the DMV. Anterograde HRP labelling was found throughout the rostro-caudal extent of the NTS and DMV, and in the caudal area postrema. These data provide evidence of direct reciprocal connections between the NTS and PVH, and suggest that these connections may be part of long loop reflex pathways through the hypothalamus involved in altering the flow of afferent information from the cardiovascular system.
(Supported by Ontario Heart Foundation).

14.3 DIRECT PROJECTIONS FROM VENTROLATERAL MEDULLARY PRESSOR REGIONS TO THORACOLUMBAR SYMPATHETIC AREAS IN THE CAT. M. M. Caverson and J. Ciriello. Department of Physiology, University of Western Ontario, London, Canada, N6A 5C1.

Recently we have identified neurons in the ventrolateral medulla (VLM) which relay cardiovascular afferent information directly to regions of the thoracic cord containing sympathetic preganglionic neurons. However, the precise site of termination of these neurons within sympathetic areas of the thoracolumbar cord remains equivocal. The present study was done to determine the spinal projections of neurons in VLM pressor regions using the autoradiographic method. Initially, pressor regions in the VLM were identified using electrical stimulation (monopolar electrodes; 10 s train; 50 uA; 100 Hz; 0.2 ms) in chloralosed, paralyzed, artificially ventilated, sinoaortic and vagal denervated cats. To determine whether the pressor responses were due to the activation of cell bodies or fibers coursing through the VLM, microinjections of L-glutamate (0.5 nmoles in 100 nl) were made throughout the region of the VLM shown to elicit pressor responses during electrical stimulation. L-glutamate injections elicited pressor responses from regions of the VLM lying close to the ventral surface between 1 and 4 mm rostral to the obex. In these regions, injections of a mixture of ^{3}H-leucine and proline (20 nl; 100 uCi/ul) were made. After a survival period of 14-28 days, frozen transverse sections of spinal segments T_1-L_3 were cut and processed using the autoradiographic method. Anterograde labeling was observed in the thoracolumbar cord primarily in the regions of the intermediolateral nucleus (IML), central autonomic area (CA) and nucleus intercalatus (IC), bilaterally, with an ipsilateral predominance. In addition, some labeling was found bilaterally with an ipsilateral predominance in the dorsolateral aspect of the ventral horn, and in the lateral and ventrolateral funiculi. Labeling in the IML was most dense at the T_1-T_3, T_8-T_9, T_{12} and L_1 levels, whereas the greatest density of labeling in the CA was observed between T_2-T_5. The greatest density of labeling in the IC was at T_5, T_9-T_{10}, T_{12} and L_1. These pathways likely represent the neuroanatomical substrate by which neurons in the VLM exert a direct influence on sympathetic preganglionic neurons involved in the control of vasomotor tone. Finally, the uneven distribution of these VLM projections to sympathetic centers of the cord suggests that the VLM may exert a preferential control over different vascular beds.
(Supported by the Ontario Heart Foundation)

14.4 PARAMEDIAN RETICULAR NUCLEUS NEURONS RELAY INPUTS FROM VESTIBULAR NUCLEUS DIRECTLY TO THE REGION OF THE INTERMEDIOLATERAL NUCLEUS. K. Elisevich* and J. Ciriello (SPON: W.R. Brown). Department of Physiology, University of Western Ontario, London, Canada N6A 5C1.

It has previously been suggested that neurons in the paramedian reticular nucleus (PRN) integrate carotid sinus nerve (CSN), fastigial nucleus (FN) and vestibular afferent inputs. Recently we have demonstrated electrophysiologically that PRN neurons relay cardiovascular afferent inputs from the CSN and pressor sites in the FN directly to the region of the intermediolateral nucleus (IML; Fed. Proc. 43: 401, 1984). In addition, HRP placed in the PRN has been shown to label neurons in the vestibular nuclear complex (VNC; Anat. Rec. 208: 51A, 1984). In this study, the convergence of VNC and CSN inputs onto PRN neurons projecting directly to the region of the IML was studied in chloralosed, paralyzed and artificially ventilated cats. To eliminate the possibility that stimulation of the VNC would result in the activation of FN-PRN fibers coursing through this region, both the FN and surrounding tissue were bilaterally removed surgically 18-60 days before the experiment to allow time for degeneration of these fibers to occur. Fifteen single units histologically verified in the PRN were excited orthodromically by stimulation of VNC (mean latency, 11.3 ± 1.0 ms). Of the 15 responsive units, 6 were antidromically excited by stimulation of the IML region with a mean latency of 2.5 ± 0.8 ms corresponding to a mean conduction velocity of 59.8 ± 14.8 m/s. None of the 15 units responding to VNC stimulation responded to stimulation of the CSN. These data suggest the existence of a population of PRN neurons which relay VNC afferent information directly to the IML region and which are likely involved in mediating vestibulo-sympathetic reflexes.
(Supported by the Ontario Heart Foundation).

14.5 EVALUATION OF THE CARDIOVASCULAR REGULATORY MECHANISMS IN THE MEDULLA OBLONGATA OF THE RAT WITH LOW-INTENSITY STIMULATION AND SINGLE-NEURON RECORDING. Samuel H.H. Chan. National Univ. of Singapore, Fac. of Med., Kent Ridge, Singapore 0511.

Surprisingly few investigations are devoted to delineating the central cardiovascular regulatory machinery in the rat. It is generally assumed that our current concepts on central circulatory control, derived primarily from cats, can be extrapolated to the rodent. As such generalization has serious limitations, I have re-evaluated the classical wisdom on cardiovascular regulatory mechanisms in the rat medulla oblongata, using pentobarbital anesthetized (40 mg/kg, i.p.) male Sprague-Dawley rats.

Low-current intensity (10-50 μA) stimulation of both nucleus reticularis parvocellularis (NRPC) and nucleus reticularis gigantocellularis (NRGC), respective representive of classical 'pressor' and 'depressor' area in the medulla, produced two basic patterns of cardiovascular changes. Pattern-one entailed hypertension, accompanied by a reflex reduction in cardiac contractility and rhythm. Pattern-two included hypotension, together with a reflex positive inotropic effect and a further decrease in heart rate. The former was readily evoked from the NRPC with a low current strength (10-20 μA) over a wide range of train pulse frequencies (25-800 Hz), whereas the latter was more easily obtainable at optimal train pulse frequencies (100-200 Hz) and/or intensities (50 μA). The reverse was essentially true when the NRGC was stimulated. These findings suggested that neurons responsible for promoting hyper- and hypotension may exist in two intermingled populations within both reticular nuclei, a feature that is different from the cat.

60% of the 531 spontaneously active NRGC single-neurons could be classified as cardiovascular neurons, with two sub-types. Type-one's changed their activities in the same, while type-two's in the opposite, direction as induced or spontaneous arterial pressure fluctuations. They thus manifested characteristics similar to their counterparts in the feline. 28% of the NRGC neurons exhibited definite relationship with either systolic or diastolic phase of the cardiac cycle, at a predominant peak activity rhythm of 6-8 Hz. As such, they were reminiscent of cells that were hitherto recorded from the classical 'pressor' areas in the cat medulla oblongata.

It is concluded that the cardiovascular regulatory mechanisms in the rat medulla oblongata may not necessarily be the same as their counterparts in the cat.

(Supported by NUS [RP 133/82, 78/83] and SNHA).

14.6 **CARDIOVASCULAR NEURONS IN THE NUCLEUS PARAGIGANTOCELLULARIS LATERALIS IN THE RAT.** **D. Lea Brown and P. G. Guyenet. Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, Virginia 22908.**

The intermediate portion of the nucleus paragigantocellularis lateralis (PGCL) is involved in controlling sympathetic efferent activity. The purpose of the present study was to explore the cardiovascular function of this structure at the single cell level.

All experiments were done in Sprague-Dawley rats (350-400 g) anesthetized with urethane, paralyzed and artificially ventilated. The PGCL was found to contain a population of spontaneously active units with activities of 1.5 to 50 spikes/s at low mean arterial pressure (MAP). These cells could be totally silenced by raising MAP to 125-185 mm Hg and are referred to as cardiovascular (CV) neurons. The activity of all these units was strongly pulse-modulated, but exhibited little or no respiratory rhythm. Pulse-modulation was observed only at elevated MAP and was increasingly pronounced as MAP increased. More than 80% of PGCL-CV neurons could be antidromically activated from the ipsilateral thoracic spinal cord (T3) with latencies of 5.8 to 92 ms, corresponding to conduction velocities of 0.4 to 8.9 m/s. All PGCL-CV neurons were completely silenced by stimulating the superior laryngeal nerve (SLN, 50 Hz, 1-5 s) and the inhibition was time locked to the associated decrease in MAP. Neighboring cells, unresponsive to MAP elevations in the 120-150 mm Hg range, were unaffected by SLN stimulation. Posterior hypothalamic stimulation (100 Hz, 1-2 s) produced both a powerful excitation in 40% of PGCL-CV neurons and a sharp rise in MAP. Single shocks to the same locus also excited these cells (latency 10-15 ms, 0.25-2 spikes/stimulus); the efficacy of driving was dramatically reduced at elevated MAP, indicating an antagonism between hypothalamic excitation and baroreceptor-mediated inhibition.

The powerful and consistent pulse-modulation of PGCL-CV neurons, as well as their inhibition by SLN stimulation demonstrates that these spinally projecting units receive a prominent baroreceptor input. These cells probably represent sympathetic excitatory premotorneurons and their spontaneous activity at low MAP could be the origin of basal sympathetic tone. **(Supported by HL 28785).**

14.7 USE OF GLUTAMIC ACID AS A TOOL TO LOCALIZE CELL BODIES IN THE VENTROLATERAL MEDULLA THAT INFLUENCE CARDIORESPIRATORY ACTIVITY. Philip J. Gatti*, Wesley P. Norman and Richard A. Gillis*, Depts. of Pharmacology and Anatomy, Georgetown Univ. Schs. of Medicine & Dentistry, Washington, DC 20007.

In our previous study we demonstrated that bilateral application of L-glutamic acid (GA) to the intermediate area of the ventral surface of the medulla (Schlaefke's area) in the chloralose-anesthetized cat increases arterial blood pressure (BP), heart rate (HR) and respiratory minute volume (RMV) (Fed. Proc. 43: 9904, 1984). The increase in RMV was due to an increase in tidal volume (V_T). The purpose of this study was to determine whether the neuronal elements activated by GA were on or near the medullary surface or below the medullary surface, specifically in nucleus paragigantocellularis lateralis (PGCL). This was achieved by comparing cardiorespiratory responses obtained with medullary surface application of GA (at the intermediate area (5 μl of 1 M solution) to cardiorespiratory responses obtained with microinjections of GA (200 nl of 1 M GA) deposited approximately 0.5 to 1.0 mm beneath the intermediate area (an area corresponding to PGCL). Unilateral application of GA using a 3 mm diameter cottonoid pledget to the medullary surface (N = 5) produced increases in BP (+41 ± 12 mm Hg; $P < 0.05$) and V_T (+5.6 ± 3.0 ml). No change in the HR was observed. In contrast, unilateral pressure microinjections of GA produced decreases in BP (-26 ± 6.6 mm Hg, $P < 0.05$) and HR (-10 ± 3.2 beats/min, $P < 0.05$). In addition, no change in V_T was observed; instead there was a significant ($P < 0.05$) decrease in respiratory rate (-1.2 ± 0.5 breaths/ min). These results indicate that cardiorespiratory responses elicited from application of GA at the medullary surface are due to excitation of neuronal elements at or near the surface, and are not due to excitation of neurons of the PGCL.

14.8 ELECTROPHYSIOLOGY OF NEURONS IN CAT VENTROLATERAL MEDULLA (VLM) PROJECTING TO INTERMEDIOLATERAL SYMPATHETIC NUCLEUS (IML). G.L. Gebber and S.M. Barman. Depts. of Pharmacol. and Physiol., Mich. State Univ., E. Lansing, MI 48824.

Although a direct projection from the VLM to the IML has been established, the electrophysiological properties of neurons in this pathway have not been defined. Caverson et al. (JANS 9: 451-475, 1983) claimed to identify cat VLM neurons that project to the thoracic IML and excite sympathetic nerves. Whether these neurons control sympathetic nerve discharge (SND), however, is open to question. First, no attempt was made to determine whether the activity of VLM neurons was correlated to SND. Second, the mean threshold current (448 μA) required to antidromically activate VLM neurons from IML was high. Furthermore, no attempt was made to determine whether the neurons could be antidromically activated with lower stimulus current from sites outside of IML. Thus, it is problematic whether the neurons studied by Caverson et al. terminated in IML. Third, mean axonal conduction velocity (C.V.; 19.6 m/s) for VLM neurons was 4-6X that usually attributed to bulbospinal sympathoexcitatory pathways.

In the current study, we used spike-triggered averaging of inferior cardiac nerve activity and antidromic mapping of the second thoracic spinal segment to identify spinally projecting VLM neurons controlling SND. VLM neurons (n=22) were classified as sympathoexcitatory on the following bases. First, their spontaneous activity was correlated to SND. Second, their longest latency antidromic responses were elicited, with the lowest threshold current (47±9 μA), from sites in the IML. Thus, these neurons presumably terminated in IML. Mean axonal C.V. for VLM neurons with activity related to SND was 5.2±0.5 m/s, a value 4-fold less than that for the neurons studied by Caverson et al. Third, these neurons were inhibited in parallel with SND during the pressor response produced by i.v. norepinephrine infusion. Fourteen VLM neurons with activity unrelated to SND were also antidromically activated from IML. However, the mean threshold current (222±58 μA) required for antidromic activation was lowered to 49±22 μA when the stimulating electrode was moved to other spinal gray sites. These VLM neurons presumably did not control SND. Mean axonal C.V. for these cells was 27.4±7.6 m/s, a value close to that for the neurons studied by Caverson et al. We conclude that VLM sympathoexcitatory neurons can be distinguished from non-sympathetic neurons on the bases of spinal termination site, axonal conduction velocity and whether they exhibit activity correlated to SND.

(Supported by NIH Grant HL13187 and an AHA/MI Grant-in-Aid.)

14.9 WIDESPREAD AND RESTRICTED AXONAL BRANCHING OF CAT VENTROLATERAL MEDULLARY SYMPATHOEXCITATORY NEURONS PROJECTING TO SPINAL INTERMEDIOLATERAL NUCLEUS (IML). S.M. Barman and G.L. Gebber. Depts. of Pharmacol. and Physiol., Mich. State Univ., E. Lansing, MI 48824.

We previously located neurons in the cat ventrolateral medulla (VLM) with spontaneous activity correlated to inferior cardiac sympathetic nerve discharge (as demonstrated with spike-triggered averaging). Among these cells is a group of sympathoexcitatory neurons (inhibited by baroreceptor reflex activation) whose axons project to the thoracic IML (as determined with antidromic mapping). An unresolved fundamental question is whether VLM neurons projecting to IML exert regional or global influences on sympathetic nerve discharge. To answer this question, we attempted to antidromically activate the same VLM neuron from the IML of two or more thoracic spinal segments (T2, T6, T11). When successful, time-controlled collision of spikes evoked by stimulation at two spinal levels allowed us to distinguish activation of an axonal branch in the more rostral thoracic segment from that of the main axon passing through to the caudal segment. Activation of an axonal branch is indicated by a maximum collision interval greater than the difference between the latencies of the antidromic responses elicited from the two spinal segments plus the axonal refractory period.

The main axons of 9 VLM neurons that branched in the T2 IML descended at least as far caudal as T11. Three of 4 neurons in this group also had an axonal branch in the T6 IML. Four VLM neurons with an axonal branch in T2 could be antidromically activated from T6 but not from T11. Four additional VLM neurons were antidromically activated by stimulaton in T2 but not in T6 or T11. These data demonstrate the existence of VLM neurons with restricted and widespread spinal axonal branching patterns, thereby supporting the view that the VLM is capable of regional as well as global excitatory control over sympathetic nerve outflow.

Antidromic mapping in T2 was also used to define the funicular trajectories of the main axons of VLM sympathoexcitatory neurons. The position of the main axon was assumed to be at the site in the T2 white matter from which the shortest latency antidromic response was elicited with the lowest threshold current. The axons of VLM sympathoexcitatory neurons were located in either the dorsolateral or ventrolateral funiculus. Thus, the sympathoexcitatory influences of the VLM are mediated over two spinal pathways. (Supported by NIH Grant HL13187 and an AHA/MI Grant-in-Aid.)

14.10 NEURONS OF THE ROSTRAL VENTROLATERAL MEDULLA MEDIATE CARDIOVASCULAR CHANGES TO STIMULATION OF MEDULLARY CHEMOSENSITIVE ZONES AND ARE INVOLVED IN NTS HYPERTENSION. E.E. Benarroch*, A.R. Granata, D.A. Ruggiero and D.J. Reis (SPON:A. Del Bo), Laboratory of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

An area of the rostral ventrolateral medulla (RVL) containing adrenaline (C1) neurons participate in the tonic (Ross et al., 1983) and reflex (Granata et al., 1983) control of arterial pressure (AP). We sought to determine whether neurons of this area: (a) mediate changes in AP known to be initiated from chemosensitive zones of the ventral surface of the medulla (VSM); and (b) are necessary for hypertension elicited by bilateral lesions of the nucleus tractus solitarius (NTS). Rats were anesthetized, paralyzed and ventilated. Solutions (100 nl, 10^{-5} to 10M, pH 7.2) of drugs were applied via pipettes to the VSM. L-glutamate (L-glu) elicited a dose-dependent increase of AP and heart rate (HR). The threshold was 100 pmol , max 1 umol and the site highly localized (1-1.5 mm rostral to the XIIth nerve, 1.5-2 mm lateral to the midline). Topical application of cholinergic agonists (carbachol, physostygmine or oxotremorine, 0.1 nmol to 100 nmol) increased AP. GABA and glycine (gly) elicited dose-dependent decreases of AP, while bicuculline increased it. With electrical stimulation, the site of lower threshold pressor responses (10 ± 2 uA) corresponded to the same zone and precisely overlapped the distribution of the rostral C1 group as defined in the same animals with antibodies to PNMT. Moreover, projections from rostral C1 neurons were seen reaching the subadjacent VSM. Unilateral electrolytic lesions of the C1 area or of PNMT labeled descending pathways in the medulla significantly reduced (by 60-70%) the changes of AP elicited by ipsilateral application of L-glu (n=8), gly (n=5) or GABA (n=5) to the active zone of the VSM. Lesions of surrounding structures had no effect. Bilateral lesions of the NTS increased AP (181 ± 7 vs 115 ± 5 mmHg, n=15, $p < 0.001$), which was profoundly lowered by: (a) bilateral application of GABA (-99 ± 10 mmHg, n=5, $p < 0.001$) or gly (-83 ± 5 mmHg, n=4, $p < 0.02$) to the VSM; (b) unilateral application of GABA combined with contralateral C1 lesions (-115 ± 10 mmHg, n=4, $p < 0.002$); or (c) bilateral C1 lesions (-111 ± 10 mmHg, n=4, $p < 0.002$). We conclude that neurons of the C1 area of the RVL: (a) mediate changes of AP initiated from a restricted area of the VSM similar to Schlafke's or glycine sensitive areas of the cat; (b) are necessary for expression of NTS hypertension; and (c) are tonically inhibited by GABAergic neurons.

14.11 ROSTRAL VENTROLATERAL MEDULLA AREA CONTAINING C1 ADRENALINE NEURONS MEDIATES SYMPATHETIC CARDIOVASCULAR RESPONSES ELICITED FROM BRAIN STEM AREA CONTAINING A1 NORADRENERGIC NEURONS. A.R. Granata, Y. Numao, M. Kumada and D.J. Reis, Lab. of Neurobiology, Dept. Neurology, Cornell Univ. Med. Coll., New York, NY 10021

In rat, electrical stimulation at low stimulus frequencies of the area of the caudal ventrolateral medulla containing the A1 noradrenergic neurons (A1 area) lowers arterial presure (AP); lesions of the area increases it (Imaizumi et al., Fed. Proc. 42:583, 1983). The pathway mediating the sympathoinhibition from the A1 area is unknown. However, neurons of the A1 area bilaterally innervate the area of rostral ventrolateral medulla tonically exciting sympathetic discharge and containing C1 adrenergic neurons (C1 area). We sought to determine whether the A1-C1 pathway mediates the sympathoinhibition from the A1 area.

Rats were anesthetized with urethane (1.5 g/kg, i.p.), paralyzed, and ventilated. AP, heart rate (HR) and renal nerve activity (RNA) were recorded. Kainic acid (KA) microinjected bilaterally into the A1 area (400 pmole in 0.2 µl) significantly ($p < .02$; n=8) reduced AP, HR and RNA (by -51 ± 5 mmHg, -92 ± 8 bpm, and -70 ± 6%). By fifteen min these values were significantly ($p < .001$; n=8) increased (to +110 ± 6 mmHg, +145 ± 42 bpm, and +1300 ± 190%). Bilateral electrolytic lesions of or microinjection of tetrodotoxin (TTX) into the C1 area abolished all responses elicited by KA in the A1 area, while TTX microinjected into the C1 area after KA was injected into A1 area reversed the increased AP, HR and RNA. Responses to KA in the A1 area were not modified interrupting other projections of A1 neurons by midcollicular decerebration (n=4), or by bilateral lesions of nucleus tractus solitarii (n=4).

Electrical stimulation of C1 area evoked an excitatory (E) response in the left RNA with a latency of 54 ± 2 msec (n=6). Stimulation of the A1 area elicited an initial excitation (latency 64 ± 3 msec) followed by inhibition. The inhibitory, but not excitatory response, from A1 was greatly diminished by injection of KA in the A1 area. Tyramine (3 nmole, 0.2 µl) or clonidine (1 nmole) microinjected into the C1 area elicited dose dependent decreases of AP, HR and RNA. The effect of tyramine was blocked by DMI. We conclude that: (a) the sympathoinhibition from the A1 area depends upon the integrity of neurons from the C1 area; (b) the tonic sympathoinhibition appears mediated by A1 neurons releasing noradrenaline upon adrenergic receptors in the C1 area. (Supported by Grant HL18974).

14.12 MIDLINE MEDULLARY AND CEREBELLAR LESION EFFECTS ON RESPIRATORY MODULATION OF SYMPATHETIC ACTIVITY. C. A. Connelly and R. D. Wurster*. Department of Physiology, Loyola University Medical Center, Maywood, IL 60153 and Hines VA Rehab. Research and Development Center, Hines, IL 60141

We previously reported (FED. PROC., Vol. 43: 685, 1984) that midline medullary lesions disrupt respiration (phrenic and laryngeal nerve activities) and the respiratory modulation of sympathetic activity (RMSA). Two purposes of the present study were 1) to determine if medullary inspiratory cells could be extracellularly recorded from the nucleus tractus solitarious (nTS) region before and after midline medullary lesions disrupted RMSA and 2) to determine if midline cerebellar lesions would similarly disrupt RMSA. An effect on RMSA was postulated since stimulation of middle cerebellar nuclei, i.e., fastigial nuclei, affects sympathetic nerve activity and blood pressure. Phrenic and inferior cardiac sympathetic nerves were recorded in twenty alpha-chloralose anesthetized, vagotomized, paralyzed cats. RMSA was determined using spectral analysis and/or respiration triggered computer summation of sympathetic nerve activity. Blood pressure and sympathetic nerve responses to bilateral carotid occlusions tested baroreceptor reflex integrity through the nTS region before and after lesions. Medullary inspiratory cells were extracellularly recorded in 12 cats before and 7 of the 12 cats after midline medullary lesions. Although rhythmic nTS units were recorded after midline lesions, they were more difficult to find than control respiratory units. Whether or not rhythmic nTS units were located after midline lesions, phrenic nerve activity and RMSA were eliminated by midline medullary lesions. In contrast, phrenic activity and RMSA were not affected by midline cerebellar lesions in 8 cats. Blood pressure and bilateral carotid occlusion responses were also not affected by either midline medullary or cerebellar lesions. In conclusion, midline medullary lesions disrupted RMSA even when some ipsilateral nTS rhythmic units were active. A decreased population of rhythmically oscillating respiratory cells after midline medullary lesions may explain these and previous results. In contrast, middle cerebellar nuclei had no apparent influence on the RMSA. (Supported by NIH Grant HL 27612)

15.1 IMIPRAMINE BINDING TO PRIMARY CULTURES OF ASTROCYTES. R.A. Waniewski, D.M. Katz* and H.K. Kimelberg (Spon: A.J. Popp). Ctr. for Labs & Res., NYS Dept. of Health, Albany, NY 12201.

The high affinity binding of imipramine (IMI) to brain membranes is thought to be associated with inhibition of neuronal uptake of serotonin. C6 glioma cells have recently been reported to bind IMI with high affinity but this site does not appear to be associated with antidepressant inhibition of serotonin uptake by these cells. High affinity, sodium-dependent serotonin uptake by primary cultures of rat cortical astrocytes has been found to be potently inhibited by IMI (IC_{50} = 200nM; H.K. Kimelberg and D. Katz, this volume). Therefore we have examined the binding of ^{3}H-IMI to these cells.

Rat cortical astrocytes were maintained in culture for 4 to 5 weeks before use in binding studies. Intact cells were examined in multiwell trays under conditions nearly identical to those used for serotonin uptake except that inhibitors were omitted and binding incubations were carried out for 60 min at 0°C instead of 37°C. Astrocyte membranes were prepared from cells grown on 100mm dishes and examined for IMI binding with the methods described by Raisman et al. (Nature, 281:148, 1979). IMI was used instead of desmethylimipramine as the displacing ligand. Several experiments with membranes and intact cells failed to reveal any saturable binding in the concentration range of 0.1-40nM. Displacement experiments with 5nM ^{3}H-IMI and varying concentrations of unlabeled IMI (10nM-100µM) showed no specific binding with concentrations less than 500nM. The displacement curves fit a single binding component with an IC_{50} of 962nM for intact cells and 2.15µM for membranes. Membranes were incubated with 8nM - 5µM ^{3}H-IMI in the presence or absence of 100µM IMI. The data were computer fit by the LIGAND program (P.J. Munson and D. Rodbard, Anal. Biochem., 107:220,1980). The best fit was obtained by a single site model and the binding constants obtained were K_D = 1.66 ± 0.15µM and B_{MAX} = 1.10 ± 0.08nmol/mg protein. ^{3}H-IMI concentrations ranging from 8-50nM did not show saturation and could not be fit to a second site. Finally, cells were exposed to 1µM IMI for 0, 1 or 21 days and their membranes examined for binding. Acute exposure increased specific binding to 137% of control membranes. Chronic exposure reduced specific binding to 49% of control ($p<.001$).

These findings suggest that IMI binding to brain astrocytes may be an important site of antidepressant action.

15.2 LOCALIZATION OF MUSCARINIC AND NICOTINIC ACETYLCHOLINE RECEPTORS IN NEURONS ISOLATED FROM CHICK EMBRYO RETINA W.M. James and W.L. Klein. Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60201.

Isolated CNS neurons from chick embryo retinas (days 13-19) were used to view by autoradiography the localization of muscarinic and nicotinic receptors. 3H-Propylbenzilylcholine mustard was used to label muscarinic receptors, and 125I-alpha-bungartoxin was used to label nicotinic receptors. Fixation of neurons within minutes after dissociation preserved the varied dendritic morphologies that exist in vivo. This approach allowed us to identify the classes of cells having receptors and to see clearly the positioning of receptors in their dendritic trees.

3H-PrBCM-labeled neurons were unipolar and resembled amacrine cells, while 125I-toxin-labeled neurons typically had two or more processes and could be identified as ganglion and bipolar cells. Some neurons resembling amacrine cells also had nicotinic receptors. The identified cholinoceptive cells were those potentially capable of making synaptic contacts with the knowncholinergic amacrine cells.

In comparing receptor positioning over the surfaces of isolated cells, we found that muscarinic receptors were exclusively localized to the amacrine cell dendrites, even as early as E13. Localization of nicotinic receptors was even more restrictive, with a preferred positioning at fine branches found at the distal ends of dendritic trees. An emerging principle of nerve cell surface differentiation is that neuroreceptor molecules develop specific patterns of localization within dendritic arbors. (Supported in part by NIH grant no. NS18490 to WLK.)

15.3 MUSCARINIC RECEPTORS ON HUMAN DIPLOID FIBROBLASTS: DIFFERENCES ASSOCIATED WITH DEVELOPMENTAL STAGE AND TISSUE TYPE. D. Van Riper*, M.P. Absher*, and R.H. Lenox. Neuroscience Research Unit, Dept. of Psychiatry and Dept. of Medicine, Univ. of Vermont Coll. Med., Burlington, VT 05405.

Cultured human lung fibroblasts (IMR-90) possess muscarnic receptors that inhibit adenylate cyclase (AC) activity in response to cholinergic stimulation. However, most investigations have been limited to fetal lung fibroblasts. We have initiated studies to examine and compare muscarinic receptor characteristics in human fetal and adult fibroblasts from skin and lung. The muscarinic receptor population was characterized using carbachol-mediated inhibition of stimulated cyclic AMP accumulation in intact cells and the binding of [3H]QNB to membranes derived from these cells.

Fetal lung and skin fibroblasts were obtained from IMR. Adult lung and skin fibroblasts were isolated from explants of primary tissue at UVM. Fibroblasts were cultured in MEM with 10% FBS. 10^5 cells were seeded in 35 mm culture dishes in 3.0 ml medium and, at stationary phase, were labelled with 2.0 uCi [3H]-Adenine for 1 hour. The enzyme reaction was initiated by the addition of either PGE_1, forskolin or epinephrine, and terminated after 10 min at 37°C. The [3H]cAMP formed was isolated using a modification of the sequential chromatography method of Salomon et al. For binding experiments stationary cultures in T-75 flasks were harvested, homogenized and washed. Binding studies were performed at 25°C for 30 min in the presence of [3H]QNB and terminated by the addition of cold buffer and rapid filtration over GF/B filters.

We found PGE_1, forskolin and epinephrine to be potent stimulators of cyclic AMP accumulation in the fetal lung fibroblast, and the inhibitory response to carbachol (10µM) was robust (65%). [3H]QNB binding was saturable, yielding a Kd of 0.04nM, a Bmax of 2180 fmol/mg and approximately $7.5x10^4$ sites/cell. Adult lung cells were less responsive to agonist stimulation of cyclic AMP accumulation, showed a diminished response to carbachol inhibition but appeared to have similar Kd and Bmax values. In the fetal skin cells, all the above parameters were greatly reduced as compared to the fetal lung. In the adult skin cells there was good response to agonist stimulated cyclic AMP accumulation, but little evidence for either a muscarinic-mediated inhibition of the adenylate cyclase or the presence of muscarinic receptors as determined by [3H]QNB binding. These findings indicate that the distribution and functioning of muscarinic receptor populations on human fibroblast may vary according to the developmental stage and tissue type.

15.4 DIFFERENTIAL DESTABILIZATION OF TYPE I AND TYPE II RECEPTORS FOR ADRENAL STEROIDS FOLLOWING DEXTRAN-COATED CHARCOAL PRETREATMENT OF BRAIN CYTOSOL. S.M. Emadian*, W.G. Luttge and C.L. Densmore. Department of Neuroscience, Coll. of Medicine, University of Florida, Gainesville, FL 32610.

Current research on the endocrine basis of salt appetite in rodents suggests the potential for a differential intracerebral action of mineralo- and glucocorticoid hormones in brain. Studies outlined here were designed to characterize the properties of mineralocorticoid (Type I) receptors in cytosol from mouse whole brain. To eliminate the nonlinearities produced by the contribution of glucocorticoid (Type II) receptors in Scatchard plots for Type I receptors, the combined use of dextran-coated charcoal (DCC) pretreatment of cytosol and a highly specific synthetic glucocorticoid, RU 26988, were employed. Parallel studies investigating the effects of DCC pretreatment of cytosol on Type II receptors were also conducted. Initial results revealed a 50% reduction in the apparent maximal specific binding of Type II receptors following DCC pretreatment. This loss of binding was not restorable by addition of molybdate to the DCC-pretreated cytosol. In contrast, DCC pretreatment produced a 3-fold increase in the apparent magnitude of Type I receptor binding obtained in the presence of 50-fold RU 26988. DCC-pretreatment was also found to reduce the thermostability of both Type I and Type II receptors. Scatchard analyses revealed that the apparent reduction in Type II receptor binding was due to a 3- to 8-fold increase in the dissociation constant of this receptor for both triamcinolone acetonide and dexamethasone. In contrast, there was no change in either the dissociation constant or the maximal binding of Type I receptors for aldosterone. Note that whereas a 50-fold excess of RU 26988 proved sufficient to block nearly all aldosterone binding to Type II sites in non-DCC pretreated cytosol, overtly non-linear Scatchard plots were obtained in the presence of this glucocorticoid in the DCC-pretreated cytosol. Addition of a 500-fold excess of RU 26988, however, was sufficient to saturate the Type II sites (and hence linearize the Scatchard plots) even after DCC pretreatment. These results suggest that the apparent increase in the binding of Type I receptors following DCC-pretreatment was due to the spurious contribution of the low-affinity Type II receptors that escaped saturation with RU 26988. (Supported by N.I.A.D.D.K.D. Grant AM 31837.)

15.5 BINDING OF ^{125}I-TETANUS TOXIN TO NEURONAL CELL LINES IN CULTURE: EVIDENCE FOR INTERNALIZATION OF TETANUS TOXIN, G.C. STAUB*, T. NICHOLS*, R. BAICHWAL*, AND T.R. ROGERS. Department of Biological Chemistry, University of Maryland, School of Medicine, Baltimore, M.D. 21201.

We have previously reported that the retinal ganglia-neuroblastoma hybrid N18 RE105 displays a high-affinity tetanus toxin receptor. We have now extended these studies and report that there is an apparent energy- and temperature-dependent internalization of tetanus toxin into N18 RE105. Binding experiments were carried out at 37°C or 0°C, either with or without a combination of metabolic inhibitors; oligomycin (4 mg/ml), rotenone (4 μM) or 2-deoxyglucose (20 mM), NaN_3 (20 mM). Experiments with metabolic inhibitors were conducted using cells in suspension as cells plated rapidly lost their ability to adhere to the plastic dishes. ^{125}I-Tetanus toxin "binding" to N18 RE105 cells in suspension was inhibited 25-50% within 2 hours at 37°C. In contrast no inhibition was observed at 0°C. Further metabolic inhibitors did not affect ^{125}I-tetanus toxin binding at 0° or 37° to membranes prepared from N18 RE105 cells. N18 RE105 cells incubated in suspension bind more radiolabeled toxin (per mg of protein) at 37°C than at 0°C. In contrast N18 RE105 membranes preparations bind the same amount of tetanus toxin at 37°C or 0°C. Treatment of cells with metabolic inhibitors reduced ATP levels by 80% of control within 5 min and by 90% of control within 2 hours at 37°C. Control ATP experiments indicated that N18 RE105 cells in suspension lose 76% of their ATP content by 2 hours at 37°C and 36% by 2 hours at 0°C. Cells plated on 24 plates however lose only 20% of their ATP during a 2 hour incubation at 37°C. NH_4Cl and CH_3NH_2•HCl have been shown to inhibit the cellular internalization of some proteins. Neither compound inhibited "binding" of ^{125}I-tetanus toxin to N18 RE105 cells grown on 24-well plates. We have also devised a method to label surface bound tetanus toxin with rabbit anti-toxin and ^{125}I-labeled Protein-A. Preliminary results indicate that this method gives an adequate specific signal and that over a period of time some bound toxin becomes protected from antibody. We have also observed that another neuroblastoma hybrid line, NCB-20 also binds tetanus toxin with high affinity. Further studies are in progress. (Supported by USARMDC grant DAMD17-83-C-3114).

15.6 DISCRETE STAGES IN THE FORMATION OF ACETYLCHOLINE RECEPTOR (AChR) AGGREGATES ON CULTURED MYOTUBES. A. Olek*, J.G. Krikorian, and M.P. Daniels. Lab. of Biochemical Genetics, NHLBI, NIH, Bethesda, Maryland 20205

AChR aggregation on cultured myotubes has been studied as a model system for the organization of AChR at the developing neuromuscular junction. Low light level fluorescence microscopy revealed that embryonic brain extract (EBX), induces aggregation of diffusely distributed AChR in distinct morphologic stages: 1) emergence of small (<1-1.5μm) punctate "microaggregates" 2) evolution of larger (2-10μm) irregularly shaped aggregates (Olek et al., Cell 34:255). We now report data on the effect of temperature and on sodium azide sensitivity indicating that these stages are mechanistically different steps in the formation and stabilization of aggregates.

Aggregate formation (in 4h) was temperature dependent, being optimum at 36° with a Q_{10}~5 from 24 to 36°, and blocked at 38°. At lower temperatures only microaggregates formed, with a Q_{10}<2 from 18 to 24°. With longer incubations at 23° (6-10h) microaggregates did not greatly increase in size, but the number and brightness did increase. In sequential observations of cells, clouds of microaggregates formed within 4h at 23° and then rapidly increased in density to form aggregates with a shift to 36°. Formation of both microaggregates and aggregates was reversibly blocked by addition of 5mM azide, suggesting a requirement for energy (ATP). Once formed, microaggregates dispersed rapidly (in 1.5h) upon withdrawal of EBX or addition of azide. However, aggregates persisted for 6-10h after these treatments. While aggregates persisted in the presence of azide they did not grow larger or brighter. The stability of aggregates was also temperature dependent. Aggregates persisted longer at 23° (>12h) upon EBX withdrawal, and were rapidly (but reversibly) dismantled upon a shift from 36 to 38° (Krikorian et al., Am. Soc. Cell Biol. Abst. 1984).

No mechanism for the transition from microaggregate to aggregate is known. However an ultrastructural study revealed a preferential association of dense cytoskeletal structure and basal lamina with aggregates compared to microaggregates (Olek, Ling, and Daniels, in prep.). Possibly, aggregates evolve when one or more components about the plasma membrane become associated with clouds of microaggregates. This temperature sensitive step renders the aggregate more stable (i.e., less dependent on EBX or energy) than its precursor, the microaggregate cloud.

15.7 IMMUNOCYTOCHEMICAL LOCALIZATION OF TRANSFERRIN RECEPTORS IN DEVELOPING CHICKEN MUSCLE. T. H. Oh, G. J. Markelonis and P. Azari*. Dept. of Anatomy, Univ. of Maryland Sch. of Med., Baltimore, MD 21201, and Dept. of Biochem., Colorado State Univ., Fort Collins, CO 80523.

The serum iron-binding protein, transferrin (Tf), is required for myogenesis and myotube survival in vitro (Oh and Markelonis, Proc. Nat. Acad. Sci. USA, 77: 6922, 1980; J. Neurosci. Res., 8: 535, 1982). While Tf is known to be internalized by receptor-mediated endocytosis, little is known about the specifics of this process in skeletal muscle. In order to better understand the receptor-mediated internalization of Tf and the factors which control this process, we investigated the distribution of transferrin receptors (TfRs) in cultured chicken embryonic muscle at the light microscopic level. Muscle cultures were fixed in paraformaldehyde-glutaraldehyde and TfRs were visualized immunocytochemically by an unlabeled peroxidase-antiperoxidase (PAP) method using rabbit antibodies against chicken erythrocyte TfRs. A reaction product was evenly distributed on cultured myotubes. The staining reaction was more intense in unfused myoblasts and small myotubes than in large multinucleated myotubes. There was a weak reaction product seen in contaminating fibroblasts. Control cultures which had been incubated with preimmune IgG showed no reaction product. Anti-TfR IgG also stained paraformaldehyde-glutaraldehyde fixed developing muscles from chicken embryos. Interestingly, the addition of chicken embryonic neural tissue explants (e.g., spinal cord) to muscle cultures caused the disappearance of TfRs in myotubes although a few small patches of staining reaction could be seen in these co-cultured myotubes. Our results thus showed that TfRs appear on developing muscles in vitro and in vivo. Our preliminary studies indicate that neural tissues may influence the expression and distribution of TfRs in developing muscle. In view of the importance of Tf in muscle development, we suggest that the Tf/TfR interaction may play a critical role during muscle development. Supported by the NIH (NS 15013 and NS 16076).

16.1 SERUM- AND NGF-DEPENDENT EFFECTS OF GM1 GANGLIOSIDE ON NEURITIC GROWTH FROM PC12 CELLS. R. Katoh-Semba*, S.D. Skaper and S. Varon. Dept. of Biol., Sch. of Med., Univ. Calif. San Diego, La Jolla, CA 92093.

Gangliosides are normal membrane constituents which are localized in the outer leaflet of the plasma membrane. Specific roles in the nervous system have been suggested since gangliosides are abundant in nerve cells and increase in parallel with neuronal development. Exogenous gangliosides have been reported to influence neuronal behaviors, including the promotion of axonal sprouting in vivo, neuritic regeneration in vitro and de novo neuritic extension in clonal cell lines of both neuronal and chromaffin origin.

In the present study, we investigated the effects of ganglioside GM1 (GM1) on proliferation and neuritic growth of PC12 pheochromocytoma cells. PC12 cells grown in 10% fetal calf serum (FCS) and 5% horse serum were reseeded in serum-free medium on polyornithine-coated dishes and switched just 2 hr after seeding to the desired medium with serum in the absence or presence of Nerve Growth Factor (NGF), with or without GM1. Total cells and neurite-bearing cells were followed with time in culture. In the absence of NGF, but not in its presence, a reduction in the numerical growth of PC12 cells was first observed after 4 and 6 days of culture with 10^{-6}M GM1 in 0.1% FCS and with 10^{-3}M GM1 in 10% FCS, respectively. GM1 did not affect the limitation of cell growth by NGF (4-6 days). The neurite recruitment induced by NGF was inhibited by FCS in a concentration-dependent manner both with regard to its onset and its subsequent rates. The influence of GM1 on neurite recruitment varied as a function of FCS concentration in three respects: i) GM1 reduced the lag but not the rate reduction imposed by FCS; ii) the effect became more pronounced with increasing FCS concentration, reaching a maximum with 1% FCS or more; iii) the effective GM1 concentration decreased with decreasing FCS concentration, being optimal at 10^{-4}M with 10% FCS and only 10^{-6}M with 0.3% FCS. The results suggest that serum delays and slows down the NGF-induced neurite recruitment of PC12 cells by two probably independent mechanisms, only one of which--the onset lag--is addressed by exogenous GM1 administration.

16.2 **MICROTUBULE ORGANISATION IN NGF ACTIVATED PC12 PHEOCHROMOCYTOMA NEURITES.**
J. Roger Jacobs and John Stevens, Playfair Neuroscience, University of Toronto and Toronto Western Hospital, Toronto, CANADA, M5T 2S8.

Microtubule (MT) organisation in NGF activated PC12 neurites was examined with complete reconstructions of serial EM sectioned material. Cultures were exposed to NGF for 3, 8 and 43 days. No changes in MT to MT distance or microtubule length are found. However, older neurites show a decline in the density of membranous organelles, and an increase in the density of MT per neurite.

Cultures osmicated in the prescence of 1% $K_4Fe(CN)_6$ demonstrate circular exclusion areas surrounding each MT profile. These exclusion areas did not stain with conventional EM stains, and appear clear against background staining of the cytoplasm. These exclusion areas can also be seen as fenestrations through sheets of membrane in the neurite. Reconstructions demonstrate the continuity of these membrane sheets with neuritic agranular reticulum.

The MT exclusion area has a diameter of 56 nm (including the 24nm MT diameter) which does not vary with the age or internal structure of the neurite. Exclusion zones are not penetrated by other MT or other organelles. If all MT to MT neighbour distances are calculated, a mode of 55 nm is found, almost exactly equivalent to the summed exclusion zone radii from two MT. The mode rises (70 nm) if a large number of membranous organelles are present, such as in neuritic varicosities. A few MT are 36-55 nm apart, but never less than 36 nm. A similar MT organisation has been described for ganglion cell dendrites (Sasaki et al, Brain Res.259:193-206, 1983).

We propose that the exclusion area is composed of some structure (possibly MAPs) that mediates MT contact with other neuritic structures. MT exclusion areas also determine the limits of maximum MT packing and hence, minimum neurite diameter as well.

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16.3 TAXOL AND GANGLIOSIDE INDUCED ABERRANT NEURITE OUTGROWTH FROM NEUROBLASTOMA AND CNS NEURONS CULTURED IN MICROFILAMENT-LIMITED CONDITIONS. D.A. Spero* and F.J. Roisen (SPON: R. Duvoisin). Dept. of Anatomy, UMD-Rutgers Medical School, Piscataway, NJ 08854.

Previous studies in this laboratory have shown that exposure of Neuro-2a neuroblastoma cells to mixtures of exogenous bovine brain gangliosides (BBG) increased neuritogenesis (Science 214: 577, 1981) and stimulated the formation of a complex network of microfilament (MF) bundles (Dev. Brain Res. 13: 37, 1984). We also demonstrated that BBG produced aberrant neurite development in Neuro-2a cells grown in the presence of cytochalasin D (cyto-D) at a concentration known to disrupt MF function. Neurite initiation, but not growth, was observed in the presence of the microtubule (MT) disruptive agent colcemid (0.25 ug/ml). These results suggested that MTs were sufficient to support neurite elongation during MF-limited conditions. To examine the cytoskeletal basis of BBG-mediated neurite formation, the MT promoting and stabilizing effects of taxol on Neuro-2a and dissociated embryonic chick cerebral neurons were determined. Neuro-2a cells and chick cerebral neurons were prepared as described by Roisen et al. (Science 214: 577, 1981) and Sennsenbrenner et al. (Dev. Neurosci. 1: 90, 1978), respectively. Cultures were grown in medium containing cyto-D (2 ug/ml) and either BBG (250 ug/ml) or taxol (1 uM) for 24h and processed for SEM and TEM. Neurons grown in serum-deprived medium or solely with BBG formed thick, highly arborized processes. In contrast, cells treated simultaneously with BBG and cyto-D produced long, thin, unbranched neurites which lacked typical growth cones. These aberrant processes grew in a circular pattern, contained many MTs and occasional bundles of neurofilaments and were deficient in MFs. The simultaneous application of taxol and cyto-D resulted in similar long, thin, unbranched neurites that were densely packed with MTs. Cultures treated solely with taxol or cyto-D did not form processes. These results demonstrate that transformed and primary neurons are able to produce neurites under MF-limiting conditions. They suggest that MTs may provide the motive force for neurite extension, while MFs appear essential for arborization and growth cone formation.

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16.4 THE EFFECT OF PERIPHERAL NERVE HOMOGENATE IMPLANTS ON REGROWTH OF CHOLINERGIC AXONS AFTER FIMBRIA TRANSECTION IN THE ADULT RAT. J. S. Wendt*(SPON:E. Drust). Department of Neurology, Dallas VA Medical Center and University of Texas Health Science Center, Dallas, TX, 75216.

Peripheral nerve tissue implanted into a site of hippocampal fimbria transection in the adult rat is extensively innervated by acetylcholinesterase (AChE) stained axons, derived largely from the septohippocampal pathway. In the present study peripheral nerve homogenate was implanted into a site of fimbria transection to examine possible neurotropic or neuronotrophic stimuli for regenerating cholinergic neurites. One week after fimbria transection, Gelfoam soaked with either peripheral nerve homogenate (from fresh or pre-degenerated nerve) or saline was implanted into the transection site. In another group of animals homogenate-soaked Gelfoam was inserted into a 1 - 1.5 mm segment of Silastic tubing, which was then implanted into the transection cavity. Animals receiving Silastic tubing with saline-soaked Gelfoam or no treatment after fimbria transection served as controls. After 1½-14 weeks animals were sacrificed, and frozen brain sections were stained for acetylcholinesterase (AChE) and counter-stained with cresyl violet. Histologic specimens were evaluated for position of the implant, AChE-stained axonal sprouting in the lesion site and axonal growth toward and within the implant.

There were no differences in axonal growth into the lesion site between controls and either fresh or pre-degenerated homogenate implants. Axonal growth preferentially occurred in regions occupied by clusters of glial cells rather than within acellular Gelfoam implants. Extensive axonal growth occurred in and around one Silastic implant with pre-degenerated homogenate which was closely apposed to the septum and was extensively invested with glial cells. It is concluded that (1) implants of peripheral nerve homogenate do not substantially alter the axonal growth response after injury and (2) native glial cells provide a preferred substratum for axonal growth.

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16.5 INFLUENCE OF PLASMA MEMBRANE FRACTIONS FROM VARIOUS TISSUES ON SENSORY NEURITE GROWTH IN VITRO. D. V. Sinicropi and N. K. Wessells*. Department of Biological Sciences, Stanford University, Stanford, CA 94305.

The central and peripheral axons of dorsal root ganglion neurons in most vertebrates can regenerate after injury in vivo within peripheral nerves and spinal roots, but not within the spinal cord. We have examined whether spinal cord, dorsal root ganglion, and heart tissues of 14-day chicken embryos contain factors that influence sensory neurite regeneration in vitro. In initial experiments, monolayers of dissociated cells cultured from the various tissues were fixed with dilute glutaraldehyde, washed exhaustively, and treated with bovine serum albumin to inactivate residual fixative. Dorsal root ganglion explants were then cultured for 48 hours on the surface of the fixed cells. The length of neurites from explants cultured on dorsal root ganglion and heart cell monolayers was similar, whereas neurite length from explants cultured on spinal cells was markedly reduced. In a second series of experiments, plasma membrane fractions of heart and spinal cord were prepared by differential and sucrose-gradient centrifugation of tissue homogenates. Explants of dorsal root ganglia dissected from 14-day chicken embryos were cultured for 60 hours in plastic dishes incubated previously with the membrane fractions. Sensory neurites frequently extended for long distances beyond the outgrowth of non-neuronal cells over the surface of dishes coated with heart plasma membranes. In dishes coated with spinal cord plasma membranes, neurites rarely extended over the adsorbed particulate material and most growth cones were in contact with non-neuronal cells. After 3 days in vitro, the maximum length attained by neurites elongating on culture surfaces coated with heart plasma membrane fractions (2137 ± 133 [3] μm) did not differ significantly from that of neurites on uncoated substrata (2220 ± 60 [4] μm). Neurites migrating on spinal cord plasma membranes were 35-40% shorter (1380 ± 56 [8] μm, $P < 0.001$) than those on heart membranes or uncoated culture dishes. These observations indicate that plasma membrane preparations of embryonic heart and spinal tissues contain factors that either facilitate or impede sensory neurite outgrowth. (Supported by NIH grant HD 04708)

16.6 STIMULATION OF NEURITE OUTGROWTH FROM CHICK EMBRYO RETINAL AND SPINAL CORD NEURONS IN VITRO DURING DEVELOPMENT. J.M. Thompson, P.-P. Yang, B. S. Thompson, A.-H. Lu and L. Velasquez. Dept. of Anat. Sci., Neural and Behav. Biol. Prog. and Coll. of Med., Univ. of Illinois, Urbana, IL 61801.

The regulation of neurite outgrowth by extrinsic substances during neuronal development is an important step in synaptogenesis. Developmental decreases exist in neurite outgrowth in vitro from chick embryo ganglionic neurons (Ebendal, Devel. Biol., 72:276, 1979), and retinal and spinal cord neurons (Thompson and Rapoport, Devel. Biol., 84:244, 1981). This developmental decline in neuritogenesis correlates with a developmental decline in synaptogenesis in vitro between retinal neurons and muscle cells and spinal cord neurons and muscle cells (Ruffolo et al., Proc. Natl. Acad. Sci. 75:4977,1977; Thompson et al., Int. J. Devel. Neurosci. 1:25, 1983). Neurite outgrowth from neurons of relatively young embryonic ages can be stimulated using extracellular soluble factors: optic lobe extract (Carri and Ebendal, Soc. Neurosci. Abstr. 7:547, 1981); brain extract (Dribin and Barrett, Devel. Biol. 74:184, 1980); corneal epithelial conditioned medium (Chan and Haschke, J. Neurosci. 1:1155, 1981). We have examined the effects of several extracellular factors on neurite extension from dissociated chick embryo retina and spinal cord neurons from various aged donors. At 1, 3 and 5 days in vitro, the percent of cells with neurites and the length of neurites were determined. Although optic lobe extract stimulated neurite outgrowth, it also stimulated growth of glial cells which may have supplied a substrate or released factors necessary for neurite outgrowth. Glial conditioned medium stimulated growth of neurites when tested, suggesting release of a soluble neurite promoting factor from glia. Corneal epithelium conditioned medium had the most dramatic effect, stimulating neurite extension at all ages of retina tested (6-16 days in ovo). Spinal cord neurites were only slightly stimulated throughout development by an extract of 16-day chick embryo brain. The effect of these factors on the stimulation of synapse formation will be the goal of future experiments.

16.7 EFFECTS OF TUMOR PROMOTERS ON NEURITE OUTGROWTH FROM GANGLIONIC EXPLANTS. L. Hsu. Department of Anatomy, University of Medicine and Dentistry of New Jersey, School of Osteopathic Medicine, Piscataway, NJ 08854.

Tumor promoters are compounds that are neither mutagenic nor carcinogenic but will promote the formation of tumors when applied repeatedly after a subthreshold dose of an initiating agent. 12-O-tetradecanoyl-phorbol-13 acetate (TPA), a phorbol ester isolated from croton oil is the most potent promoter of skin carcinoma. In a number of cell systems, TPA and related promoters have been found to regulate a wide range of activities including proliferation, gene activation, alterations in differentiation, enzyme induction and cell surface changes. Binding activity of the tumor-promoting analog of TPA, [^{3}H] phorbol 12,13,dibutyrate ([^{3}H]PDBU), was notably concentrated in brain tissue (Dunphy et al, Canc. Res. 40:3635, 1980) and appeared to be associated with sites of extending cellular processes in the neural tube of rat fetus (Murphy et al, Science 222:1036, 1983). In the present study, we report that the addition of TPA to the growth medium of explanted ganglia elicited a rapid outgrowth of neurites and produced morphological changes in nerve bundle formation.

Sensory, sympathetic and parasympathetic ganglia from chick embryos were explanted on collagen or polyornithine coated wells and maintained in a defined serum-less medium consisting of Ham's F12 with glutamine supplemented with insulin, transferrin, selenium and progesterone. In control media, neurite differentiation was minimal. With the addition of TPA, both neurite outgrowth and non-neuronal cell proliferation were significantly enhanced. At low concentrations, TPA elicited the development of long, fine neurites within 2 days. With increasing concentrations of TPA (100-300 ng/ml), heavy outgrowths of neurites were induced but the overall extent of axons was markedly reduced. Treatment with such high concentrations of TPA typically produced thick fascicles of short neurite bundles. These effects of TPA are independent of other hormonal or growth factors in the medium or the presence of non-neuronal cells. Neurite-promoting activity was found to correlate with tumor promoting activity. Active tumor promoters such as phorbol-12,13,didecanoate and mezerein also stimulated neurite development while inactive derivatives such as phorbol or 4-α-phorbol-12,13,didecanoate were ineffective.

16.8 PURIFICATION OF RAT SCHWANNOMA NEURITE PROMOTING FACTOR G.E. Davis, M. Manthorpe and S. Varon. Dept. Biol., Sch. of Med., Univ. of Calif. San Diego, La Jolla, CA 92093

Cultured rat RN22 Schwannoma cells release into their growth medium a large, acidic macromolecule that when bound to polyornithine coated substrata will stimulate neuritic regeneration from previously axotomized peripheral and central neurons. Here, we report on the purification of this polyornithine-binding neurite promoting factor(PNPF). The purification procedure involves the processing of 1.5 liters of serum-containing conditioned medium through DE52 ion-exchange chromatography, cesium chloride equilibrium gradient and sucrose density gradient centrifugation steps. About 10-15 μg of purified PNPF is obtained (representing a 15,000-fold purification over the starting conditioned medium) and it has a specific activity of 3×10^5 Units/mg. Using Sepharose 4B gel filtration chromatography, the PNPF activity elutes in a peak corresponding to a molecular weight of 1.2 million daltons and after 7.5% SDS-PAGE with reduction, two major bands are visualized at 200 Kd and 190 Kd.

We have recently reported that purified rat yolk sac tumor derived laminin, a large basement membrane glycoprotein that on SDS-PAGE gives 400 and 200 Kd subunits, is a potent polyornithine-binding neurite promoting factor (J. Cell Biol., 97: 1882-1890, 1983). Antibodies to rat laminin block the neurite promoting activity of laminin in our assay, but fail to block the activity of purified PNPF. These antibodies, however, bind very strongly to the 200 Kd PNPF band after electrophoretic transfer to nitrocellulose. They also immunoprecipitate the purified PNPF activity from solution. Interestingly, others have reported that laminin obtained from immunoprecipitation of rat schwannoma, rat schwann and mouse skeletal muscle cell conditioned medium consisted of a 200 Kd subunit on SDS-PAGE without a visible 400 Kd subunit, thus suggesting its similarity to PNPF (J. Cell Biol., 96: 1218-1226, 1983; PNAS, 80: 3850-3854, 1983; Dev. Biol., 93: 344-354, 1982). Further experiments are in progress comparing the biochemical and biological properties of PNPF and laminin. Supported by NS 16349.

16.9 EFFECTS OF INSULIN ON PHOSPHORYLATION IN CULTURED HUMAN NEUROBLASTOMA CELLS. J.F. Mill and D.N. Ishii. Pharmacology Department and the Cancer Research Center, Columbia University, New York, NY 10032.

We have previously found that physiological concentrations of insulin can increase neurite formation in sensory, sympathetic, and human neuroblastoma SH-SY5Y cells. It has only recently come to light that insulin can have direct effects on neurons derived from the peripheral nervous system. We studied whether alteration in phosphorylation might be associated with neurite formation directed by insulin in cloned SH-SY5Y cells. Insulin can increase neurite formation in cells cultured in serum-free medium, a condition in which cells survive without loss in numbers for at least a week. After 1 day in serum-free medium, insulin caused a dose-dependent and reversible increase in total phosphorylation in intact cells, as revealed by the autoradiographic pattern following SDS PAGE. The large increase in total phosphorylation was evident even when corrected for the increased protein content of insulin-treated cells. The curve for the dose-dependent increase in total phosphorylation was superimposible with that for the dose-dependent increase in neurite formation. There was a corresponding reversible increase in the amount of $^{32}PO_4$ taken up by the cells cultured in insulin. Insulin also increased total phosphorylation in cells cultured in medium with serum. The pattern of increased total phosphorylation was not observed when the homogenates derived from insulin-treated cultures were studied, using ^{32}P-ATP. These observations suggest that insulin caused an increase in the uptake of phosphate from the medium. In addition, the studies with the homogenates revealed increased phosphorylation of specific proteins. These studies suggest that insulin may enhance neurite formation by increasing the overall phosphorylation of cellular proteins and altering phosphorylation of select proteins. Nerve growth factor and tumor promoters can enhance neurite formation in SH-SY5Y cells. It is known that nerve growth factor can alter phosphorylation in target cells and that the tumor promoter receptor is closely associated with a protein kinase activity. It is possible that a common event for neurite formation is the altered phosphorylation of specific cellular components. (Supported in part by NIADDKD grant RO1 AM32841 and NINCDS grant RO1 NS14218).

16.10 ELECTRIC FIELDS AFFECT ACTIVE TRANSPORT OF PROTEINS IN NEURONS. Bruce N. Mayes*, and John A. Freeman. (SPON: D. Buxbaum). Vanderbilt University, Nashville, Tn. 37232.

Small DC electric fields influence many basic neurophysiologic processes. A dramatic example is the accelerated growth of nerve fibers produced by small cathodal fields (Patel & Poo, J. Neurosci., 1982). Because many of a nerve terminal's functional macromolecules are supplied by rapid axonal transport, any change in function of a nerve terminal may reflect a change in the pattern, quantity, or velocity of actively transported proteins. Thus an effect of small intra-axonal voltage drops upon active transport must be considered in any explanation of field-regulated neurite extension. To determine whether axonal active transport is sensitive to local changes in electric field, we imposed small longitudinal electric fields upon excised bullfrog DRGs/sciatic nerves which were actively accumulating radiolabelled protein against a ligature. First the DRGs were incubated for 3 hrs in preoxygenated frog Ringers (pH 7.4) containing ^{35}S-Met (200 uCi/500 ul); their ligated nerves were passed through a 1 mm silicone grease barrier into an external bath (1mg/ml Met, frog Ringers). Then the ganglia and nerves were transferred to chambers which allowed application of a steady electric field and constant perfusion with oxygenated Ringers. The nerve fibers remained excitable throughout the application of the fields. We estimated the intracellular axial voltage gradient and changes in transmembrane potential produced by a given extracellular electric field, and confirmed these estimates by making intracellular voltage measurements in crayfish giant axons exposed to different field strengths. After six hours the nerves were sectioned into 1.0 mm segments and processed for liquid scintillation counting. We found that anodal fields as small as 5 mV/cm increased accumulation by as much as 20%. This increase remained constant for fields up to 75 mV/cm, and thus was not an electrophoretic effect. Instead, applied electric fields appear to affect active transport directly. Above 75 mV/cm, this effect becomes more pronounced. We conclude that relatively weak electric fields affect accumulation of transported proteins at a ligature, possibly by acting on the substrate- transport coupling mechanism, or on the rate of transport. This effect occurs at field strengths known to exist in embryos and in regenerating tissue, and might provide a mechanism whereby endogenous electric fields influence development and regeneration in the nervous system. Supported by NIH Grants EY-01117 and NS-18103.

16.11 FIBRONECTIN-LIKE IMMUNOREACTIVITY IN THE DEVELOPING NEOCORTEX OF THE MOUSE. A.L. Pearlman, G.R. Stewart, and J.P Cohen*. Depts. of Physiology and Neurology, Washington University School of Medicine, St. Louis, MO 63110

Fibronectin (FN) is a large (mw-460,000 d), dimeric glycoprotein that is a major component of extracellular matrices. In the developing embryo it appears to be involved in cell adhesion, proliferation, migration and differentiation. In the nervous system, FN plays a critical role in migration of neural crest cells and subsequent formation of autonomic ganglia, and may be involved in the migration of cerebellar granule cells; it has thus far not been demonstrated in the developing forebrain except in association with the vasculature and pia. In tissue culture, FN promotes neuritic outgrowth and is expressed by astrocytes. Since neuronal migration, aggregation into laminae, and process formation are critical features of neocortical development, we have examined the neocortex of the mouse for the presence of immunoreactivity to anti-fibronectin antibodies.

The brains of mice ranging in age from embryonic day 11 (E11) to adult were fixed in 4% paraformaldehyde and sectioned on a cryostat. The sections were incubated with an affinity-purified rabbit anti-human plasma fibronectin (aFN) antibody provided by Dr. J. McDonald. Binding of aFN to sections was visualized indirectly with a fluorescein-conjugated goat anti-rabbit IgG. As controls, aFN was either replaced with non-immune rabbit IgG or pre-absorbed with FN.

Blood vessels and pia stain brightly at all ages, serving as an internal control for aFN binding. Prior to delineation of the cortical plate (E11-E13), faint staining extends radially from the proliferative zone to the pial surface with additional patchy staining just beneath the pia. Over the next three days (E14-E16) the cortical plate (CP) forms and widens along lateral to medial and posterior to anterior gradients. During this time bright bands of aFN staining appear directly beneath the CP within the subplate, and in the marginal zone. Both of these regions are relatively acellular and contain developing fiber systems. After E16 all aFN binding within the neuropil of the neocortex rapidly dissipates. The spatial and temporal distribution of aFN binding thus closely parallels the formation of the cortical plate; binding occurs prominantly in the regions occupied by afferent and efferent axons, and thus may be important in process formation. (Supported by research grant EY00621 form NEI).

16.12 DISTRIBUTION OF LAMININ AND FIBRONECTIN DURING EARLY AXONAL GROWTH IN THE CHICK PNS. S.L. Rogers, S.C. McLoon and P.C. Letourneau*. Dept. of Anatomy, University of Minnesota, Minneapolis, MN 55455.

Axons that innervate peripheral targets traverse heterogeneous extracellular pathways early in development. The composition of these pathways has yet to be defined, but the spatial and temporal distribution of adhesive molecules may provide important guidance cues for the growing axons. We have shown previously that the adhesive glycoprotein laminin supports neurite extension by both central and peripheral nervous system neurons in vitro whereas under similar conditions only peripheral neurons responded to the related glycoprotein fibronectin. Fibronectin is present along paths of neural crest cell migration (Newgreen and Thiery, Cell Tiss. Res. 211:269, 1980), but it has not been clear whether either fibronectin or laminin areassociated with the earliest paths of axonal growth. Using immunocytochemical techniques, we are studying the distribution of these two molecules along pathways that may be followed by axonal growth cones emerging from the ventral spinal cord and developing sensory ganglia. Chick embryos between stages 17 and 25 of development were fixed with paraformaldehyde, frozen, and cut in either cross or longitudinal section. The sections were incubated with affinity purified rabbit antibodies to FN or LAN (gifts of Dr. Leo Furcht) followed by incubation with fluoresceinconjugated goat anti-rabbit IgG. To visualize the early nerve fibers, the same sections were treated with a mouse monoclonal antibody specific for neurofilaments (EC8, a gift of Dr. James Weston) and a rhodamine-conjugated secondary antibody. Dense deposits of fibronectin but not laminin were found in intersomitic spaces. It is not yet clear whether these regions form portions of axonal pathways. In contrast, the ventral root pathway (VRP) contains laminin but not fibronectin. At all stages examined, laminin in the VRP was associated with the external limiting membrane of the neural tube, with the cellular matrix between the neural tube and somites, and along the surface of the somites. Thus, growth cones of ventral horn neurons may be in contact with a laminin substratum throughout their entire migration from spinal cord to peripheral muscle masses. Laminin, but not fibronectin, is also present in newly formed sensory ganglia. In conjunction with our in vitro studies, these results raise the possibility that laminin provides adhesive guidance cues along early axonal pathways.
(Supported by EY05371 and EY0372 from the NIH).

16.13 LAMININ SUBSTRATES ENHANCE NERVE GROWTH FACTOR (NGF) INDUCED NEURITOGENESIS AND REGENERATION IN PC12 CELLS. K. Tomaselli* and L.F. Reichardt. (SPON: K.L. Valentino). Div. of Neurobiol., Dept. of Physiol., UCSF, S.F., CA 94143.

When immobilized on a tissue culture substratum, the basement membrane glycoprotein, laminin, is a potent inducer of neurite regeneration from both central and peripheral neurons. Laminin can also modulate neuronal responses to the trophic factor, NGF, which itself affects survival and neurite outgrowth of sympathetic neurons. The rat pheochromocytoma cell line PC12 also responds to NGF by extending neurites over the course of one week. PC12 cells primed with NGF for one week and mechanically divested of their neurites regenerate them rapidly ($<$ 24 hrs.) when reexposed to NGF. The ability of laminin to influence both de novo neurite outgrowth and neurite regeneration in the PC12 cell line was investigated.

PC12 cells were grown on poly-D-lysine coated polystyrene tissue culture plates pretreated with or without laminin at 10 ug/ml. Cells grown in the presence of NGF initiated neurite growth sooner and to a greater extent on laminin treated substrates. An altered dose-dependent response to NGF was observed for cells grown on laminin. Four- to six-fold less NGF was needed for a given extent of neurite outgrowth.

In addition, PC12 cells that had been primed with NGF for more than one week and then had their neurites mechanically sheared were able to regenerate neurites rapidly on laminin treated substrates even in the absence of NGF. In contrast, comparably primed cells grown on poly-D-lysine alone required NGF for neurite regeneration. Such NGF induced neurite regeneration was further enhanced on laminin treated substrates.

The apparent synergy of laminin and NGF with respect to neurite outgrowth might reflect the enhancement, by laminin, of NGF's action at the cell membrane or convergent intracellular pathways underlying the effects of these molecules.

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16.14 "NEURITE OUTGROWTH-PROMOTING FACTORS" IN CONDITIONED MEDIA ARE COMPLEXES CONTAINING LAMININ. A.D. Lander*, D.K. Fujii*, D. Gospodarowicz*, and L.F. Reichardt. Depts. of Physiology, Obstetrics & Gynecology, and Cancer Research Institute, University of California, San Francisco, CA 94143.

Many cells release into conditioned medium (CM) a factor which will adsorb to polycationic substrata and render them powerful stimulators of neurite outgrowth by many types of neurons. Studies on bovine corneal endothelial (BCE) CM and other CM's indicate that this factor is a complex containing a heparan sulfate proteoglycan (HeS-PG) and other proteins[1-3].

In an attempt to identify an active component, several groups have screened purified extracellular matrix molecules for similar activity, and found that laminin (LN) is similar to the CM-derived factors in its effect on neuronal growth and response to trophic factors[3-6]. However, antibodies against LN block the activity of LN-treated substrata, but not that of CM-treated substrata[2,3,5-7]. Antibodies against a partially purified CM-factor block that factor's activity but not that of LN[6]. While these data indicate that LN and the CM-factors are not identical, the CM-factors might still contain LN, but in a form (e.g. a complex with other molecules) in which antibody binding to its active domains is altered or blocked. We now present evidence that this is the case:

When purified to homogeneity the factor in BCE-CM contains LN, in association with a HeS-PG (LN is known to bind HeS) and a sulfated protein of $\sim$150kD, tentatively identified as entactin (a LN-binding protein[8]). During purification of the factor, biological activity, LN-immunoreactivity, and the presence of a band that comigrates on SDS gels with purified LN, all follow each other. Immunoprecipitation of BCE-CM with affinity-purified anti-LN removes this band, and with it, all biological activity. Immunoprecipitation also removes all activity from other CM's (RN22 rat Schwannoma, PC12 rat pheochromocytoma and C-2 mouse myoblasts). Like BCE-CM, the activity of these CM's is not blocked by anti-LN. Evidence from purification indicates that LN, HeS-PG and entactin are also associated with the factors in these CM's. We are seeking to learn how HeS-PG and entactin might interact with LN to prevent the access or binding of antibodies that block the activity of isolated LN. (Supported by grants GM07618 (NIH) and BNS8100342 (NSF)). / [1]Lander et al. J. Cell Bio. 94 574. [2]Lander et al. Neurosci. Abstr. 9 208. [3]Lander et al. C.S.H. Symp. Quant. Biol. 48 611. [4]Baron-Van Evercooren et al. J. Neurosci. Res. 8 179. [5]Manthorpe et al. J. Cell Bio. 97 1882. [6]Edgar and Thoenen, personal comm. [7]Coughlin et al. Neurosci. Abstr. 9 10. [8]Carlin et al. J. Biol. Chem. 256 5209.

16.15 LAMININ IMMUNOREACTIVITY AND EXTRACELLULAR SPACES IN CHICK EMBRYO NEURAL RETINA. Janice Jerdan,* James D. Lindsey, Ruben Adler and A. Tyl Hewitt*. The Wilmer Institute, The Johns Hopkins University, Baltimore, Md. 21205.

It is an emerging but still controversial concept that the extracellular matrix (ECM) can influence neuronal survival and differentiation not only in the PNS but also in the CNS. Several laboratories, including our own, have reported responsiveness of cultured CNS neurons to ECM components (i.e., Rogers et al., Dev. Biol., 98:212, 1983); Manthorpe et al., J. Cell Biol., 97:1882, 1983). In our study (Adler and Hewitt, J. Cell Biol., 97:97a, 1983) survival and differentiation of cultured retinal neurons were strongly stimulated by laminin suggesting that this glycoprotein may play a role in retinal development. In support of this possibility, we now report that laminin can be detected in the embryonic retina by means of ELISA and immunocytochemistry.

The avascular neural retina from 8-day chick embryos was cleanly dissected from other retinal tissues including the vitreous and pigment epithelium. After a 1 hr incubation in PBS to remove interphotoreceptor matrix, the retinas were extracted in 0.5M NaCl. The salt extract reacted strongly with a specific anti-laminin antibody by ELISA. This positive reaction was inhibited by purified laminin but not by other ECM molecules. Only low levels of laminin could be detected in the PBS wash and none was found in vitreous extracts.

Eight-day embryonic eyes were lightly fixed in paraformaldehyde, embedded in acrylamide and sectioned with a cryostat. Using avidin-biotin immunocytochemistry, laminin immunoreactivity was detected in the inner limiting membrane region as well as at the interphase between the retina and the pigment epithelium. Some staining could also be detected around the cells which make up the retinal neuroepithelium at this stage, although more accurate localization of the staining was difficult with the level of resolution offered by light microscopy. Transmission electron microscopy of 8-day retinas showed the presence of abundant extracellular spaces between retinal neuroepithelial cells. EM immunocytochemistry will now be performed to determine whether laminin is present in these spaces.

This finding of laminin immunoreactivity in vivo indicates that this survival- and differentiation-promoting molecule is well positioned to play an important role in retinal development.

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16.16 WIDESPREAD REACTIVITY OF THE MONOCLONAL ANTIBODY HNK-1 WITH CELLS OF THE CENTRAL NERVOUS SYSTEM. R.C. McGarry*, R.J. Riopelle, S. Mirski* and J.C. Roder* (SPON: H.B. Dinsdale). Depts. of Microbiology & Immunology and Medicine, Queen's University, Kingston, Ontario, Canada K7L 3N6.

HNK-1 (Leu 7) is a monoclonal mouse IgM antibody which was produced against a membrane antigen from the cultured T cell line HSB-2. HNK-1 recognizes a differentiation antigen present on approximately 15% of normal peripheral blood lymphocytes. These cells are principally large granular lymphocytes and have been shown to represent much of the natural killer cell activity of the peripheral blood. We have previously shown that HNK-1 recognizes myelin associated glycoprotein (MAG) (McGarry, R.C. et al., Nature 306:376, 1983) which is a minor glycoprotein found in both the central and peripheral myelin sheaths and comprises 1% or less of the total protein present. Immunofluorescent staining of cultured cells from the rat central nervous system has indicated that all of the galactocerebroside-positive oligodendrocytes and about 1/3 of the GFAP-positive astrocytes and astroblasts from newborn rat corpus callosum react with HNK-1. In addition, 100% of the tetanus toxin binding cells in cultures of 14d embryonic rat neurons derived from rat spinal cord and dorsal root ganglia reacted with HNK-1. Similar reactivity was noted with central and peripheral neurons from 7d chicken embryos. The antigen on dissociated cells from both rat and chick spinal cord was found to be trypsin sensitive with regeneration inhibited by cycloheximide. Staining of formalin-fixed paraffin sections of 14d rat embryos confirmed the widespread reactivity within the developing nervous system. Gel electrophoresis and immunoblotting of lysates of embryonic brain and cultured neurons indicated that in addition to a molecule which comigrated with MAG, several higher molecular weight glycoproteins reacting with HNK-1 were present. This indicates that, in addition to MAG, HNK-1 recognizes either immature forms of MAG or related glycoproteins of higher molecular weight. Taken together, the cellular distribution and gel data presented here indicate that MAG and a related family of glycoproteins may be widely distributed within the CNS.

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16.17 SOME SUBSTRATE INTERACTIONS OF NEURONS IN VITRO ARE MEDIATED BY A FAMILY OF SURFACE PROTEINS RELATED TO AND INCLUDING MYELIN ASSOCIATED GLYCOPROTEIN. R.J. Riopelle, R.C. McGarry*, S. Mirski* and J.C. Roder*. Depts. of Medicine and Microbiology & Immunology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

A mouse monoclonal antibody [HNK-1(Leu 7)] that recognizes an epitope on human myelin associated glycoprotein (MAG) (McGarry, R.C. et al., Nature 306:376,1983) has been shown (i) to be widely distributed in the developing nervous system, (ii) to react with proteins on the surface of cultured nervous system cells from chick and rat central and peripheral nervous systems, and (iii) by immunoblot analysis of cultured neuron lysates, to recognize a family of proteins, one of which co-migrates with MAG (McGarry, R.C. et al., this meeting). Previously it was demonstrated that the neuronal molecular species with a MAG-shared epitope could promote neuronal anchoring and rapid neurite extension on immobilized HNK-1 (McGarry, R.C. et al., Soc. Neurosci. Abst. 9:346, 1983). To determine if these molecular species were involved in neurite-promoting activity on biological substrates, HNK-1 and MAG were used in experiments designed to perturb neuronal attachment to, and process formation on, a variety of cell-derived substrate attached molecules (SAM's). Both HNK-1 and MAG exhibited dose-dependent inhibition of performance of chick CNS and PNS neurons on SAM's derived from conditioned media of cultures of neurons (Riopelle, R.J. and Cameron, D.A., Dev. Brain Res., 1984, in the press), a neural crest tumour (Riopelle, R.J. et al., Cancer Res. 43:5184, 1983), and another non-neural crest cell line, but did not influence the interaction of the neurons with laminin or fibronectin.

A family of related proteins, one of which is MAG, may be spatially and temporally heterofunctional in the nervous system: in early neurogenesis some of these proteins may regulate neuronal interactions involved in patterning; later the same or modified proteins may subserve axon-glia interactions.

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16.18 NEURITE FORMATION AND ELONGATION AT AN AIR-FLUID INTERFACE. R.W. Gundersen. Biomedical Research Institute, Univ. of Wisconsin-Parkside., Kenosha, WI 53141.

Studies of in vitro neurite formation and elongation involve solid substrata such as glass and tissue culture plastic which have been further modified with collagen, polyamino acids, laminin, fibronectin, or other surface adsorbed molecules. In addition, 3-dimensional matrixes of hydrogel, collagen and agar have also been utilized. All of these substrata are solid, which raises the question of whether or not neurite formation and elongation require a solid substratum. In order to address this question, dorsal root ganglia from 7 d chick embryos were floated on the surface of a tissue culture medium consisting of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 ng/ml 7S NGF. In this floating system, if a solid substratum was necessary for neurite formation elongation, no neurites would be observed. However, after 24 hr in vitro a neurite halo surrounding the floating ganglia was observed at the air-culture medium interface. The neurites were straight over their entire length and each had a terminal growth cone with microspikes. If the culture dish was gently agitated, or a micropipette used to displace a neurite, rapid neurite retraction occurred (<3 min). During neurite retraction, the neurites exhibited a typical helical pattern. Treatment of the floating neurites with cytocholasin B(1 µg/ml) inhibited neurite elongation, but did not cause neurite retraction. Addition of colchicine to the medium (1µg/ml) produced rapid neurite retraction. Both cytocholasin and colchicine blocked neurite formation. Decreasing the surface tension by supplementing the medium with fetal calf serum (10%) caused the neurites of floating ganglia to retract. Microgradient application of an elevated concentration of NGF (50 ng/ml), which elicited a chemotaxic response from substratum attached neurites within 20 min, produced no chemotaxic response from floating neurites during a 3 hr observation period.

These results indicate that neurites do not appear to require a solid substratum, as indicated by the observation that the physical interactions, such as surface tension, present at an air-fluid interface could support neurite formation and elongation. Microtubules may provide internal cytoskeltal support for neurites, while high surface tension provides necessary external support. However, changes in neurite direction apparently require a solid substrate, since floating neurites were unable to exhibit a chemotaxic response to NGF. Supported by NIH #NS18214.

16.19 PATTERNS OF AXONAL BUNDLES FORMED BY MOUSE RETINAL GANGLION CELLS IN VITRO. I. Shalaby, S. Price* and R.W. Guillery. Department Pharmacological and Physiological Sciences, University of Chicago, Chicago, Illinois 60637.

A method of growing cells from mouse retina in vitro in a manner that will allow the new formation and study of axon bundles has been developed. The aim has been to study the size of the axon bundles, their growth directions, and the interactions between bundles, so that tissues obtained from different mutant strains and grown under different conditions can be compared. The long term aim is to develop a situation where the relationship between melanin, the absence of which is associated with visual pathway abnormalities, and patterns of axonal outgrowth from the retina can be studied.

We chose not to study retinal explants since some of the patterns of bundle formation are already determined at the time of explantation. Instead, we initially used the method described by Garber and Moscona (Develop. Biol. 27:217-234, 1972). Retinal Cells from 14 day fetal C57Bl/6J mice were dissociated and then allowed to reaggregate in rotating flasks to form small, spherical, organized cell aggregates. The aggregates formed from the mouse tissues, in general, resemble those described for the chick (A. Moscona, In: Cells and Tissue Culture, vol. 1, E. Willmer (ed.), Academic Press, 1965). Rich axon bundles develop in a layer immediately beneath the surface, where they form extensive intertwinings and crossings. Melanin containing cells survive well in the central parts of the aggregates, generally at some distance from the fiber bundles, although fiber bundles can also be seen immediately adjacent to melanin bearing cells.

In order to display the axon bundles more conveniently, and to study the relationships between melanin bearing cells and such bundles, we have transferred the aggregates onto polylysine coated coverslips after they had developed in the flasks for 2-8 days. The aggregates flatten and axon bundles of varying sizes form two rather distinct systems. One system is formed by axons growing radially, the other forms circumferential axons growing at some distance from the aggregate. In these preparations one can define the relationships of the bundles to each other and to pigment epithelial cells, one can modify the production of melanin, and combine tissues from mutants having different developmental histories. These types of studies can clarify the interactions that may occur between pigment epithelial cells and cells of the neural retina during development.

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16.20 THE EMBRYONIC DEVELOPMENT OF AXONAL ARBORIZATIONS IN NUCLEUS LAMINARIS OF THE CHICK AUDITORY SYSTEM. S. R. Young* and E. W Rubel. Dept. of Otolaryngology, Box 430, Univ. of Va. Med. Center, Charlottesville, Va., 22908

The development of terminal fields of individual axons from n. magnocellularis (NM) neurons to n. laminaris was studied. Nucleus laminaris (NL) is a monolayer of neurons in the avian brain stem that receives segregated, binaural, 2nd order auditory input to distinct dorsal and ventral dendritic fields. The major input to NM is from the ipsilateral cochlea. Each NM neuron projects to both the dorsal dendritic field of the ipsilateral NL and to the ventral dendrites of the contralateral NL. Both nuclei are tonotopically organized. Individual axons of NM neurons were filled with HRP in chicks from embryonic day 6 (E6) to post-hatch day 30 (P30). Stained axons were drawn with a camera lucida and reconstructed by aligning their cut ends in adjacent sections. The terminal fields were plotted on a planar projection of NL.

The mature NM axonal arborizations form narrow bands oriented perpendicularly to the axis of the tonotopic organization (Young, S. R. and E. W Rubel, J. Neurosci., 3: 1373, 1983). By E14-15, dorsal and ventral terminal fields are identical in shape and orientation. Dorsal and ventral afferents differ, however, in their developmental histories. At all ages studied the contralateral terminal arborizations form bands in the ventral NL dendritic region. In contrast, at E9, the dorsal projection forms a punctate terminal field. The position of this field in the dorsal neuropil of NL topographically matches the position of the NM cell body within NM. The transition from punctate dorsal terminal fields at E9 to an oriented band of terminals at E14 occurs via a gradual elongation and narrowing of the E9 fields.

Examination of younger embryos may elucidate the origin of the punctate E9 terminal fields. At E6, ipsilateral branches of NM axons terminate as growth cones in the nearby ventricular zone. By E7 these growth cones have left the ventricular zone and are extended perpendicularly from it towards the developing group of neurons which is the presumptive NL. We suggest that the specific, punctate, arborizations seen at E9 are the result of specific connections made by NM growth cones on presumptive NL cells in the ventricular zone. As the NL neurons migrate to their final site, their ipsilateral afferent axons are towed behind.

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16.21 LOCAL FEATURES DIRECT PATTERNED NEURITE OUTGROWTH. K. W. Tosney* and L. T. Landmesser, Physiology Section, Biological Sciences Group, The University of Connecticut, Storrs, CT. (SPON: R. J. Wyman).

To investigate features that may dictate the gross anatomical pattern of motoneuron outgrowth in the chick hindlimb, we totally or partially ablated the early hindlimb bud and determined how the subsequent pattern of nerve outgrowth related to the distribution of tissue remnants. In addition, in normal embryos, we correlated the patterns of outgrowth and alcian blue staining, which shows the distribution of glycosaminoglycans (GAG).

Our results suggest that the gross anatomical pattern of outgrowth is directed by local elements, since: (1) Determinates of individual pathways can be selectively removed without altering the nerve pattern elsewhere. (2) Neurites will enter the ventral crural nerve pathway in the leg when the target for this nerve is absent. (3) The position where the major nerve trunks enter the leg is dictated by the portion of the pelvic girdle precursor that lies between the plexus region and the base of the leg. When gaps are made in this central region of the girdle precursor, neurites traverse the gaps and form novel nerves into the leg, but when other regions of the girdle precursor are removed, the neurites remain in their normal pathways, even when these are not the shortest routes into the leg. (4) Neurites do not enter regions that are rich in GAG. For instance, the precursor of the pelvic girdle is identifiable with alcian blue staining by the time that growth cones reach the base of the leg. In addition, neurites in the spinal nerve pathways grow out between areas that stain darkly and lightly for GAG.

These results imply that the local environment during outgrowth is the primary determinate of the gross anatomical nerve pattern. We suggest that tissues along the neurite pathways differ in their ability to support axon elongation, and that tissues high in GAG are lowly permissive for growth cone advancement. The disposition of these less permissive regions may serve to restrict outgrowth to defined regions so that a consistent anatomical nerve pattern will develop, regardless of the specificity of innervation.

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16.22 IS TIMING IMPORTANT IN THE GUIDANCE OF AXONS OF IDENTIFIED NEURONS? B. Mendelson. Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

Identified reticulospinal (RS) neurons were studied in the larval zebrafish in order to learn if their stereotypic axonal pathways are correlated with specific differences in their times of neuronal development. I have previously shown that dorsally positioned RS neurons (Mauthner, MiD1 and MiD2) are born and initiate axonal outgrowth before ventrally located RS neurons (MiV1 and MiV2) (Mendelson, B., Soc. Neurosci. Abstr. 9: 445.4). We recently observed that there are two distinct types of MiD1 and MiD2 neurons (C. Kimmel, W. Metcalfe, and B. Mendelson unpublished results). The MiD1c and MiD2c neurons are very similar in morphology to the Mauthner neurons in that they have large somata located dorsally with respect to other RS cells and axons which decussate to descend contralaterally into the spinal cord. The MiD1i and MiD2i are similar to their counterparts in morphology and position with the important exception that their axons project ipsilaterally. Does timing correlate only with cell body position or does timing also determine axonal projection?

I compared the timing of development of these four types of MiD neurons. I determined by 3H-thymidine incorporation and autoradiography that all four cell types are born between 8 and 10.5 h after fertilization. MiD1c and MiD2c neurons were first unlabeled with 3H-thymidine at 8 h while MiD1i and MiD2i cells were first unlabeled at 8.5 h. But I have also observed animals in which both MiD1c and MiD1i are very lightly labeled as if they were leaving the cell cycle together. I also have determined the earliest developmental times that these cells can be retrogradely filled with HRP from a lesion performed about 200 um caudal to their somata in order to learn when their axons are present. I found that these cell types show no significant differences, within less than an hour, in the time that their axons grow to the lesion site. From these data I conclude that if a timing mechanism is important in the axonal guidance of these identified neurons, it is operating at a level of resolution below that detected. (Supported by NIH grant NS 17963 and NIH GM 07257.)

16.23 CRYOPROTECTIVE METHODS FOR PRESERVATION OF FETAL CENTRAL NERVOUS SYSTEM TISSUES FOR TISSUE CULTURE. J.C. Kawamoto* and J.N. Barrett. Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, Florida 33101.

Several central nervous system tissues obtained from fetal rats on days E_{14} to E_{18} of gestation were stored either in the cold (+2°C) or frozen (-20° or -90°C) prior to tissue culture in a serum-free synthetic CSF-like medium. Phase contrast microscopy was used to determine the initial number of cells adhering to the collagen-polylysine substratum, and the number of cells surviving after one day. At time periods from 3 days to 4 weeks, the number of apparently viable cells with neuronal characteristics were distinguished from those with non-neuronal appearance. The activity of choline acetyltransferase in spinal cord cultures was determined by radioenzymatic assay. These parameters were correlated with variations in storage conditions.

Effects of the following storage parameters were analyzed in this study:

1) ionic composition of the "hibernation medium" in which tissues were stored (Na^+, K^+, Ca^{++})
2) storage temperature
3) duration of storage
4) type and concentration of cryoprotectant used
5) size of tissue pieces
6) osmolality
7) pH
8) glucose concentration

These studies demonstrate that a "hibernation medium" which minimizes ionic stresses is important for prolonged storage of CNS tissues in the cold. We have found that in a medium containing 50mM KH_2PO_4, 10 mM NaCl, 0.5 mM EGTA, 24 mM $KHCO_3$, 22 mM glucose adjusted with sorbitol to 300 mosmol and to pH 7.0, whole CNS tissues remain viable for at least 7 days in the cold. If 5-10% DMSO is added to the medium dissociated neurons may be frozen for several weeks.

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17.1 ONTOGENESIS OF GABA-LIKE IMMUNOREACTIVITY IN THE CEREBRAL AND CEREBELLAR CORTICES OF THE RAT BRAIN . A.Privat, M.Geffard, G.Le Gal La Salle and F. Sandillon(SPON M.C. Calvet)INSERM U-249 Montpellier and U-259 Bordeaux France

Direct visualisation of Gaba containing cells has been made possible recently through the use of antibodies directed against Gaba(Geffard 1983) . In the present experiment, rats ranging from 14 days foetal to adult were used. Foetuses were fixed by immersion in a mixture of glutaraldehyde 5% and sodium metabisulfite 1% in cacodylate buffer, and newborn to adults were perfused with the same fixative. Vibratome sections were performed and treated according to the PAP method after Sternberger. For electron microscopy, sections were postfixed with OsO4 and flat embedded in araldite . In the cerebral cortex, immunoreactivity was present as soon as the 14th foetal day, under the appearance of coarse subpial processes progressing from the rostral pole and sparing the ventricular layer. At the 16th foetal day, three plexuses were seen : a very dense plexus located under the pia, made of horizontal cells with small perikarya and irregular processes A second plexus was located under the cortical plate ; it was less dense than the subpial plexus and made of small cells with horizontal and oblique processes . A third plexus, located above the ventricular layer was made essentially of immunoreactive perikarya without processes. In addition, a few isolated cells were seen throughout the cortex, and in the ventricular layer, where they extended horizontally. In the newborn animal, the subpial plexus was thinner, but still very dense, and preliminary EM observations indicate that it is made of small cells with irregular contours, devoid of synapses, and different of Cajal-Retzius cells . The second plexus had disappeared, and the third one was still present as a very loose network of horizontal cells . Besides these plexuses, many radially oriented cells were found throughout the cortex. Ten days after birth, the picture was that of the adult, with however a loose array of cells at the surface of the corpus callosum. In the cerebellum, a dense reticulum of immunoreactive cells was found in the 16th days foetus, occupying most of the rhombic lip and sparing the EGL . In the newborn, immunoreactive elements concentrated in the IGL and Purkinje layer. At 10 days, Golgi neurons were strongly reactive, Purkinje cells faintly stained, and a few basket-stellate cells were seen . At 20 days, the picture was that of the adult. The relevance of this specific pattern for brain morphogenesis is now under study .

17.2 TRANSIENT ACETYLCHOLINESTERASE (AChE)-REACTIVITY IN DEEP CEREBELLAR NUCLEI OF NEONATAL RAT. N.A. Martin-MacKinnon* and D.A. Kristt (SPON: J.S. Williston). Stanford Univ. Med. Ctr., Stanford, CA 94305

The observation of transient AChE reactivity in some noncholinergic cell groups of infant rat brain (Neurosci., 10:923, 1983) may provide clues as to the possible roles of this glycoprotein in neuronal maturation. We report here the finding of transiently AChE-positive neurons within the **deep cerebellar nuclei (DCb nu.)** of infant rat, despite the putatively noncholinergic nature of these cell groups. At birth the medial and lateral nuclei contain stained cells; by the fourth day neonatally (4dpn) neurons in all four nuclei are reactive. Generally, these neurons are first densely packed and nonreactive, become positively stained, then progressively more loosely packed, and finally they lose their stainability. The maturational decrease in cell density is presumably due to dendritic proliferation and/or cell death. Both large and small stained cells are dispersed homogeneously or are focally clustered within each nucleus. Differences in these patterns of cell distribution distinguish each nucleus; characteristic maturational changes also occur in these patterns of cell distribution within each nucleus. By 32 dpn very few positive-staining cells remain. The AChE seen in these neurons is contained within the perikaryal cytoplasm, extending centrifugally into the proximal processes, suggesting that the DCb nu. neurons transiently synthesize AChE. The background neuropil is relatively unstained. The possibility of other AChE-positive elements of cerebellum contributing to this staining was considered. Although the fibers of the inferior cerebellar peduncle (ICP) are AChE-positive at birth, they are fasciculated and pass dorsal to the deep nuclei without giving off detectable fibers to the nuclei. AChE-reactivity of Purkinje cells arises after that of the deep nuclei, and therefore seems unlikely as a source of this staining.

In light of studies documenting the transient synthesis of AChE by thalamic neurons, our data suggest that 1) the presence of transient AChE-reactivity in noncholinergic neurons is not limited to thalamus, 2) both thalamus and DCb nu. show specific temporal-spatial patterning of AChE staining, and 3) in both sites, the staining appears to be post-migrational and begins to disappear with the proliferation of cell proceses. In general terms, it appears that the phenomenon of transient AChE reactivity occurs with similar properties in sites not directly related, either in terms of neural connectivity or embryological development. Support: NSF Grant BNS 81-40895.

17.3 TEMPORAL AND REGIONAL DIFFERENCES IN EXTRACELLULAR MATRIX OF EMBRYONIC BRAIN STAINED WITH LECTINS. M.D. Shaw and M.E. Hatten. Dept. Pharmacology, N.Y.U. Medical Center, New York, NY 10016.

Extracellular matrix glycoconjugates are involved in the morphogenesis of many embryonic structures. Lectins, affinity ligands that bind specific carbohydrate sequences, can be used to detect extracellular glycoconjugates. Biotinylated lectins were used to stain frozen sections of embryonic mouse brain. The staining patterns obtained from concanavalin A (Con A), wheat germ agglutinin (WGA), and Ricinis communis agglutinin (RCA-1) are all different and change significantly with the developmental phase of the animal.

On embryonic day 12 (E12, plug =E1), Con A -staining material appears to accumulate at motor nuclear anlagen. Because it is present so early and disappears soon afterward, this is more likely to be a marker for the future site of the nucleus than a product of neuroblast differentiation. On E13, there is a particularly dense layer of Con A staining just external to the ventricular layer - the region into which new neuroblasts must migrate. On E14, Con A accumulates in the cell sparse core of the cerebellum, an area through which extensive migration will soon take place. In later embryos (E17), these layers melt into an overall, homogeneous extracellular staining. WGA also stains the juxta-ventricular layer most darkly at E14, but it is distributed in patches. This distribution may relate to the blood supply; in areas of little extracellular staining (E14 dorsal cerebrum), the blood vessels stain clearly with WGA, but in areas of denser matrix staining, blood vessels are not distinguishable. RCA-1 at all ages studied stains blood vessels intensely. At E14, there is also an intriguing accumulation of RCA-1 in the midline region of cerebellum, brainstem and spinal cord. Also at E14, there appears to be specific staining of sensory axon tracts. Although at E14 there is homogeneous staining of the cerebral cortex, by E17 there are two distinct layers, one lightly stained near the pia and one more darkly stained near the ventricle. Other lectins tried so far did not stain neural tissue. The results indicate that the staining patterns of these lectins are distinctive, change with developmental stage and region, and appear to correlate with developmental processes of importance, such as migration, axon tract formation, and histological organization. Supported by NIH grant NS 15429.

17.4 DEVELOPMENTAL AND REGIONAL ASPECTS OF LECTIN-BINDING IN THE MOUSE BRAIN. N.G.F. Cooper* and D. A. Steindler (SPON: B.J. McLaughlin). Dept. of Anatomy, University of Tennessee Center for the Health Sciences, Memphis, TN 38163

In order to characterize membrane glycoconjugates of differentiating neurons, we have sectioned brains of 1, 3 and 7-day postnatal and adult animals, and incubated them in lectin-peroxidase conjugates. Mice were anesthetized, perfusion fixed with aldehydes, and the whole brains were then vibratome sectioned (20-50 um). The sections were buffer washed, pretreated with BSA and incubated in Concanavalin-A (CON-A), Wheat Germ Agglutinin (WGA), or Peanut Agglutinin (PNA). After peroxidase cytochemistry they were mounted for light microscopy. Peroxidase reaction product within the sections indicates the presence of mannose, N-acetylglucosamine or sialic acid, and galactosamine containing glycoconjugates. Using the intensity of reaction product as a measure of lectin-binding, several trends could be detected which were indicative of developmental and regional variabilities. In the cerebrum of 1 day postnatal animals CON-A and WGA label layers 1, 3 and 4 of visual cortex, layers 1 and 4 in more rostral sensory/association cortical areas, and layers 1 and 3 in frontal cortex. PNA labels the same cortical layers maximally at postnatal day 3 whereas the other lectins label maximally on postnatal day 1. This laminar pattern decreases in intensity with postnatal age, and cannot be detected with PNA at day 7 whereas CON-A and WGA label just layer 1. No laminar pattern is seen in the adult cerebrum although Con-A and WGA labeled cells are observed. In the postnatal day 1 cerebellum, the Purkinje cell layer is most intensely labeled with PNA but this decreases with age. CON-A labeling of this layer is seen at this age but its intensity increases with postnatal age. WGA labeling of this layer is always less distinct. In the adult, perisomatic, punctate, labeling is detected with all 3 lectins within the deep cerebellar nuclei. White matter fasicles label with all 3 lectins at early postnatal times. CON-A and WGA labeling of white matter decreases with age whereas PNA labeling is retained in the adult. It is likely that the patterns of labeling observed here reflect specific alterations in membrane glycoconjugates of migrating and differentiating neurons and glia, growing axons, and forming synapses. These observations provide a basis for future microsocpic and molecular characterizations of neurogenesis. Supported by USPHS Grants EY02708 and NS15931.

17.5 MONOCLONAL ANTIBODIES POINT TO NOVEL FEATURES OF LEECH SEGMENTATION. B. Zipser and T. Flanagan. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

Physiologically and anatomically homologous neurons that repeat from segment to segment along the leech nerve cord have been identified in previous studies using monoclonal antibodies. Now we are reporting on antibody-stained neurons that are assigned to specific locations within leech CNS according to the following supersegmental counting mechanisms: 1) 1 type of neuron is assigned to even-numbered ganglia; 2) 1 type of neuron is assigned to ganglia 7 and ganglia 2 x 7 = 14; 3) 1 type of neuron is assigned to the second cephalic and second midbody ganglion.

We began to characterize these neurons that are distributed according to supersegmental counting mechanisms. So far we have studied 2 of the 3 cell types and shown that they are intersegmental interneurons. Another feature these neurons have in common is that their antibody-stained processes are beaded in a way that is reminiscent of beaded processes stained by antisera to peptides such as enkephalin.

Previous studies of the leech central nervous system had focused on the ganglion as a segmental unit. Our mabs are pointing to novel organizing principles that divide the nerve cord into different supersegments. Comparing the developmental history of neurons reiterated in all ganglia to those assigned restrictively according to a supersegmental counting mechanism may shed light on the control of segmentation in the leech.

17.6 A PURKINJE CELL ANTIGEN REVEALS SAGITTAL BANDS IN THE RAT CEREBELLAR CORTEX. R. Hawkes, N. Leclerc, M. Colonnier, Laboratory of Neurobiology, Laval University, Québec, Canada G1K 7P4.

We have produced a library of monoclonal antibodies which recognize antigens expressed during the development of the rat cerebellar cortex. One of these, mabQ113, reacts on Western blots with a single polypeptide, apparent molecular weight 120,000. Immunostaining of sagittal sections of rat cerebellum shows that Q113 is confined exclusively to the Purkinje cells. Reaction product is from throughout the cell with the entire dendritic tree including the dendritic spines, the cell body and the axons and their collaterals all stained. Electron microscopy confirms this distribution and reveals that in the larger dendrites, labelling is associated with the microtubules while in the dendritic spines label appears as flocculent deposits in the cytosol.

Not all Purkinje cells are labelled. In horizontal sections, the labelled and unlabelled Purkinje cells form parasagittal rows which run throughout the cerebellar cortex. The pattern of bands is highly conserved from individual to individual and between rat and mouse. This suggests a biochemical parcellation of the cerebellar cortex into parasagittal modules which perhaps corresponds to the sagittal organization of the afferent inputs.

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17.7 HISTOGENESIS OF THE PERIAQUIDUCTAL GREY IN THE MOUSE . E. Taber-Pierce and L.K Laemle. Dept. Anatomy, Harvard Med. Sch., Boston, MA 02115 and Dept Anatomy, N.J. Sch. of Med. and Dent., Newark, N.J. 07103.

Three periaquiductal grey zones are delineated in the midbrain, dorsalis, lateralis and ventralis. The three zones vary in their density of neurons-compact in the dorsal zone, dispersed in the lateral zone and in the ventral zone the cells are dispersed or interspersed among designated nuclei. Immediately surrounding the aquiduct the three zones are characterized by a relatively cell free zone.

Neurons arise on days 9-15. Peak time of cell origin occurs on days 11 and 12. Three gradients are present- one ventral to dorsal, a second is rostral to caudal and a third, less obvious, is inside-out. Attention has been directed to recording the time of origin of the various cell types. Data collected indicate that the time span of origin, although specific for each cell type, ranges broadly among the specific cell types.

Female mice BALB/c Gn mated to SJL males were given one injection of ^{3}H-Thymidine subcutaneously on a known day of gestation, 5 u Ci/g body weight. The offspring were killed 2-3 months later by perfusion through the heart with 10% acrolein and were processed for autoradiography using Kodak bulk emulsion (NTB_2). Sections were cut at 10 u and stained with toluidine blue. Position of labeled cells was plotted by camera lucida with every 20th section mapped at 200X.

17.8 A TRANSCELLULAR FILAMENT NETWORK THAT INTERCONNECTS CELLS IN TISSUES. Mark H. Ellisman. Laboratory for Neurocytology, Dept. Neurosciences, U.C.S.D., La Jolla, CA 92093.

A highly developed system of interconnected transcellular fibers has been observed within the electric organ of Electrophorus electricus. The existence of this system was first noted when 1-2μm thick sections were viewed with the aid of high voltage EM (HVEM). Confirmation of its extensive distribution and the macromolecular identity of some of its constituents has now been obtained. This network appears to be composed of 9-10nm filaments, alpha-helically wound, forming interconnected and branching cables of larger diameters. Within the cell, this system includes components that radiate from the nucleolus, traverse the nucleoplasm, the nuclear envelope and thereafter ramify extensively throughout the cytoplasm. Forms of extrusions beyond the plasma membrane include: Simple projections, of from two-four 9-10nm filaments, twisted in the form of a multiply-coiled alpha helix ending freely with closed loop terminations or re-entering the same cell; Connections with extracellular matrix, in the form of ordered associations with collagen; and Transcellular connections, which in this tissue include filamentous continuities between nerve and electrocyte at the endplates. Why the distribution and nature of this pervasive and clearly important "transcellular filament system" (TCFN) had not been observed previously appears to relate to the physical properties of the network's molecular constituents and the principles by which structures are imaged in the electron microscope. These structures are not positively contrasted by conventional electron dense stains, rendering them refractory to direct electron microscopic visualization. Further, the electron scattering of these structures is lower than surrounding epoxy resin. These factors combine yielding a network delineated in micrographs by its lack of electron contrast or negative image thus difficult to recognize except when viewed in 3-D. Methods used to both enhance the visibility of the TCFN and verify its distribution include: increasing the electron scattering of the epoxy matrix further, relative to that of the TCFN; attaching intermediate filament antibodies as specific vectors for contrast enhancement; and replication techniques. The general features of this network appear ubiquitous and have been observed in association with cells of both vertebrate and invertebrate tissues. The distribution, ubiquity, subunit dimensions and immunoreactivity of this filament system suggest that intermediate filaments are one of the central constituents.

17.9 EFFECTS OF CIS-HYDROXYPROLINE ON THE DEVELOPING NEUROEPITHELIAL BASAL LAMINA: A CORRELATED SLICE CULTURE AND WHOLE EMBRYO CULTURE STUDY. K.S. O'Shea* (SPON: S.P. Hicks). Dept. Anat. and Cell Biol., Univ. Mich. Sch. Med., Ann Arbor, MI 48109.

The neuroepithelial (NE) basal lamina (BL) may play a crucial role in determining structural stability, cell polarity, and cell-cell interactions during neurulation. To examine the role of collagen (a structural component of BL), whole embryos or midbrain slices were exposed to cis-hydroxyproline (CHP), a proline analogue that inhibits collagen deposition in the BL.

Embryos and slices were cultured either prior to cephalic neural fold (NF) elevation (1-2 somites) or shortly after elevation had begun (6-8 somites). At least 16 CHP embryos and 44 CHP slices were cultured at each stage, and similar numbers of controls received PBS alone. Whole embryos were cultured in medium containing 1 ml rat serum, 1 ml FC emulsion, 20 ug/ml garamycin, 50 ug/ml CHP. Slices (approx. 0.5 mm) were cut from the future midbrain, 4-5 placed into shaker culture in flasks containing 2.5 ml DMEM with 0.01% BSA, 20 ug/ml garamycin and 50 ug/ml CHP. Cultures were gassed with 5% CO_2 in air and maintained at 37°C for the 4 h culture period. Tissues were fixed in 1% glutaraldehyde in 0.1 M phosphate buffer with 1% tannic acid for 1 h, then embedded in Epon-Araldite, sectioned, and examined using TEM.

The NF of CHP embryos were wavy and irregular and often did not fuse in the midline, unlike controls. In 6-8 somite embryos, the posterior neural folds were especially affected. Ultrastructurally, the control NE BL was composed of a mat of collagen fibrils that interfaced with a smooth lamina densa. Collagen fibrils increased in number during this period, and there was a slight increase in collagen deposition in explant BL. The NE BL of CHP-treated tissues was very wavy and incomplete, with few associated collagen fibrils. With early CHP exposure, NE cells herniated through the patchy BL. Current studies are in progress to localize BL components using TEM immunocytochemistry.

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17.10 MORPHOMETRIC ANALYSES OF SHAPING AND BENDING OF THE AVIAN NEURAL PLATE. G.C. SCHOENWOLF, M.L. POWERS* and M.V. Franks *. Department of Anatomy, University of Utah, School of Medicine, Salt Lake City, Utah 84132.

Bending of epithelial cell sheets is a common morphogenetic event involved in the formation of a number of organ rudiments. The neuroepithelium is an excellent model for studying shaping and bending of an epithelial cell sheet. Changes in the volume, length, width, and height of the neuroepithelial were examined in stage 4 to 11 chick embryos to gain insight into the mechanisms of neurulation and to obtain normal parameters for comparison with data obtained from embryos with neural tube defects. Data were collected on an Apple II+ computer and Hipad with a program called DIG-TRACE-EZE ("digitizing and tracing made easy"--developed in collaboration with Dr. Dennis Schweitzer, Creative Computer Consulting, Salt Lake City, Utah). During stages 4-11, the volume of the neuroepithelium increases 4.9 fold, its length increases 8.7 fold, and its lateral height increases 1.3 fold. Concomitantly, the median height of the neuroepithelium decreases 23%, and its width decreases 46% basally and 58% apically. It is clear from these measurements that a one to one correlation between changes in the width and height of the neuroepithelium does not exist in chick embryos (i.e., the increase in lateral height of the neuroepithelium is too small to account for the dramatic decrease in its width). Thus, either the number of cells spanning the width of the neuroepithelium must decrease progressively or cell volumes must decrease to compensate for this difference. Several paradigms have been constructed to explore the possible effects of changes in neuroepithelial cell volumes, heights, and numbers on shaping and bending of the neural plate. Research was supported by grants HD 15231, NS 18112, and HD 18143 to G.C.S. from the National Institutes of Health.

17.11 COMPUTER-AIDED STUDY OF CELLULAR FORMS IN MOUSE CEREBELLUM J.H. Rho* (spon. T.O. Fox) Departments of Neuroscience, Children's Hosp. & Neuropathology, Harvard Med. Sch., Boston, Mass., 02115.

Central to any study of CNS organization is an understanding of the morphological relationships among the constituent cells. To gain a more precise understanding of the spatial relationships among cells of the adult mouse cerebellum, we have been developing methods to combine intracellular microiontophoretic techniques with computer graphical manipulation in order to assemble images of various morphological structures in relationship to other neurons, tissue boundaries, and cytoarchitectonic contours. Cerebellar tissue slices were prepared by fixing the brain with sequential perfusion of saline, 4% paraformaldehyde, and saline, then sectioning the cerebellum into 200 um slices on a Vibratome. To visualize the fine morphological detail of Purkinje (PC) dendrites, Stellate (St) cells, Bergman (BG) fibers, etc. 5% Lucifer Yellow (LY) was pulsed (2/sec) with brief hyperpolarizing current (2nA) from a hydralically mounted microelectrode (50 Mohms) into the small dendrites and axons (backfilling the somata also) that are encountered in the molecular and granular layers . When neuronal processes appeared, under flourescent compound optics, to be fully filled, dye ejection was stopped and processes of other neighboring cells were penetrated. Consistent morphological patterns and orientations shared by groups of LY filled neurons reveal much about the underlying polarities in the local neuropil. For example, the fundamentally radial orientation of a curving folium is delineated by BG fibers, in relation to which the PC dendritic trees maintain orderly planar patterns not obvious when isolated PC are visualized. To quantitate such morphological details, cerebellar slices were mounted with a glycerol/carbonate buffer and then examined with a flourescent compound microscope whose stage movements in the XYZ dimensions are controlled remotely through a computer. The 3-D structures of LY filled neurons are converted into numerical form by superimposing an image traced on a digitizing tablet onto the image in the microscope field of view and quantifying vectorial relationships that model the 3-D shapes of anatomical material. The digital data files are entered into a VAX 11/780 computer; cellular dimensions, vectors of dendritic and axonal processes, and angular displacement of the dendrities with respect to folial contours, are elicited by software manipulations. The reconstruction of the 3-D neuronal form is then displayed on a graphics terminal in register with other cells and folial contours that were also digitized. Reconstruction of cerebellar cytoarchitecture is expected to yield a more precise understanding of structural-function relationships among cerebellar cellular elements. Supported by NIH. grants NS 06965, HD 18655, and RR 01393.

17.12 A CORRELATIVE GOLGI - INTRACELLULAR IONTOPHORETIC LABELING AND COMPUTER IMAGE ANALYSIS OF THE CEREBELLUM IN THE SWAYING, SW, MUTANT MOUSE. J. Quattrochi*, P. Dikkes* and J. H. Rho* (SPON: R. L. Sidman). Depts. of Neuroscience, Children's Hospital and Neuropathology, Harvard Medical School, Boston, MA 02115.

This autosomal recessive mutation displays a severe cerebellar malformation of the anterior vermis superficially resembling human Dandy-Walker malformations. The cerebellum is split midsagittally and contains diffuse ectopic islands of cells separated by aberrant fascicles of white matter (Sidman, Development of Interneuronal Connections in Brains of Mutant Mice. In Physiological and Biochemical Aspects of Nervous Integration, Carlson, F. (Ed), 1968). We now examine the postnatal homozygous mutant cerebellum by performing Golgi impregnations (Stensaas and rapid) and intracellular microinjections of fluorescent dye in 200 um sagittal cerebellar slice preparations, followed in each case by 3-D computer image analysis. The swaying (sw/sw) cerebellum is found to differ significantly from normal (+/+) controls.

Abnormal Purkinje cells are distributed widely throughout the mutant cerebellar cortex. Loss of somatic spines is first evident at P20 in swaying, but occurs earlier at P10 in controls. Focal areas of multilayered Purkinje cells are common. In the anterior vermis, ectopic granule cells are observed in the molecular layer with no evidence of associated glomerular structures. Some Purkinje neurons display abnormal spatial orientation, rotated 90 degrees in the y axis; however, parallel fiber orientation does not appear to be disturbed. In an attempt to elucidate correctly the morphology and orientation of these cells in relation to each cortical layer within a folium, Lucifer Yellow was iontophoretically injected into specific cell bodies or dendrites and their 3-D arborization and position was computer digitized. The Purkinje primary dendrite in swaying has a unique "swan neck" configuration and extends linearly in the sagittal plane approximately 64 um compared to 30 um in controls. Purkinje cell dendritic territory measures approximately 1200 um at its fullest extent in the sagittal plane in both swaying and controls; however, the mutant displays fewer branching units with a sparse dendritic arborization. Computer image rotation of 90 degrees demonstrates that in the transverse plane the thickness of Purkinje arbors in the mutant is approximately half that of controls between P10 and P25.

Our analyses suggest that the swaying mutation causes abnormal morphogenesis and a disturbed histogenetic pattern in the cerebellar cortex not seen with other mutations. Supported by NIH grants HD18655, NS20820, NS07017.

17.13. **A STEREOLOGICAL STUDY OF NEOCORTICAL MATURATION IN THE PRECOCIAL MURID RODENT Acomys cahirinus.**

P. C. Brunjes, Department of Psychology, University of Virginia, Charlottesville, Virginia, 22901

Studies of animals with diverse patterns of early maturation may help us understand basic ontogenetic processes. We have been examining brain growth in a relative of the laboratory mouse which, unlike its altricial cousin, is born in a mature state. Acomys cahirinus (the spiny mouse) emerges after a 38 day gestation period with open and functional ears and eyes and good locomotor skills. Studies of the olfactory bulb (Dev. Brain Res., 8, 335, 1983) and hippocampal formation (Brain Behav. Evol., 24, 58, 1984) indicate that developmental timetables differ between Acomys and the lab rat and mouse. Observations are extended here to include Area 17.

Despite Acomys' late age at birth, substantial growth occurs in Area 17, with cortical depth increasing 14% between birth and day 20. The numerical densities (Nv) of neurons and glia in newborn, 10, 20, and 60 day postpartum pups were determined using the techniques of O'Kusky and Colonnier (J. Comp. Neur., 210, 278, 1982). Total neuronal density decreased 40% between birth and day 60, with 2/3 of the change occurring in the first 10 postnatal days. Marked alterations were found in layers 2-3 and 4, where neuronal density declined 35% between days 0-10. While total glial density remained relatively constant, density decreased 30-40% in layers 1, 2-3 and 4 during days 0-20. ^{3}H-thymidine treatment at birth revealed little neuronal proliferation, although many endothelial cells and some glia were labelled.

Comparisons of Acomys with the mouse on the basis of postpartum age yield interesting similarities: both show an initial rapid decline in neuronal density followed by a stable period, suggesting that the pattern is an adaptive feature of cortical histogenesis. Comparisons based on post-conception age yield interesting differences: rapid declines in neuronal density occur in Acomys when levels have nearly stabilized in the mouse, indicating that altricial and precocial species differ in the overall timing of cortical maturation.

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17.14 DEVELOPING MOTOR NEURONS OF THE TAIL SPINAL CORD OF XENOPUS. R.H. Nordlander. Dept. of Oral Biology, Case Western Reserve Univ., Cleveland, OH 44106.

The purpose of this study was to characterize and follow the morphogenesis of motor neurons innervating tail myotomes of larval Xenopus. Motor neurons were visualized by retrograde filling with horseradish peroxidase applied to identified tail myotomes of early and middle larval stages. Specimens were examined as wholemounts or in transverse sections.

Initially, the cells filling from a single myotome are few in number and exhibit a simple morphology and a uniform position. As development proceeds, the number of filled motor neurons increases and cells vary more in position and configuration. The unfolding of these variations involves the addition of new motor neurons, displacement as neighboring cells differentiate, and the progressive elaboration of dendrites into the growing lateral fasciculus.

By mid-larval stages the column of neurons filled via a single myotome extends for a distance equivalent to four myotome segments. Along this column two groups of neurons can be distinguished. At rostral positions in the column most cells show the classical characteristics of primary motor neurons; large cell bodies, extensive dendritic spreads, and stout axons. These represent more mature versions of the simple neurons filled from primitive levels of the tail. More caudal cells in the column are smaller with generally finer and less extensive processes. These are probably secondary motor neurons. Both cell types ocassionally display commissural processes.

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17.15 CHANGES IN AXONAL NUMBERS IN THE TRACT OF LISSAUER DURING DEVELOPMENT. Kyungsoon Chung and Richard E. Coggeshall. Dept. of Anat. and Physiol. and Biophys. and the Marine Biomed. Inst., Univ. of Tex. Med. Branch, Galveston, TX 77550.

More neurons are produced early in development than survive into adulthood. Simultaneous with or shortly after the formation of these neurons, there is a wave of neuronal death that results in the adult number of neurons. Despite our knowledge about the proliferation and death of neurons in the developing nervous system, there is relatively little data on changes in axonal numbers in central pathways during development. Since axons are primary neuronal communicating channels, it is important to determine how their numbers change during development.

Pregnant albino rats were obtained from Texas Inbred Mice Company. The day of birth was marked and rats were sacrificed 1 day, 2 weeks and 1 month following, and in adulthood. After perfusing with 3% glutaraldehyde, 3% paraformaldehyde, and 0.1% picric acid in pH 7.4 cacodylate buffer, the second sacral segment of spinal cord was removed and processed for electron microscopy. Thin sections were cut, montages were made and all axons, myelinated and unmyelinated, were counted.

The following table shows average axonal numbers in the tract of Lissauer of the S2 segment of rat spinal cord at various times of development.

	1 DAY	2 WEEKS	1 MONTH	ADULT
MY	30	276	616	722
UN	9529	6205	3816	2759
TOTAL	9559	6481	4432	3481

Thus there is a 20 fold increase in myelinated fibers from birth to adulthood. By contrast, there is approximately a three fold decrease in total number of fibers during this same period of time. It seems clear from these data that the factors that determine the number of ganglion cells are not the same as the factors that determine axonal numbers. It is our opinion that the factors that determine axonal numbers are as important as those that determine cell numbers.

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17.16 ANALYSIS OF DENDRITIC BUNDLES IN THE CERVICAL AND LUMBOSACRAL SPINAL CORD OF THE ADULT AND NEONATE RAT. D. Lorton and W.J. Anderson. Indiana Univ. Sch. Med., Terre Haute Ctr. for Med. Educ., Terre Haute, IN 47809.

Dendritic bundling in cervical segments, C3, C4, and C5, and in lumbar segments L6 and S1 were examined using histological staining techniques, Golgi-Cox method and electron microscopy. Epon-embedded sections revealed several differences in the organization of the bundles in the cervical and lumbar region. Dendritic bundling in the lumbar region was much more extensive and compact, consisting of about 1200-1600 dendrites per bundle. In the cervical region, bundles consisted of about 10-20 dendrites and the bundles were less discrete. Cell bodies, probably motoneurons, appeared dispersed within the lumbosacral bundles, but not within the cervical bundles. Axon terminals were more frequently observed in the cervical bundles. Size of dendrites forming the cervical bundles appeared to be much smaller, than those forming the lumbosacral bundles. The Golgi-Cox technique revealed a much greater diversity in soma shape of cells contributing to the cervical bundles. The size and arrangement of dendrites forming cervical and lumbosacral bundles were studied quantitatively using camera lucida drawings. Dendritic bundle formation in the lumbosacral region of the rat was also examined. Rat pups were sacrificed at 1, 5, 10, 15, 20, 25, 30, 60, and 90 days of age. The lumbosacral cords were removed and prepared using the Golgi-Cox method. Dendritic bundle formation occurred postnatally. At birth cell bodies and dendrites were fairly well developed; however, the dendrites had not achieved their total length and there was no evidence of bundling. At 5 days, dendritic shafts began to arrange themselves into the saggital plane and by 10 days of age dendritic bundling was apparent. Dendritic growth and bundling appeared to be complete by about 2 months of age. The pattern of ramification, arrangements, and lengths of dendrites forming this bundling complex were also examined quantitatively to gain insight into the active growth pattern of these dendrites, and to map out the specific time sequences of their differentiation. Correlation of dendritic bundle formation with motor development supports the Scheibel's hypothesis that the bundling complex functions as a central program site for controlling integrated patterns of hindlimb activity.

17.17 "BIRTHDATES" OF LOCAL CIRCUIT NEURONS IN RAT VISUAL CORTEX. Michael W. Miller and Mary Ann S. Fernandez*. Dept. of Anatomy, Tulane Univ. Sch. of Med., New Orleans, LA 70112.

Autoradiographic evidence shows that pyramidal (projection) neurons migrate into cerebral cortex according to an inside to outside pattern. The migratory schedule for local circuit neurons, however, is not known. This study examines the time when local circuit neurons cease mitotic activity and begin to migrate into the cortex (neuronal "birthdates") with a combined immunocytochemical-autoradiographic procedure.

Pregnant hooded rats were injected with [^{3}H]thymidine on gestational day 18 and the pups were perfused on postnatal day 90. Day 18 was chosen in order to determine the birthdates of neurons in mid-cortical layers where local circuit neurons are most abundant. After sacrifice, peptidergic local circuit neurons were stained immunocytochemically with a primary antibody directed against somatostatin (SRIF), cholecystokinin (CCK), or vasoactive intestinal polypeptide (VIP). Subsequently, the tissue was prepared by standard autoradiographic techniques in order to identify the neurons born on the day of the injection.

The population of autoradiographically labeled neurons can be described by a bimodal distribution composed of heavily- and lightly-labeled neurons. Presumably the heavily-labeled neurons represent those which underwent only one cell division before migrating, whereas lightly-labeled neurons probably divided two or more times. About 78% of the heavily-labeled cells were located in deep layer II/III and in layer IV, and 83% of the lightly-labeled cells were distributed throughout layers II/III and IV.

Double-labeled neurons, i.e., immunoreactive neurons with autoradiographic silver grains over their nuclei, are in all layers of cortex. For SRIF-positive neurons, 82% of the autoradiographically heavily-labeled neurons are located in layer IV and 76% of the lightly-labeled neurons are in layers II/III and IV. Thus, the distribution of double-labeled neurons is similar to the distribution of autoradiographically single-labeled neurons. A similar pattern was seen with CCK-positive and VIP-positive neurons.

Based on these results, we conclude that local circuit neurons follow an inside to outside pattern of migration. Furthermore, it appears that local circuit and projection neurons in the same cortical layer are born concurrently.
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17.18 THE MIGRATION OF PYRAMIDAL CELLS TO AREA CA3c OF THE HIPPOCAMPUS OF MICE CARRYING THE MUTATION "HIPPOCAMPAL LAMINATION DEFECT". R.S. Nowakowski. Dept. of Anatomy, Univ. Miss. Med. Ctr., Jackson, MS 39216.

In area CA3c of the hippocampus of mice homozygous for the mutation "Hippocampal lamination defect" (provisional gene symbol: Hld) late-generated pyramidal neurons occupy the deepest portion of the pyramidal cell layer, sandwiched between an intrapyramidal mossy fiber layer and the stratum oriens. In contrast, in mice with normal (i.e., wild type or +/+) lamination of the pyramidal cell layer of area CA3c, late-generated pyramidal neurons occupy the superficial-most portion of the pyramidal cell layer, just below the supra-pyramidal mossy fiber layer.

As a first step in determining the sequence of events leading to the development of this difference in disposition of late-generated pyramidal neurons, the time that migrating neurons arrive at their final position in Hld/Hld and +/+ mice was established. Late-generated pyramidal neurons were labeled with tritiated thymidine (10 µCi/g) given on either embryonic day 15 (E15) or E16; subsequently, the path of migration of both CA3 and CA1 neurons was followed by sacrificing labeled fetuses (prenatally) or offspring (postnatally) at various times afterwards and processing them for autoradiography.

At birth (postnatal day 0 or P0) late-generated pyramidal neurons have not yet reached their final position in either CA1 or CA3. By P4, however, late-generated CA1 neurons have reached the top of the pyramidal cell layer, whereas late-generated CA3 neurons are still in the intermediate zone. In +/+ mice late-generated CA3 neurons reach the top of the cortical plate by P7, but in Hld/Hld mice they remain below the previously generated ones. Moreover, during the migratory period the distance from the proliferative zone to the cortical plate of area CA3 (but not of CA1) increases greatly, presumably because of the growth of the fimbria.

These results indicate: 1) that the events which result in the failure of late-generated pyramidal neurons of area CA3c of Hld/Hld mice to bypass early-generated neurons probably occur between P4 and P7, and 2) that the distance traversed by neurons destined to reside in area CA3c is much greater than that traversed by neurons which will reside in area CA1. These observations may be of eventual significance for understanding when and how the Hld gene acts to influence the lamination of the pyramidal cell layer of area CA3c.

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17.19 PRENATAL DEVELOPMENT OF INTERSTITIAL NEURONS IN THE "WHITE" MATTER OF THE HUMAN TELENCEPHALON. I. Kostović, Sect. of Neuroanatomy, Dept. of Anatomy, Medical Faculty, 41001 Zagreb, Yugoslavia.

We have previously shown that white matter in infant and adult primate telencephalon contain interstitial neurons /i. neurons/ which are generated at the end of the first third of gestation /Kostović and Rakić, J. Neurocytol. 9:219, 1980./. In this study we investigated prenatal development of i. neurons by means of Golgi method and acetylcholinesterase /AChE/ histochemistry in the internal capsule, corona radiata, corpus callosum and convolutional white matter of human fetuses ranging between 9 and 34 weeks of gestation /w./. I. neurons are distinguished from migrating neurons by multiplicity of processes and from glia by smooth contours, decreasing thickness of processes and large perikarya. I. neurons with these characteristics were found as early as 13 w. in the following fibre systems: corpus callosum-subventricular zone, internal capsule-corona radiata, external capsule-external sagittal stratum. I. neurons were present in these fibre systems throughout prenatal development. After 15 w., during the enlargement of corona radiata and growth of callosal fibres, these deep neurons show adaptive changes: the alignment of dendrites parallel to the direction of surrounding fibres or "compression" of the dendritic tree. After 18 w., deep i. neurons show AChE reactivity. Arround 34 w., concomitantly with the gradual resolution of the subplate zone, another class of large polymorphic neurons with extensive dendrites appeared within the convolutional white matter and distal portions of the corona radiata. There is a spatial overlap between i. neurons situated in the convolutional white matter and neurons of the subplate zone, with continuous distribution of AChE reactive neurons. In conclusion, i. neurons of the major cortical fibre systems develop from two different cell classes: convolutional i. neurons are in developmental and spatial continuation with neurons of the subplate zone, while deep i. neurons of internal-external capsule and callosal radiation develop from the primordial cell population situated in the deep fibre systems and subventricular zone. Early origin, distinct location and common histochemical characteristics may facilitate future studies of the fate and developmental role of i. neurons.
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17.20 THE SUBPIAL GRANULAR LAYER OF HUMAN EMBRYONIC CORTEX : A CYTOLOGICAL ANALYSIS. J.F. Gadisseux$ G. Lyon* and A.M. Goffinet, Dev. Neurobiol. Univ. Louvain. B-1200 Brussels Belgium.

The subpial granular layer (SGL) of the human embryonic cortex is a transient cell population which appears during the third fetal month and vanishes before birth (Ranke, 1909, Beitr. Path.Anat.all. Path. 47:51-125) (Brun,1965, Acta Path. Microbiol. Scand.S179). This cell contingent is of recent phylogenetic origin, for it is well developed in human embryos but rudimentary, if at all present, in other species.

The cytological organization of the SGL has been studied in the human marginal zone (MZ) using usual histological stains, Golgi impregnation and electron microscopy. At least three cell types are found in the MZ at the stage of SGL development. Glial cells are recognized by the shape of their cytoplasmic extensions, the presence of end-feet and a high content in glycogen granules. Cajal-Retzius neurons, usually subjacent to elements of the SGL, are large (nuclear diam.8-10 µm) and have a prominent cytoplasm with immature Nissl bodies. On Golgi impregnation, they show numerous radial extensions (see Marin Padilla,1982, Anat.Embryol. 164: 161-206). By contrast, cells of the SGL have small (diam. 4-5 µm) nuclei with dense chromatin clumps and prominent nucleoli. Their cytoplasm is sparse and poor in organelles. Long horizontal extensions stem from the cell body. They are filled with microtubules showing a tendency to fasciculate but contain few organelles and no intermediate filaments. On Golgi preparations, some of the horizontal extensions have an axonal differentiation. Cells of the SGL almost never come into contact with the basal lamina. Intercellular junctions of the adhaerens type are seen between cells of the SGL as well as between other elements. Synapses are not present on SGL cells or on Cajal-Retzius neurons. Cells with features reminiscent of those in the SGL, but with a more radial differentiation, are seen in the depth of the MZ and at the periphery of the cortical plate, and might correspond to elements of the SGL engaged in radial migration towards the cortex.

These observations suggest that cells in the SGL are principally neurons which migrate tangentially over the neocortex. These neurons might contribute to the cortical population following a radial inward migration analogous to that of the external granule cells of the cerebellum.

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17.21 UNFOCUSED LASER ILLUMINATION KILLS DYE-TARGETED MOUSE NEURONS BY SELECTIVE PHOTOTHERMOLYSIS. J.D. Macklis* and R. Madison, Departments of Neuroscience and Neuropathology, Children's Hospital and Harvard Medical School.

Selective photothermolysis (SP) is a novel technique by which brief, unfocused laser pulses are selectively absorbed by, and cause selective thermal damage to, endogenously pigmented structures. Anderson and Parrish first described SP for use within the field of dermatology (Science: 220: 524-527, 1983). **Megawatts of laser energy at an appropriate wavelength can penetrate several millimeters of unpigmented tissue without absorption or damage.** This approach is quite different from any existent methods of effecting cellular damage using laser microbeams, fluorescent dye injections, or focused fluorescent beams. The localization of damage can be as precise as with microbeam techniques, but thousands to millions of targets can be damaged simultaneously without precise aiming. **This abstract reports the first demonstration of SP using an exogenous chromophore, procion blue (PB).** Dorsal root ganglia (DRG) neurons targeted in vitro with PB conjugated to wheatgerm agglutinin (WGA-PB) were selectively damaged by SP. An experimental system is described to allow the "real-time" monitoring of the health of DRG neurons over many hours to days after damage by SP. Propidium iodide (PI), a fluorescent dye that leaks through damaged membranes and binds to nucleic acids, was used in a novel way to assess progessive cellular damage. Video images of DRG neurons were recorded with a silicon-intensified camera attached to an inverted phase-contrast and fluorescence microscope. Rigorous statistical analysis of graded scores of intracellular PI fluorescence in groups of experimental and control cells demonstrated a highly significant difference in uptake of PI that was dependent on the dose of laser energy. Controls for laser, lectin, and nonspecific damage were negative. More dramatic damage occured to DRG neurons selectively filled with PB by iontophoresis and exposed to unfocused laser illumination. Experimental neurons literally melted while contiguous unlabeled cells remained intact. Selective photothermolysis will provide an experimental tool for neurobiologists in particular and for general use within the biomedical field. **Potential therapeutic applications of SP targeted via exogenous chromophores include situations in which it is desirable to damage selectively pathological cells embedded within normal tissue.** Supported by NIH grant EY05317

17.22 SUPERNUMERARY RETINA FORMATION DURING REGENERATION IN EMBRYONIC XENOPUS. C.F. Ide. Dept. of Biology, Tulane University, New Orleans, LA 70118.

When challenged to regenerate, Xenopus embryonic one-third sized eyebud fragments show two external healing patterns, a rounding-up pattern which correlates with restoration of the normal visuotectal projection, and a second pattern involving cell movements from the remaining fragment into the region of the ablation to form a supernumerary retina. Within 3 days, the supernumerary fuses with the remaining fragment; this healing pattern correlates with pattern duplication of the visuotectal projection. In the same manner, lens duplication arises via cell movements during the first 24 hours of healing. In some cases, external pattern does not correlate with the final projection type. This may be explained by anatomical data showing that 1) a minority of externally scored "rounded-up" fragments actually include a cryptic supernumerary retina, undetectable due to absence of a secondary pigmented retinal epithelium, and, 2) some externally scored supernumerary retina are actually excluded from the ciliary margin of the fused eye within 3 days post-surgery. Variables such as temperature, pH, and genotype influence the occurrence of external scoring errors.

That visuotectal pattern duplication may be instigated by supernumerary retina formation is consistent with 1) small grafts of genetically marked cells into the embryonic retina prior to fragment formation produces, in some cases, marked cells at mirror-image positions in both the supernumerary retina and the retina of the remaining fragment, and 2) vitamin A application (produces pattern duplications during limb regeneration) during early healing causes supernumerary retina formation in 2/3 sized fragments which normally never form supernumeraries. These data are consistent with the idea that cell migration and supernumerary formation underlie pattern duplication.

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17.23 RETROGRADE LABELLING OF REGENERATED SPINAL ELECTROMOTOR NEURONS: RELATION TO NATURALLY-OCCURRING CELL DEATH. H.L. Fong*, M.J. Anderson and S.G. Waxman. Dept. Neurology, Stanford Univ. Sch. of Medicine and VA Medical Center, Palo Alto, CA 94304.

In the weakly electric fish Sternarchus albifrons, the electric organs are comprised of modified axons whose cell bodies, the electromotor neurons, are located in the spinal cord. Both spinal cord and electric organ readily regenerate after amputation of the tail (Cell Tiss. Res., 208:343, 1980; 219:1, 1981). Previous studies have demonstrated an initial over-production of electromotor neurons in regenerated cord, followed by cell death of some of these cells in older regenerates, to finally achieve a relatively normal spinal morphology (Dev. Biol., 103:443, 1984). To test the hypothesis that incorrectly-located electromotor neurons are eliminated because their axons do not reach the target area (electric organ), small detergent-soaked chips of horseradish peroxidase (HRP; J. Neurosci. Meth., 9:87, 1983) were implanted into 9-month regenerated electric organ on one side of the fish, and the regenerated spinal cord was examined 2-3 days later. Cord was fixed (2% glutaraldehyde), frozen-sectioned, and reacted with diaminobenzidine-peroxidase. Many regenerated electromotor neurons label with HRP, including those with cell bodies at the edge of the cord. Retrograde labelling of these ectopic cells demonstrates that their axons do extend into the regenerated electric organ; thus, total misdirection of the axons cannot be the cause of their subsequent cell death.

The HRP-labelled electromotor neurons were all located on the same side of the spinal cord as the injection site. In some transverse sections of regenerated cord at the region of excess electrocyte number, all of the electromotor neurons on the labelled side of the cord show HRP-reaction product. We conclude that despite the abnormal, apparently-disorganized morphology of early regenerated cord, virtually all of the regenerated electromotor neurons have axons, and those axons project to the electric organ peripherally. Selective cell death in this system thus does not reflect the absence of axonal projection to the correct target area. Supported in part by the VA and NS-15320.

17.24 ORGANIZATION AND DIFFERENTIATION OF FETAL ANTERIOR HYPOTHALAMUS TRANSPLANTED TO THE ANTERIOR CHAMBER OF THE RAT EYE. M.F. Bernstein and R.Y. Moore, Department of Neurology, SUNY-Stony Brook, Stony Brook, N.Y., 11794.

The capacity of the rat hypothalamus to differentiate in an altered environment was examined after transplantation of fetal anterior hypothalamus from embryonic day 15 or 16 to the anterior chamber of the eye in young adult rats. Light and electron microscopic techniques were used to study development from 15 to 74 days post-transplant. The transplants are visible grossly in the host anterior chamber as round to oval profiles attached to the iris. Light microscopy at 15 days post-transplant shows neuronal perikarya scattered through the transplants. The cellular morphology and organization of the neurons varies considerably among transplants but no distinctive nuclear subdivisions are apparent. In material prepared for ultrastructural analysis many growth cones, synapses and bundles of unmyelinated axons are present. In transplants studied between 24 and 74 days post-transplant, differentiation of neurons into magnocellular and parvocellular groups appears. The extent of organization achieved by transplants seems to bear little relation to the age of the transplant. In a few transplants at 24, 37 and 43 days post-transplant, an aggregation of very small neurons adjacent to an ependymal surface has the appearance of the suprachiasmatic nuclei. However, the organization of neurons into nuclei in the transplants differs markedly from intact hypothalamus. Immunohistochemical studies show vasopressin-containing perikarya and axons at 43 and 55 days post-transplant, probably representing differentiation of paraventricular nucleus neurons. In addition, axons containing luteinizing hormone-releasing hormone also are present in older transplants but no perikarya are demonstrated. The ultrastructure of older transplants shows a complex neuropil with several synaptic types including synaptic terminals characterized by spherical vesicles, pleomorphic vesicles or numerous large dense core vesicles and making symmetrical or asymmetrical axodendritic synapses. The neuropil is similar to that seen in normal hypothalamus. These data indicate that fetal anterior hypothalamus is capable of differentiating in the anterior chamber of the eye. The pattern of differentiation differs from that of normal hypothalamus in that morphologically distinctive nuclei such as the supraoptic and suprachiasmatic nuclei are poorly developed. Further studies are in progress analyzing the development of fetal anterior hypothalamus in both anterior eye chamber and after transplantation to brain. Supported by USPHS Grant NS17600.

17.25 IN VITRO PREPARATIONS OF EMBRYONIC BRAIN SURVIVE AND DIFFERENTIATE AFTER TRANSPLANTATION INTO PERIPHERAL NERVES OF ADULT RATS. L.C. Doering and A.J. Aguayo. Neurosciences Unit, Montreal General Hospital, 1650 Cedar, Montreal, Quebec. H3G 1A4.

The present study examines the fate of embryonic CNS neural precursors that have been disaggregated, grown in vitro and then transplanted into peripheral nerves of adult rats.

Embryonic E12 telencephalon and E15 neopallium were mechanically disaggregated and grown in vitro for 3 to 7 days in a modified Eagles minimal essential medium supplemented with 5% horse serum. Cells were then trypsinized, centrifuged and transplanted into previously crushed or transected peripheral nerves.

For transplantation a longitudinal incision (.5 mm) was made in the epineurial sheath of the sciatic nerve and a cell pellet (approximately 1.0 µl) was aspirated into a capillary tube and transferred to the nerve. The epineurium was closed with 10-0 suture.

Animals were sacrificed from 1 to 4 months after transplantation and the host nerves fixed with aldehyde solutions and processed for light and electron microscopy.

In transplants of E12 telencephalon the proliferation of neural precursors caused a marked increase in the volume of the transplants and a visible distention of the host nerves. Many areas of the transplants appeared organized and resembled portions of mature cerebral cortex. In these transplants we observed central lamination with neurons of different types and shape, abundant neuropil, numerous myelinated fibers, synapses, meninges, ependyma lined lumina and choroid plexus-like structures.

By comparison, transplants of E15 neopallium yielded islands of differentiated neurons, astrocytes and oligodendrocytes.

These experiments indicate that the PNS environment provides a favorable in vivo milieu for the study of certain aspects of CNS cell proliferation, differentiation, morphogenesis and long term survival of in vitro prepared embryonic CNS.

17.26 TECTAL BRAIN TRANSPLANTS: MICROIONTOPHORETIC STUDIES IN RATS. G. Golden, R. Fariello and L. Marco. VA Medical Center, Coatesville and Thomas Jefferson Univ., Philadelphia, PA 19107.

Tectal brain tissue from 15-17 days embryonic rats was transplanted adjacent to the superior colliculus of 1 day old rats. Three to 9 months after transplantation, hosts were anesthetized with subcutaneous urethane (1.2 Gm/Kg). After stereotaxic fixation, transplant responses to optic and peripheral nerve single square pulse electrical stimulation and to iontophoretic ejection of glutamate, GABA, bicuculline, ACh, and atropine were tested. Seven-barrel micropipette assemblies of 5-10 µ at the tip were used allowing one pore for recording and another for current neutralization. Conventional electronic equipment was used for recording and iontophorizing. No electrically driven activity has been elicited consistently. Over 30 units have so far been studied in three different transplants. They were recorded at depths ranging from 160 to 1500 µ into the transplants. Most of them fired spontaneously at rates ranging from 0.02 to 19.54 H_z. In 12/30 (40%), clear-cut short-delay excitatory responses to 50 nA or lower pulses of glutamate were obtained. The remainder failed to be activated by glutamate at current strengths usually up to 50 nA but sometimes even pulses of 130 nA were ineffective. GABA induced both excitatory (5/30) and inhibitory (5/30) responses in different units but ambiguous (excitatory and inhibitory) responses were not obtained in the same neuron regardless of the current strength which most frequently was at 50 nA or below. Interestingly, bicuculline dampened the activation in 2 of the 5 units excited by GABA but no bicuculline effects could be demonstrated in the other 5 cells inhibited by GABA. Such findings tend to rule out the possibility that such effects were current-induced. GABA-induced excitatory effects which were countered by bicuculline were also observed in three neurons of the superior colliculus of the host animal ventral to the transplanted tissue. These effects often changed firing rates by a factor of 2 to 10. Some units could not be influenced by either glutamate or GABA. Acetylcholine was remarkably ineffectual in its ability to influence unitary behavior despite repeated attempts with current strengths up to 100 nA. No correlation between depth of recording and neurotransmitter characterization of units has become apparent. The significance of these findings will be discussed. Supported by VA funds.

17.27 TECTAL BRAIN TRANSPLANTS: MORPHOLOGICAL STUDIES IN RATS P. F. Reyes*, G. T. Golden, and R. G. Fariello Research and Neurology, VA Medical Center, Coatesville, PA 19320 and Thomas Jefferson Medical College, Philadelphia, PA 19107.

Donor tectal brain tissue from fetal rats was transplanted adjacent to the superior colliculus of host newborn rats (1 hour to 1 day old). The donor tissue was taken from rats of 14-17 days gestation, most being of gestational age E-15. Time-mated pregnant Long Evans rats were anesthetized and their embryos removed under sterile conditions and stored for up to five minutes in ice cold F10 medium (GIBCO). Tecta were dissected from the rest of brain and overlying membranes using microscissors and dissecting microscope. The neural tissue to be transplanted was sucked into a pipette and gently injected into the host animal's brain.

Transplants were examined from 3 weeks to 1 year after transplantation. Transplants containing neurons and glia were identified in 80% of animals. The transplants were enveloped by a thin, transparent leptomeninges overlying the inferior colliculus and cerebellum. Under light microscopy, gliosis, inflammatory reaction, meningeal fibrosis and acute and chronic neuronal changes were absent. Neuronal processes were readily identified in silver preparations. Small and large neurons in the transplanted neural tissue were unevenly distributed and did not follow the usual laminar arrangement seen in the host superior colliculus. In some transplants the leptomenigeal interface was absent and neurites were seen at the periphery of the transplanted tissue. An immunoperoxidase method was utilized using glial fibrillery acetic protein to demonstrate astrocytic proliferation. Mild staining was observed in certain regions of the leptomeniges and occasionally in the subpial region. This positive reaction indicated focal increase in fibrillary astocytes in transplant, which was not evident by standard histochemical preparations.

Although the significance of this difference in cytoarchitecture is undetermined at present, we believe that brain transplantation may be an important technique which can be utilized to replace or replenish previously damaged neuronal groups in the central nervous system.

17.28 FORMATION OF TYPICAL CEREBELLAR CYTOARCHITECTURE BY DISSOCIATED, PELLETED CEREBELLAR TRANSPLANTS. E.B. Ezerman AND L.F. Kromer, Dept. of Anatomy and Neurobiology, Univ. of Vermont, Burlington, Vermont 05405.

Transplants of solid pieces of embryonic rat cerebellar primordia placed into intracephalic cavities in the CNS of an adult rat host develop many cytoarchitectonic features present in the normal mature cerebellum. Thus, cell genesis, migration, and phenotypic neuronal development occur in developing cerebellar transplants in ectopic locations in the adult host CNS. Cell suspensions in culture (DeLong, R. and R.L. Sidman, Devel. Biol. 22:584, 1970) or as injection transplants (Schmidt et al. Soc. Neurosci. Abst. 9:849, 1983) have shown that several cerebellar cell types survive the dissociation procedure and undergo some segregation and reformation of typical cerebellar organization. The present study was undertaken to investigate further whether dissociated and repelleted cerebellar cells from different developmental stages maintain their ability to reorganize. For these experiments, E13-14 and E17 cerebella were dissociated into single cell suspensions and then repelleted by centrifugation. The pellets were implanted in premade cavities in adult rat brains. At one month survival, E13-14 cerebellar pellets develop deep nuclear areas internal to or partly surrounded by cortical regions showing the typical trilaminar pattern of molecular, Purkinje, internal granule cell layers. The internal granule cell layers exhibit structures similar to glomerule formations and myelinated fibers interconnect the Purkinje and deep nuclear cells. The transplant is often oriented so that cortical areas are demarcated by fissures which are adjacent to pial-lined surfaces or coverings of the cavity. In 2 week survival transplants, the external germinal cells (EGL) are seen along the pia and around blood vessels. In some one month E13-14 transplants persistent EGL cells may be seen in these locations. Even very small pieces of E13-14 pellets show the characteristic trilaminar structure. E17 pellet tranplants are much smaller than E13-14 transplants, but contain some trilaminar organization of the surviving Purkinje and granule cells. It is unclear whether deep nuclear cells survive. These results demonstrate that laminar and nuclear organization which are dependent on glial-neuronal interactions still occur when the cells have been forced into unnatural associations in randomly organized tissue pellets. Thus, this system may be used to analyze different levels of tissue reorganization. (MOD #5-372 and NIH #NS-07289)

18.1 CENTRIPEDAL PROCESSES IN BEHAVIORAL EXTINCTION AND EVOLUTION. R. R. Provine, Dept. of Psychology, University of Maryland Baltimore County, Catonsville, MD 21228.

The loss of a motor pattern during evolution, by whatever cause will be called behavioral extinction. While there is evidence for behavioral extinction, little is known about its mechanism. The present account is offered as a possible explanation.

The initial step in behavioral extinction is the selection against the energy consuming muscles producing a behavior that is no longer adaptive. Hamburger and others have already established that experimental decreases in the peripheral muscle mass during embryogenesis reduces motonueron numbers by increasing the rate of naturally occurring motoneuron cell death. The mechanism is competition by motoneurons for limited innervation sites and/or trophic substances in the limb. The selection against muscles during evolution secondarily selects against the spinal cord motoneurons that innervate them by increasing the normally occurring motoneuron cell death during embryonic development. This will be termed phylogenetic cell death. Interneurons may also be indirectly affected by a reduction in muscle mass because they probably compete for motoneuron innervation sites and/or trophic substance just as motoneurons compete for muscles. Some affected interneurons may be pattern generating circuits for movement. Interneuronal circuits that have lost their normal motoneuron terminals may either degenerate, remain intact without motor function or, most intriguing, synapse with novel motoneurons, thereby patterning motor outflow to novel muscles. Thus, apparently regressive events may become a significant force in the evolution of new patterns of movement.

The above outside-in or centripedal sequence of events (muscle → motoneuron → interneuron) may explain how the apparently conservative motor system undergoes regressive transformations during evolution. Neuronal mutation need not be involved. Selection against muscle mass may be the necessary and sufficient initial event. The present hypothesis suggests a research program that turns to developmental neuroscience to explain evolution at the level of the neuron.

18.2 CATECHOLAMINERGIC NEURONS OF LOCUS COERULEUS PROJECT TO THE GANGLION CELLS OF THE NERVUS TERMINALIS (NT) IN GOLDFISH. R. D. Fernald and T. E. Finger. Inst. of Neuroscience, Univ. Oregon, Eugene, OR 97403 and Dept. of Anatomy, Univ. Colo. Sch. Med., Denver, CO 80262.

The terminal nerve (NT), or zeroeth cranial nerve is a relatively unknown neuronal system which is preserved throughout the vertebrate lineage. The ganglion cells for this system lie along the course of the nerve which penetrates the brain in association with the olfactory nerve or tract. Little is known regarding the function of the terminal nerve.

At least some of the ganglion cells of the NT contain LHRH and, in teleost fishes, send a process into the optic tract to terminate in the retina (Munz, et al., '82). Other cells of the NT give rise to centrally directed processes which terminate in the ventral telencephalon (Demski & Northcutt, '82). Although the NT is associated with the olfactory nerve, olfactory inputs to the NT ganglion cells have yet to be demonstrated. Other afferents to the NT system are virtually unknown.

In order to determine inputs to the NT ganglion, we injected HRP into the region of the NT cell bodies, i.e. where the olfactory nerve enters the olfactory bulb. In these cases, postmortem analysis revealed that the injections did not involve the olfactory bulb proper although they included the rostral division of the NT ganglion. These injections retrogradely label only two sites within the nervous system: NT ganglion cells contralaterally, and a few cells in the locus coeruleus (LC) bilaterally. Retrogradely labeled axons could be traced from the injection site, through the olfactory tract and median forebrain bundle, to the LC neurons. Our identification of these retrogradely labeled neurons as the catecholaminergic neurons of LC is based on three criteria: similarities in position, size, and neurotransmitter. Injections of HRP into the median forebrain bundle reveal anterogradely labeled fibers which bypass the olfactory bulb and terminate as distinctive perisomatic terminals on the NT ganglion cells. The identical terminal morphology is observed following immunocytochemical staining for tyrosine hydroxylase. The presence of tyrosine hydroxylase in the perisomatic terminals supports our contention that the catecholaminergic cells of LC project to the NT ganglion.

In summary, these data indicate a catecholaminergic input to the NT ganglion from the locus coeruleus, and secondarily, an input to the NT ganglion from its contralateral counterpart. Since the NT ganglion cells appear to possess only short dendritic processes, it is possible that this limited input which we describe constitutes the bulk of the afferents to the NT ganglion cells.

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18.3 COMPARATIVE AND EVOLUTIONARY MORPHOLOGY OF CONVEXITY NEOCORTEX IN THE DOLPHIN. M.S. Jacobs, A.M. Galaburda and P.J. Morgane. N.Y. Univ. Dent. Ctr., New York, NY 10010, Dept. Neurology, Harvard Med. Sch., Boston, MA 02215 and Worcester Foundation Exp. Biology, Shrewsbury, MA 01545.

Using Golgi material in conjunction with quantitative cytoarchitectonic analyses, we have examined the convexity neocortex of the dolphin (Tursiops truncatus) in order to assess the evolutionary stage of development of this neocortex in terms of the Sanides growth ring concepts. Cortical development has been traced outward from the archicortex medially and paleocortex laterally, through the periarchicortices and peripaleocortices, and limbic cortex proper and insular cortex into the paralimbic and parinsular cortical formations on the upper convexity of the hemisphere. We have been unable to identify foci or cores of koniocortical specialization or, in frontal areas, the area gigantocellularis cells. Golgi analyses of the convexity cortex reveal an extremely generalized homotypical cortex with neuronal elements in all laminae showing considerable undifferentiated or transitional characteristics. A general lack of specialized neuronal forms is a feature of the dolphin convexity cortex present in all laminae. Thus, convexity cortex contains many isodendritic long-radiator cells in all laminae along with numerous immature or transitional type pyramids. Stellarization is extremely limited with only approximately 12% of cells being classifiable as stellate in type. Lamina IV is either not present or at least incipient with possible layer IV cells still remaining in lower parts of lamina III.Lamina II is of a markedly accentuated character with cells that are pyramidal in type and resemble dentate granule cells with markedly extraverted apical dendrites extending into layer I (subpial dendritic arborization). Many of these cells show few or no basilar dendrites. The accentuated layer II, since it marks the periallocortical and proisocortical stages of cortical evolution, implies by such development on convexity cortex in the dolphin that this cortex is intermediate in type and has not reached the final stage of cortical evolution. It is a protoneocortical mark indicating the archaic prevailing input into layer I of the axodendritic type. Layer I is markedly developed comprising almost 1/3 of the cortical thickness. In reality, the cortex gives the impression of a paleocortical type organization in layers I and II overlying a more developed parinsular/paralimbic type cortex in the lower layers. The dolphin brain thus appears to represent one type of prototypical brain not far removed from that of terrestrial mammalian ancestors existing 70 or so million years ago when whales returned to water. (Supported by NSF Grant BNS 82-42356).

18.4 COMPARATIVE AND EVOLUTIONARY MORPHOLOGY OF THE DOLPHIN CONVEXITY NEOCORTEX. P.J.Morgane, M.S.Jacobs and A. Galaburda. Worcester Found. for Expt.Biol.,Shrewsbury, MA 01545, Dept.of Pathobiology,New York Univ.Dental Ctr.,New York,NY 10010 and Dept.ofNeurology, Harvard Medical School,Boston, MA 02215.

We have applied Golgi techniques and quantitative cytoarchitectonic analyses in examining convexity cortex of the dolphin brain in order to classify the cortices in these aquatic mammals in relation to the extent of development of cortical growth rings described by Sanides (1970,1972). These studies are the result of our recently developed Golgi techniques for study of long-term formalin fixed material. In the dolphin convexity cortex only rudimentary granularization is seen,the entire cortex being dominated by a pyramidal type of organization. Absence of layer IV indicates this cortex is of the transitional or intermediate type. Complete absence of hypergranular cores indicates that convexity cortex has not evolved to the final growth ring stage of koniocortices. The marked accentuation of layer II along with pyramidization, makes this cortex similar in structural organization to paleocortex and periarchicortex. In most placentals conspicuous layer II does not extend beyond the proisocortex(mediolimbic and insulolimbic) limits. Accentuated layer II in convexity cortex in other placentals is characteristic of neocortical growth rings I & II indicating a prevalently zonal arborization. The presence of very large stellate cells, particularly in layers II & III, is another characteristic of dolphin convexity cortex which is common in other groups of primitive mammals such as the hedgehog. Pyramidal cells in all layers are poorly differentiated and imprecise(clavate, mace shaped, etc.) and are obviously transitional in type. Long radiator type cells,having rectilinear dendrites with few branches, similar to those seen in the reticular formation, are seen in several lamina of dolphin convexity cortex giving a further sense of generalized "reticular" type of organization. In sum, these cortices appear to show signs of the "original architectonics" of the brain of the earliest mammals. Development of granularity was a feature of cortical differentiation of most terrestrial forms that occurred after the whales had returned to sea some 70 million years ago. Whales could reflect in their neocortical structure many features of archetypal mammalian brains that first showed the strati-laminated pattern typical of mammalian neocortex. Finally, as to mode of cortical evolution in the dolphin, there is an increase in the territory of neocortex, but without substantial reorganization of the 6-layered stratification which appeared in common ancestors of present day mammals(supported by NSF grant BNS 82-42356).

18.5 EVOLUTION OF GIANT AXONS IN FLIES (DIPTERA, DROSOPHILA).
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Giant axons offer a convenient model for studying neuronal specialization. Giant axons are apparently adapted for rapid conduction or reliable synaptic transmission. Since evolutionary adaptation depends on genetic alteration of a complex developmental process, one might ask how precisely the diameter of giant axons can be adaptively tuned by mutation and selection, and whether many separate axons can have their diameters independently adjusted by modification of a finite genome. The evolution of specialized neuronal pathways requires a system of genetic organization that not only can control the differentiation of individual nerve cells but can also permit the integrated evolutionary transformation of these neurons by mutation.

Tools for genetic and developmental study of Drosophila make the giant fiber system of flies most attractive for investigating how the characteristics of individual neurons are determined by genetics, ontogeny and evolution. Phylogenetic comparison of diverse dipteran species may reveal constraints upon neuronal adaptation and thus provide the basis for hypotheses concerning the genetic regulation of neural organization.

Flies which possess dorsomedial giant axons homologous to those in Drosophila belong to an apparently monophyletic assemblage (Eremoneura). Among species which lack such dorsomedial giants are a few unrelated flies (a tabanid and a psychodid) with ventral giant fibers. This demonstrates the unremarkable fact that different axons can evolve analogous specializations in different species. More interesting is the possibility of constraint such observations suggest, hinting that both dorsal and ventral giant axons may not evolve concurrently.

Still more intriguing is the pattern of axon sizes found in a syrphid, a fly which by taxonomy (in the Cyclorrhapha) might be expected to have dorsal giants. The syrphid had no giant fibers but instead displayed many large axons which are perhaps associated with the superb optomotor reflexes of such animals. That the presence of many axons specialized by size accompanies the unusual absence of giant fibers suggests that the dipteran genome might not accommodate information sufficient to specify both giant axons adapted for escape behavior and additional large axons specialized for exceptional flight performance or other skills.

Evolutionary adaptation must sometimes impose a compromise among competing demands for a limited genetic resource.

18.6 PHYLOGENETIC PATTERN OF A NEURAL CELL ADHESION MOLECULE (NCAM)
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The phylogenetic distribution of cell-cell interactions mediated by NCAM was analysed in nervous tissue membranes from major representative classes of vertebrates and invertebrates by immunoblot techniques and binding assays. Two types of antibodies were used for these studies: a monoclonal antibody which reacts with the unique sialic acid-rich moiety of NCAM, and a rabbit antiserum against mouse NCAM. Immunoreactivity was examined after fractionation of detergent-solubilized membrane proteins by SDS-PAGE, transfer of proteins to nitrocellulose, incubation with anti-NCAM antibodies, and visualization using peroxidase-linked second antibody. In this procedure, all chordates through cyclostomes and one invertebrate produced a broad band of immunoreactivity with the anti-carbohydrate antibody. The diffuse appearance of the band is characteristic of variations in the sialic acid content of NCAM. Material from these animals also had detectable immunoreactivity with a rabbit antiserum against NCAM. These results suggest that antigenic determinants on NCAM or related molecules have been conserved during at least 500-600 million years.

NCAM is a homophilic ligand, and therefore if different species express functionally-compatible NCAM, their membranes should be capable of adhering to each other. To detect such interspecies binding, a monolayer of chick neural retina cells, which are known to have cell surface NCAM, was affixed to a plastic petri dish. Fluorescent membrane vesicles from nervous tissue of each animal were then incubated with this monolayer in the presence or absence of Fab fragments from a rabbit antiserum against chick NCAM. Binding of the Fab to the monolayer alone is sufficient to inhibit binding of chick brain vesicles, and therefore Fab inhibition could be used as a control for specificity in heterotypic combinations as well. Vesicles from all chordates tested through cyclostomes gave substantial levels of binding with at least 50% inhibition by the Fab. In contrast, with the possible exception of octopus, none of the invertebrates tested displayed any binding whatsoever. These results suggest that NCAM binding function has also been highly conserved during vertebrate evolution.

18.7 PRESENCE OF TORPEDO RAY SYNAPTOSOMAL ANTIGENS IN NEURONAL AND ENDOCRINE CELL LINES.
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A collection of 141 monoclonal antibodies, which were generated in response to a Torpedo synaptosomal preparation (Kushner, J.Neurochem,43,1984) were screened using a radio-immunometric assay, against neuronal and endocrine cell lines. The synaptosomal preparation was from the electric organ which contains purely cholinergic innervation. We felt it would be informative to ask which antigens of Torpedo cholinergic terminals are common to a panel of cell lines having differing similarities with cholinergic neurons. The cell lines chosen include PC-12 (rat pheochromocytoma), NG-108 (rat neuroglioma), SY5Y (human adrenergic neuroblastoma), MC-IXC (human cholinergic neuroblastoma), GH-3 (rat prolactin and growth hormone secreting cell line of anterior pituitary), HIT (Syrian hamster insulin secreting cell line of pancreatic origin). Despite the evolutionary divergence between ray and mammals, 12 of the 141 antibodies recognized an antigen in one or more of these cell lines. Given this conservation, not unexpectedly some antibodies recognized only SY5Y cells which are adrenergic while others recognized only MC-IXC cells which are solely cholinergic as is Torpedo plax tissue. Using the approach of screening cell lines for the presence of antigens, one can discern similarities among various cell lines not otherwise evident. The major advantages, of course, using cell lines to characterize an antigen, are the ability to obtain large amounts of antigen which may be rare in central nervous system, and the potential to isolate the gene.

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19.1 TRANSIENT TECTOGENICULATE PROJECTIONS IN NEONATAL KITTENS. B.E. Stein, J.K. Harting, J.G. McHaffie*, & Huerta, M.F. Dept. of Physiology/Biophysics, Medical College of Virginia, Richmond, VA 23298, and Dept. of Anatomy, University of Wisconsin, Madison, WI 53706.

The projection of the superior colliculus (SC) to the dorsal lateral geniculate nucleus (tectogeniculate pathway) was studied in developing cats. The neonatal projection pattern was characterized by dense labelling well outside the adult terminal field and it was not until 21 days postnatal that the tectogeniculate projection acheived its adult-like pattern.

Pressure injection of of ^{3}H- leucine (0.15-0.20µl of 110µCi/µl) were made into the SC of 14 kittens (2 at 12-24 hrs., 3 at 2 1/2-3 days, 3 at 6 1/2 and 1 at 21 days), and 5 adults. Following survival periods of 42-48 hrs the animals were perfused and the tissue was blocked and post-fixed. Brains were cut frozen in either the coronal or parasaggital plane at 40µm and every section was processed according to standard autoradiographic procedures.

In adults the SC projection to the dorsal lateral geniculate nucleus (LGNd) is restricted to the most ventral C laminae (C^2 and C^3). However, in the newborn kittens tectogeniculate projections were distributed over all the laminae and interlaminar zones of the ipsilateral LGNd extending to, and sometimes slightly beyond, the apparent upper border of lamina A. This terminal field included the medial interlaminar nucleus (MIN) as well. Within the laminated portion of LGNd, the densest terminal labelling was present in the C laminae and the interlaminar zones. The labelling density was somewhat less in lamina A^1 and least in lamina A.

The projection pattern observed in the earliest newborn animals had not changed substantially in 6 1/2 day old kittens, but at 12 days postnatal changes in the terminal field were apparent as a more restricted and less dense labelling zone in A and A^1. By 21 days postnatal the terminal field appeared to have reached its adult-like configuration. Apparently, this adult-like pattern of restricted tectogeniculate projections is acheived by a selective retraction of projections during a 3 week period of postnatal maturation. It is not clear at this time whether this process involves the retraction of axon collaterals or the death of SC cells themselves.

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19.2 **ANOMALOUS GENICULOCORTICAL CONNECTIONS IN THE PRENATALLY ENUCLEATED CAT.** B.L. Shook and L.M. Chalupa. Department of Psychology, University of California, Davis, CA 95616.

Binocular interactions are known to influence the development of retinofugal projections. In monkeys (Rakic, 1981) and cats (Williams and Chalupa, 1982) removal of one eye before birth results in the maintenance of widespread, bilateral projections from the remaining eye to the dorsal lateral geniculate (LGd). In turn, the remaining eye projects to striate cortex in a continuous fashion with no signs of ocular dominance columns (Rakic, 1981; Shook et al., 1983). Here we report that prenatal unilateral enucleation in the cat results in an anomalous reciprocal connection between the LGd and area 19.

WGA-HRP was deposited into area 19 or lamina A of the LGd in normal adult cats and in adult animals which had one eye removed more than two weeks before birth. After a survival time of 24 hours sections were reacted for HRP using TMB as the chromogen. After LGd deposits in normal cats, no reaction product was visible outside of areas 17 and 18. Area 19 injections resulted in anterograde and retrograde label in the C laminae and MIN of the LGd, pulvinar, lateral posterior complex, pretectum and the central lateral nucleus. Similar LGd deposits in prenatal enucleates demonstrated retrograde label in layer VI and sparse anterograde label in layer IV of area 19. Injections into area 19 of the prenatal enucleates resulted in anterograde label, as well as in a few filled cells, in lamina A of the LGd, and in all of the nuclear groups mentioned above for normal cats. Thus, a sparse anomalous reciprocal projection exists between the LGd and area 19 in prenatally enucleated cats. One explanation of these results is that interruption of in utero binocular interactions influences cell migration in the LGd so that some neurons destined for the C laminae or MIN become situated in the A layers. These displaced neurons would then project as normal C laminae cells to area 19. The morphological characteristics of LGd cells in the A laminae are under investigation.

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19.3 THE EFFECTS OF NEONATAL ENUCLEATION ON THE RETINOGENICULATE PROJECTION IN ALBINO AND PIGMENTED FERRETS. J.E.Morgan* and I.D.Thompson* (SPON:Z.Henderson).University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, U.K.

In the newborn ferret the retinal ganglion cell projection to the lateral geniculate nucleus (LGN) is diffuse, with considerable overlap of afferents from the two eyes (Card Linden, D. et al., J.Comp.Neurol.,203:189-211, 1981). The role of competition in the refinement of this projection has been tested in other species such as the rat where neonatal removal of one eye results in marked changes in the extent of the ipsilateral retinofugal projection. We have examined the effect of neonatal unilateral enucleation on the uncrossed projection in pigmented and albino ferrets. (Normal albino ferrets have the typical reduced ipsilateral projection seen in other species.)

The distribution of the retinofugal projection was assessed in neonatally enucleated adults by the anterograde transport of ^{3}H proline (0.5 mCi in 5µl distilled water, intraocular injection). The distribution of retinal ganglion cells involved in this projection was determined from the retrograde transport of HRP after large injections (2.5-7.0 µl 40% HRP in 2% DMSO in distilled water) into the LGN and optic tract ipsilateral to the remaining eye.

In both ipsilateral and contralateral LGN, terminal labelling was restricted primarily to the appropriate laminae but with considerable spread to adjacent (inappropriate) laminae. Increases were found in the number of ganglion cells in the temporal crescent participating in the uncrossed projection. For the pigmented enucleates the mean number of such cells was 6,700 (maximum=7,797 N=3,S.D.=937) compared with 6,300 (maximum=6,863 N=3,S.D.=280) for the normal pigmented ferret: for the albino enucleate the corresponding number of cells was 1,600 (maximum=1,800 N=3,S.D.=279) compared with 1,100 (maximum=1,290 N=3,S.D.=222) seen in the normal albino. The number of labelled cells outside the temporal crescent varied considerably between animals and seemed critically dependent on the spread of HRP beyond the LGN; the effects of enucleation on these cells is under investigation.

These findings therefore suggest that enucleation augments the population of ipsilaterally projecting ganglion cells in the temporal crescent by a similar, small number in albino and pigmented ferrets. Greater changes might be produced by prenatal enucleation.

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19.4 SURVIVAL OF RABBIT RETINAL GANGLION CELLS FOLLOWING NEONATAL VISUAL CORTEX ABLATION. M.Wilkes*, G. Zingaro* and E.H. Murphy. Dept. of Anatomy, Med. Coll. of PA, Philadelphai, PA 19129

In the rabbit, most of the retinal ganglion cells project to both the dorsal lateral geniculate nucleus (dLGN) and the superior colliculus (SC). In contrast, the cat has a class of retinal ganglion cells (Beta cells) which do not project to the SC. Neonatal visual cortex ablation results in rapid and severe degeneration of dLGN and, in the cat, there is subsequent retrograde transneuronal degeneration of retinal ganglion cells. However, this degeneration is seen only in the Beta cells, not in the cells which project to both dLGN and SC. This suggests that the collateral to SC plays a role in the survival of the remaining cells. If this hypothesis is correct then all cells in the rabbit retina should be protected from transneuronal degeneration following neonatal visual cortex ablation since, in the rabbit, all retinal ganglion cell types project to SC and there is no class of cells which project only to dLGN. In order to test this hypothesis, we ablated the right visual cortex of Dutch belted pups within 24 hours of birth. These animals survived a period of 3-4 months following surgery. Lesion reconstruction confirmed the extent of cortical damage and histological examination of the thalamus revealed severe degeneration of the dLGN. The left retina of each rabbit was whole mounted and examined for cell loss.

Camera lucida drawings were made of all cells falling within an 80 x 80 um grid. The entire extent of each retina was sampled in one millimeter intervals. An electronic planimeter was used to calculate cell diameters from the camera lucida drawings. This procedure was carried out for normal retinae and the retinae of the decorticate rabbits and cell size distributions were generated for each retina. A sample area from the center of the retina, extending from the visual streak to the inferior periphery was selected to determine cell density for each retina. Each retina from a lesioned animal was randomly paired with a retina from a normal animal. Comparison of the cell size distributions and the cell density measurements indicate that there is no detectable loss of ganglion cells in the rabbit retina following neonatal visual cortex ablations.

Our results demonstrate that, in the rabbit, retinal ganglion cells survive visual cortex ablation despite severe degeneration of a principal target, the dLGN. This study supports the hypothesis that the presence of collateral axon sustains the retinal ganglion cells and prevents transneuronal retrograde degeneration.

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19.5 VISUAL CALLOSAL PROJECTIONS IN THE STRIATE CORTEX OF THE RABBIT FOLLOWING NEONATAL MONOCULAR ENUCLEATION. A.M. Grigonis, E.H. Murphy and L.H. Ostrach.* Dept. Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129 and Dept. Psychology, University of California, Davis, CA 95616.

The normal development of callosal connections between visual areas of the cortex can be altered by manipulation of afferent input. The present study investigated the effect of monocular enucleation (ME) in the neonate on the distribution of callosal cells in rabbit striate cortex. The extent of the callosal cell zone was compared in normal and neonatally enucleated rabbits following injections of HRP in the contralateral cortex. In the normal rabbit, the cell bodies of callosal efferent neurons which originate in the visual cortex are confined to the lateral extent of the striate cortex, in the region adjacent to the occipital cortex. The majority of cells of callosal origin are found in laminae II-III, with relatively few occurring in laminae IV and V. Neonatal ME resulted in an abnormal radial and tangential distribution of callosal projections in the cell zone in the cortex ipsilateral to the remaining eye. The callosal cell zone is increased in size following ME, to 40% larger than the zone found in normal animals, and has expanded into the medial extent of the striate cortex, in addition to the normal distribution found in the lateral area. The altered radial pattern is seen as an 11% increase in the proportion of callosal cells in lamina IV relative to that found in a normal distribution. It has been previously reported that, in the normal neonatal rabbit, the callosal projection extends into the medial striate cortex. Our data indicate the role of afferent input in the normal postnatal retraction of this early widespread projection pattern.

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19.6 DEVELOPMENTAL CHANGES IN THE DISTRIBUTION OF TRANSCALLOSALLY PROJECTING NEURONS IN RELATION TO THE BORDER ZONE OF AREA STRIATA/OCCIPITALIS. H. Distel* and M.J. Pinilla-Morillo* (SPON: H. Holländer). Inst. Med. Psychol., Univ. München, D-8000 München, W-Germany and Dept. Anat., Fac. Med., Zaragoza, Spain.

It is well known from HRP-injections into visual cortex that the border zone of area 17/18 (striata/occipitalis) is marked by a high concentration of transcallosally projecting cells. As a part of a study on the development of intracortical connections it was attempted to define the border zone using this method in flatmount preparations of the occipital cortex of rabbits.

Multiple injections of HRP were placed in the region of the striate cortex of newborn (days 0,1,4,6,8,10,12,16,24) and of adult rabbits. The following day animals were perfused and tangential sections of the flattened cortex prepared according to Mesulam's TMB-protocol (J.Histochem.Cytochem.126:106,1978). Every second section was counterstained with thionin.

At all ages, an elongated concentration of labelled cells was found at a site corresponding to the border zone of area striata/occipitalis seen in the adult animal. However, labelled cells, not present at birth, were found by day 4 in all but the most caudo-dorsal region of the striate cortex and reached their greatest distribution by day 8 in the supragranular layers. At this time concentrations of infragranular cells first appeared at the border zone. By day 16 the pattern was similar to that of the adult animal with supragranular cells now beeing restricted to the border zone. Interestingly, a scattering of infragranular cells throughout the rostral striate cortex survives into adulthood, although the functional significance of a connection between the lower-visual-field representations remains unclear.

When the orientation of the elongated cell concentration was compared to the orientation of the thalamic and callosal fiber systems in the underlying white matter, the relationship was found to remain unaltered with age despite the considerable growth of the cerebral hemispheres. This would suggest that the relation between the orientation of the fiber systems and the striate/occipital border is determined prenatally, long before the transcallosal connections are formed.

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19.7 COMPARISON OF COMMISSURAL AND VISUAL CORTICAL MYELINATION DEVELOPMENT IN THE CAT. G. A. Looney and A. J. Elberger, Department of Neurobiology and Anatomy, University of Texas Medical School at Houston, Houston, TX. 77025.

The time of appearance and early development of myelinated fibers of the superior colliculus commissure (cSC) and white matter subjacent to Areas 17, 18 and Lateral Suprasylvian (LS) cortex was compared with the onset of myelination and its early development in other fiber bundles of the CNS. Kittens of postnatal days (PND) 15 and 18 were perfused; segments of white matter (2 mm inferior to the pial surface) in these cortical areas and the cSC were prepared for electron microscopic analysis. The tissue was thin sectioned, with the fibers in cross section, and the tissue was photographed on an E.M.

All the areas examined contain myelinated fibers on PND 15. These fibers are sparsely distributed with a density of less then 2,000 fibers/mm^2. In the following three-day period, there is a small increase in the density of the myelinated fibers in the cortical areas that were sampled. However, the cSC shows a substantial increase in the myelinated fiber density from 800 myelinated fibers/mm^2 on PND 15 to greater than 50,000 myelinated fibers/mm^2 on PND 18. During this period of rapid myelination, there is no significant change in the cross sectional areas of these myelinated fibers, nor is there a significant change in the number of myelin lamellae around the fibers.

These data indicate that Areas 17, 18 and LS cortex and the commissure of the superior colliculus have begun myelinating by PND 15 in the cat. Interestingly, the onset of myelination is at least 3 days earlier than the onset of corpus callosum myelination (Looney and Elberger, Soc. Neurosci. Abst. 9: #353.6, 1983). These data are consistent with other studies that have indicated that neurogenesis occurs in the superior colliculus and visual cortex earlier than in the callosum (Altman and Bayer, Exp. Brain Res. 42:423, 1981; Jensen and Altman, J. Comp. Neurol. 209:113, 1982). The significant increase in density of the myelinated fibers of the cSC between PND 15 and PND 18 also parallels the rapid increase in myelinated fiber density during the first three days of callosal myelination. The evidence suggests that there is a difference in the rate at which myelination progresses for commissural versus cortical axons, with the commissures myelinating more rapidly during early development.

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19.8 TOPOGRAPHY AND DEVELOPMENTAL PLASTICITY OF VISUAL CLAUSTROCORTICAL PROJECTIONS VIA THE CORPUS CALLOSUM IN CATS. A. J. Elberger, Dept. Neurobiology and Anatomy, Univ. Texas Medical School at Houston, Houston, TX 77025.

The dorsocaudal region of the claustrum (CLdc) projects to ipsilateral Areas 17, 18, 19, 20a, b, 21a and lateral suprasylvian regions of the visual cortex; more restricted contralateral projections to these cortical regions have been reported. The regions of the corpus callosum (CC) and claustrum containing these contralateral projections and their possible developmental plasticity were examined.

Five groups of cats were studied using horseradish peroxidase (HRP). 1)HRP was injected at the Area 17/18 border and the contralateral claustrum was examined. 2)HRP was applied to the sectioned posterior CC in normals, and the claustrum on the nonretracted side was examined. 3)HRP was applied as in #2, but in cats with neonatal non-visual anterior CC sections. 4)HRP was applied as in #2 to the posterior 16% or 9% of the CC in cats that had the other portion of the CC sectioned neonatally. 5)HRP was applied as in #2 in cats reared with optically induced strabismus; such cats have bilaterally expanded zones of CC soma in Area 17 (Elberger et al, Neurosci. Lett. 35:19-24, 1983).

Group 1 had a relatively restricted zone of origin but with most of the cells labeled, in the dorsalmost part of the CLdc projecting to contralateral Area 17/18. Group 2 had a larger dorsal-ventral zone of labeled soma; thus CLdc cells projecting to non-17/18 visual cortex arise from a more ventral region within the dorsal expansion of the claustrum. Group 3's results are as in Group 2; visual claustrocortical connections traverse the posterior CC. In Group 4 few claustral soma were labeled from the posterior 9% of the CC; however labeling the posterior 16% of the CC resulted in a distribution and number of cells equal to Groups 2 and 3. Thus, most visual claustrocortical cells traverse the CC region between the posterior 9 and 16% and neonatal section of the adjacent anterior CC has no effect on claustrocortical cells traversing the posterior CC. Group 5 was similar to Groups 2-4, with additional labeled cells found in the more ventral claustrum. The increased total number of labeled soma suggests that additional contralaterally projecting cells, rather than just an expanded zone of origin, resulted. Thus contralateral claustrocortical projections are altered by developmental conditions that affect corpus callosum interconnections between other visual cortical regions.

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20.1 RECEPTIVE FIELD PROPERTIES OF HUMAN OPTIC RADIATION FIBERS. Charles L. Wilson, Masako Isokawa-Akesson, and Thomas L. Babb. Brain Research Inst. and Dept. of Neurology, UCLA School of Med., Los Angeles, CA 90024.

Temporal lobe depth electrode recordings are sometimes required to localize sites of origin of seizure activity for surgical treatment of intractable epilepsy. In order to reach epileptic areas, the electrodes must pass through the white matter of the temporal lobe, which contains the portion of the optic radiations known as Meyer's loop. These visuotopically organized fibers are axons of those lateral geniculate nucleus neurons receiving input from the lower retina, and thus each temporal loop represents the contralateral upper visual quadrant.

The fiber responses described here were obtained during monitoring of microwires 15 mm lateral to the posterior hippocampal gyrus and 25 mm medial to the surface of the middle temporal gyrus. We observed monophasic, positive action potentials with durations of 0.3 to 0.5 msec from 2 to 3 fibers with amplitudes 2 to 5 times noise level. Recordings from both left and right optic radiations (LOR,ROR) were obtained in response to flashed or reversed checkerboard patterns under mesopic lighting.

PSTH onset latency for the ROR was 34 msec for flash and 39 msec for pattern-shift. The response was monocular (left eye) and occurred only during stimulation of the left lower quadrant, in contradiction to an expected receptive field (RF) site in the upper left quadrant. The presence of such a lower quadrant RF may explain the occasional hemianopsia which can occur following anterior temporal lobectomy.

The RF of the fibers recorded in LOR was located in the upper right quadrant, at an eccentricity of 7^{0} from point of fixation and 2^{0} right of the vertical meridian. Despite an onset latency of 60 msec and binocular response, the RF had other properties characteristic of lateral geniculate body neurons. Its shape was circular, with a central excitatory region 1.5^{0} in diameter and an inhibitory surround extending to a radius of 6^{0}. Responses to small spots were sustained, with continuing brisk firing to light increments 15 sec in duration. The large size of the RF may be related to foveal eccentricity or blurring caused by response of 2 or 3 fibers with adjacent RFs. Except for size, the response of this RF is comparable to those which have been described for X-cells in other mammalian visual systems.

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20.2 A GOLGI IMPREGNATION STUDY OF NEURONS IN THE RABBIT DORSAL LATERAL GENICULATE NUCLEUS. L.C. Towns and V.L. Lindley* Department of Anatomy, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501.

The morphology of neurons in the rabbit dorsal lateral geniculate nucleus (DLGN) was studied by the rapid-Golgi impregnation method. Impregnated thalami from six rabbits were sectioned either coronally or horizontally on the Vibratome and individual sections were infiltrated with plastic, mounted on slides, coverslipped with plastic and the plastic polymerized. Drawings of fully impregnated neurons were made with the aid of a microscope drawing tube and the location of each drawn neuron within the DLGN was noted on a low power representation of the section.

Three classes of neurons have been preliminarily identified. The first and most abundantly impregnated class of neuron has a relatively large soma from which extends a radially disposed dendritic tree of about 250-350 μm diameter. The second class of DLGN cell has a medium-sized cell body with a total of two to five stout proximal dendrites which further branch to form an elongated dendritic tree. This dendritic tree is arrayed evenly from either pole of the soma and its long axis is typically oriented perpendicularly to the many fascicles of fibers which arc through the DLGN. The third class of neurons, less frequently seen, has a small spindle-shaped soma with two or three principal dendrites which extend for some distance away from the soma and which have relatively few branches. A large group of heterogeneous neurons remained which could not readily be placed in one of these three classifications.

The dendrites of most cells are quite rough and show several short spines, particularly in their distal branches. Few cluster, or "grape-like", appendages on the dendrites were identified. No apparent pattern of distribution for any of the three classes of neurons within the DLGN could be discerned in this preliminary investigation as examples of each of the three classes were found in all sectors of the nucleus.

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20.3 SOME CORTICAL AND SUBCORTICAL CONNECTIONS OF THE GROUND SQUIRREL DORSAL LATERAL GENICULATE NUCLEUS. N. Lugo-García. Dept. of Anatomy and Lab. of Neurobiology, Univ. of Puerto Rico School of Medicine, San Juan, PR 00901.

The dorsal lateral geniculate nucleus (LGd) is believed to serve mainly to relay visual information from the retina to the striate cortex. However, recent studies have elucidated connections between LGd and such other visual nuclei as the superior colliculus and pretectum. In order to examine connections of LGd in the 13-lined ground squirrel (Spermophilus tridecemlineatus) this nucleus was iontophoretically injected with horseradish peroxidase (HRP, Sigma type VI, 10% in 0.01 M NaCl). After survival periods of 3 days brain sections were processed with benzidine dihydrochloride according to the method of de Olmos and Heimer (Neurosci. Lett. 6: 107-114, 1977).

Most of the labeled structures were found on the side of the brain ipsilateral to the injection. A few lightly labeled neurons, along with many labeled terminals, were found in the anterior and posterior pretectal nuclei; well-labeled cells and terminals were observed in the nucleus of the optic tract. Both labeled cell bodies and terminals were seen in the thalamic reticular nucleus. HRP-filled cells were seen in both rostral and caudal sectors of nucleus lateralis posterior. In the superior colliculus labeled cell bodies were found throughout the stratum griseum superficiale and in the upper portion of stratum opticum. Labeled pyramidal neurons were mainly located in layer VI of striate cortex, although in some sections isolated HRP-filled cells were seen in layer V. Labeled neurons were found bilaterally in the mesencephalic reticular formation. There the number of cells labeled and the intensity of the label were greater ipsilaterally.

These observations indicate connections between the dorsal lateral geniculate nucleus and several other visual and non-visual structures. The connections effected by these other structures may modify information transmitted along the retino-geniculo-cortical pathway, thereby suggesting a more complex role for LGd in the processing of visual information.

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20.4 LIGHT (LM) AND ELECTRON MICROSCOPIC (EM) EXAMINATION OF THE CONNECTIONS OF THE THALAMIC RETICULAR NUCLEUS (TRN) WITH THE DORSAL LATERAL GENICULATE NUCLEUS (LGNd) AND THE VENTRO-BASAL COMPLEX (VB) IN THE RAT STUDIED USING DEGENERATION AND INTRACELLULAR LABELLING TECHNIQUES. Peter T. Ohara, Department of Anatomy, University of California, School of Medicine, San Francisco, CA 94143.

Studies have shown that different sectors of the TRN establish reciprocal connections with particular dorsal thalamic nuclei. This study addresses the question of how precisely organized those connections are and the mode of termination of the dorsal thalamic nuclei projections to the TRN. Wistar strain albino rats were subjected to either of two procedures. (1) Physiologically characterized cells in the TRN, LGNd or VB nucleus were labelled by intracellular injection of HRP, and the axons of filled cells examined at the LM and EM levels. (2) Electrolytic lesions were made in the LGNd under stereotaxic control and the TRN examined at the EM level for terminal degeneration at varying survival times.

Axons from HRP-filled VB or LGNd neurons could be traced from the nucleus of origin, through the TRN and eventually into the internal capsule. No recurrent collaterals were observed. Within the TRN all terminations of LGNd or VB axons arose as collaterals from the traversing fibers of passage and terminal-like aborizations of the collaterals were confined to a region near the main axon.

Intracellularly-filled TRN cells with projections to the LGNd or VB were located in the areas which received axons from those respective nuclei. Axons of TRN cells exhibited few collaterals within the nucleus and no further branching occurred until upon reaching their termination within VB or LGNd the axons bifurcated into a number of branches each of which gave rise to a small terminal arborizations close to the site of bifurcation.

Following ablation of the LGNd, a sparse population of degenerating terminals were found in the dorso-caudal portion of the TRN. These terminals were 0.5 - 1 um in diameter, contained spherical synaptic vesicles and established Gray type I contacts principally on dendrites and dendritic spines.

The lack of extensive axonal arborization associated with the projections between the TRN and the LGNd and VB support the suggestion that the connections between the TRN and dorsal thalamic nuclei have a relatively precise organization.

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20.5 ULTRASTRUCTURE AND SYNAPTIC RELATIONS OF GLUTAMIC ACID DECARBOXYLASE (GAD)-IMMUNOREACTIVE NEURAL ELEMENTS IN THE DORSAL LATERAL GENICULATE NUCLEUS OF THE CAT. V.M. Montero and W. Singer*. Dept. Neurophysiology, Waisman Center, Univ. of Wisconsin, Madison, WI 53705, and Max-Planck-Institute für Hirnforschung, Frankfurt, West Germany.

The cat LGN was examined by light and electron microscopy after immunocytochemistry for GAD (the synthesizing enzyme of the inhibitory neurotransmitter GABA), to identify GABAergic cells and processes. GAD+ perikarya were found distributed in the A and C laminae, constituting a moderate proportion of cells in LGN. They were characterized by small size (8-12 µm diameter), scant cytoplasm, a relatively large nucleus with common indentations, small mitochondria, few organelles and few strands of rough endoplasmic reticulum. The origin of a cilium was seen in a GAD+ perikaryon. Unlabeled cells (in the reactive zone) were of large, medium and small size. GAD+ terminals were identified as F1 and F2 types (Guillery's nomenclature) on the basis of their synaptic relations and ultrastructure (pleomorphic synaptic vesicles). Labeled F2 terminals, which have been identified as processes from dendrites (Famiglietti and Peters '72), were seen postsynaptic to RLP boutons and presynaptic to unlabeled dendrites in synaptic glomeruli. Labeled F1 terminals made synapses on unlabeled somata and dendrites, and on labeled dendrites and F2s. Terminals from the visual (perigeniculate) segment of the thalamic reticular nucleus in rat LGN have been identified as F1 type (Montero and Scott '80, '81). RLP (retinal) and RSD (cortical) boutons remained unlabeled in the reactive zone. These terminals made synapses with labeled and unlabeled dendrites and with labeled F2 boutons. The ultrastructural features of GAD+ somata correspond to those of interneurons in cat LGN defined in a separate study by lack of retrograde transport of HRP from the visual cortex (to be reported elsewhere). These results provide immunocytochemical and morphological evidence suggesting that postsynaptic inhibition of different types that have been observed on relay cells in cat LGN (Singer '77) are mediated by GABAergic intrinsic and extrinsic (perigeniculate, Montero and Singer '84) interneurons. The ultrastructural features and synaptic relations of GABAergic cells and processes in cat LGN are similar to those of equivalent neural elements in LGN of rat and monkey (Ohara et al. '83; Hendrickson et al. '83), suggesting a general morphological cell type and circuitry for GABAergic neurons in LGN of different mammals.

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20.6 THE CHOLINERGIC INNERVATION IN THE DORSAL LATERAL GENICULATE AND PERIGENICULATE NUCLEUS OF THE CAT. AN EM-IMMUNOCYTOCHEMICAL STUDY. A.D.de LIMA*, V.M.MONTERO, W.SINGER*. MPI für Hirnforschung, Deutschordenstr.,46, 6000 Frankfurt 71, West Germany.

Neuropharmacological evidence suggests that the modulatory control of transmission in the dorsal lateral geniculate nucleus (dLGN) is mediated by a cholinergic mechanism. This prompted our search for morphological evidence of a cholinergic innervation in this nucleus and in the perigeniculate nucleus (PGN), the latter participating in inhibitory mechanisms to relay cells in the LGN. The morphology and distribution of cholinergic profiles were investigated in the cat dLGN and in the PGN using a monoclonal antibody against Choline acetyltransferase (Eckenstein-Boehringer), the rate limiting enzyme for the synthesis of acetylcholine, and with both PAP and Avidin-Biotin immunocytochemical procedures. Reacted sections were flat-embedded in Ducurpan to permit light microscopic and subsequent electron microscopic evaluation. In the region of the dLGN the immunoprecipitate was associated with fine fibers distributed over the main laminae A, A1 and C and over the PGN. We found no evidence of labeled cell bodies in these regions. The labelled profiles in the A-laminae and in perigeniculate nucleus are small axons, most of them unmyelinated, distributed throughout the neuropil. They formed in the dLGN synaptic complexes with dendrites both inside and outside the glomeruli. Similar labeled synaptic terminals are found on the dendrites of the neurons situated in the perigeniculate nucleus. Since the cells in the perigeniculate nucleus are GABAergic (Montero and Singer, Exp.Brain Res., in press), our data thus provide direct evidence for a cholinergic control of both GABAergic interneurons and of LGN relay cells.

20.7 DIFFERENTIAL DISRUPTION OF CHOLINESTERASE ACTIVITY WITHIN THE DORSAL LATERAL GENICULATE NUCLEUS OF TREE SHREW (TUPAIA GLIS) WITH KAINIC ACID. K. M. Horn and R. G. Carey. Div. of Neurobiology, Barrow Neur. Inst., Phoenix, Az 85013.

Cholinergic activity within the dorsal lateral geniculate (LGN) of the tree shrew exhibits a laminar pattern of activity comparable to that seen in other species. High acetylcholinesterase (AChE) and moderate butylcholinesterase (BuChE) activity is localized within layers 4 and 5, with the entire nucleus demonstrating higher than background activity levels. Fitzpatrick and Diamond (J. Comp. Neur., 194, '80) demonstrated that the laminar AChE activity in the Galago LGN was dependent upon descending projections from the striate cortex. In the present study we utilized kainic acid (KA) injections in the LGN, eye enucleations, or lesions of the striate cortex with survival periods of 1-9 days to determine the origin of the AChE and BuChE activity within the tree shrew LGN. AChE and BuChE activity were demonstrated on alternate sections using modifications of Karnovsky and Roots protocol (J. Hist. Cyto., 12, '64) in the presence of the appropriate inhibitor (iso-OMPA or BW284C51).

The KA injections in the LGN resulted in massive gliosis and neuronal loss with little involvement of surrounding structures. Survival periods as short as 2 days following KA injections resulted in a mild disruption of AChE activity in the LGN. With longer survival periods, the AChE activity was reduced by approximately 50%; however, the dense bands of activity found in layers 4 and 5 remained intact. Regardless of the survival period, KA lesions produced a near total obliteration of BuChE activity, and gave the nucleus a blanched appearance over the effected region. Again these effects appeared time dependent with extended survival periods leading to a more severe reduction of BuChE activity within the LGN. No changes in the AChE or BuChE activity were found following either eye enucleations or lesions of the striate cortex.

These results indicate that in the tree shrew LGN, the AChE activity arises from both endogenous and exogenous sources, while the majority of the BuChE activity arises from within the geniculate body. The fact that the AChE activity continued to deteriorate with the longer survival periods following KA injections may indicate a disruption of AChE positive terminals within the LGN due to a long-term effect of KA within the LGN.

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20.8 **NEONATAL UNILATERAL ENUCLEATION IN THE CAT RESULTS IN ALTERED TECTO-GENICULATE PROJECTIONS** L. H. Ostrach and L. M. Chalupa. Dept. of Psychology and the Physiology Graduate Group, University of California, Davis CA 95616.

Removal of one eye in the neonatal cat induces a limited number of retino-geniculate axons to invade the denervated laminae of the dorsal lateral geniculate nucleus (dLGN) as demonstrated by Guillery (1972). We have found that this manipulation also causes a surprisingly robust innervation of the denervated dLGN layers by the superior colliculus. The tecto-geniculate pathway was examined using the anterograde transport of WGA-HRP in normal cats and in adult animals which were unilaterally enucleated on the second or third day after birth. Single or multiple electrophoretic deposits of this tracer were made into the superficial layers of the colliculus, a 20 to 24 hr. survival period was employed, and the tissue was processed using the highly sensitive TMB method (Mesulam, 1978). In agreement with previous studies, in normal animals the heaviest label in the dLGN was seen in the C3 layer, with a substantial amount of label in the other C laminae. Additionally, very sparse label was also observed in the A and A1 layers, as well as in the interlaminar zones. In the neonatally enucleated cats anterograde label in the dLGN ipsilateral to the removed eye was very dense in all C layers and substantial label was also found in the overlying denervated A1 layer. Contralateral to the removed eye injections of WGA-HRP in posterior regions of the colliculus (representing the visual field periphery) heavily labeled the C laminae and also resulted in substantial label in the monocular segment of the denervated A layer. The reorganization in the tecto-geniculate pathway following neonatal eye removal may represent new growth into regions of the dLGN vacated by retinal fibers or the maintenance of exuberant tectal projections which may be present during early development. We are currently studying the tecto-geniculate pathway of neonatal cats in order to distinguish between these alternatives.

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20.9 VISUOTOPIC ORGANIZATION IN THE ADULT TAMMAR WALLABY LATERAL GENICULATE NUCLEUS. J. Wye-Dvorak*, W.R. Levick[1] and R.F. Mark (SPON: R.F. Mark). Dept. of Behavioural Biology, R.S.B.S., Australian National University and [1]Dept. of Physiology, J.S.C.M.R., Australian National University, Canberra, 2601, Australia.

A spatial map of the visual world is represented in an orderly pattern in the dorsal lateral geniculate nucleus (LGNd) in the adult wallaby. Information from both eyes is projected via the retina onto 9 cytoarchitecturally separate laminae. There are 2 distinct segments, alpha and beta. Tritiated proline was injected into one eye and label was transported to four contralateral laminae and 5 ipsilateral laminae. This study focusses on the topographic representation of the visual field by relating receptive field center locations and types of receptive fields with laminar location in the LGNd. Single units were recorded from cells in the LGNd of wallabies anaesthetized with Surital and maintained for several days with amino acids and light anaesthesia. One hundred fifty single units were characterized. The majority (97%) were found to be concentrically organized. Fifty-two percent of these were excited by a black spot moved into the field center (off-center) while 47% were excited by a white spot moved into the field center (on-center). In each of the cellular laminae of the α segment of the LGNd, on-center and off-center cells were intermingled. Evidence for an orderly visuotopic projection was obtained. The superior temporal (monocular) field is located in the dorsal section of the rostral geniculate while the inferior temporal field is located on the same track further ventrally. The wallaby has 50-60^{o} of binocular overlap. This area of visual space is represented in the mid geniculate region.

20.10 COMPARISON OF RESPONSE PROPERTIES OF GANGLION AND GENICULATE X- AND Y-CELLS. A.K. Sestokas, S. Lehmkuhle* and K.E. Kratz. Dept. of Psychology, Brown Univ., Providence, RI 02912 and Dept. of Anatomy, Louisiana State Univ. Medical School, New Orleans, LA 70112.

We recorded the spike discharges of retinal ganglion cells (RG) in the optic tract and dorsal lateral geniculate cells (LGN) in the A laminae of anaesthetized, paralyzed cats. Cells with receptive fields within 20° of area centralis were classified as X or Y by conduction latency to optic chiasm stimulation and linearity of spatial summation. They were then tested with sinewave gratings presented 24 times for 100 msec, at a rate of 1 presentation/sec. Instantaneous discharge frequency (IF) profiles were analyzed on a trial-by-trial basis.

Mean baseline discharge rates for RG X- and Y-cells were generally higher than their LGN counterparts. The standard deviation (SD) of the baseline IF about its mean value was comparable for RG X-, RG Y- and LGN X-cells. LGN Y-cells had significantly higher baseline variabilities. RG X- and Y-cells responded more reliably than LGN X- and Y-cells to briefly presented stimuli. Specifically, the evoked responses of ganglion cells exceeded a criterion amplitude window set 8 SD's above baseline mean on many more trials than did responses of LGN cells. For X-cells, this difference between RG and LGN was more pronounced at low spatial frequencies. RG Y-cells, in contrast to LGN Y-cells, exceeded the criterion on nearly all the trials for most stimuli.

Response rise times from a 2 SD criterion to the peak discharge were generally longer for RG than LGN cells, as were response fall times. In addition, response durations (response onset to offset at the 2 SD window) were longer for RG cells. RG Y-cells had longer durations than RG X-cells. LGN X- and Y-cells had comparable durations. Response onset latencies of RG cells were shorter than those of LGN cells, but latencies of peak responses were similar. Y-cells generally had shorter latencies than X-cells for lower spatial frequencies.

These observations clearly indicate that there are significant transformations of visual information arriving at the LGN, which may be intrinsic to the retino-geniculate connections and/or due to extraretinal influences.
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20.11 QUANTITATIVE ANALYSIS OF RETINAL X-CELL TERMINATIONS IN THE CAT LATERAL GENICULATE NUCLEUS. Mary F. Kritzer and Mriganka Sur. Sect. of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

We have analyzed the retinogeniculate terminations of single, physiologically characterized X-cell axons intracellularly injected with horseradish peroxidase. Contralateral axons, terminating in lamina A, and ipsilateral axons, terminating in lamina A1, yielded totals of 378-1018 boutons. Every axon traced through the optic tract bifurcated, sending a medialward branch toward the brachium of the superior colliculus. Five axons traced into the medial interlaminar nucleus terminated there with 7-71 boutons. Terminal distributions within the A laminae of 13 axons were assessed in further detail. Laminae A and A1 were each divided into dorsal and ventral halves, or into sublaminae I,II and III, comprising dorsal, middle and ventral thirds. Statistical inter- and intragroup comparisons employed the Mann-Whitney U-test and a modified ANOVA. Contralateral and ipsilateral axons had greater numbers of boutons in the dorsal half of lamina A or A1. Specifically, the middle third of lamina A and the dorsal 2/3 of lamina A1 had significant bouton concentrations. Interestingly, ipsilateral axons had greater total bouton numbers. The terminal distribution in lamina A1 differed most markedly from that in lamina A in sublamina I, which was consistently richer in boutons for lamina A1. Comparison of ON and OFF center cells revealed no significant differences in the proportion of boutons within sublaminae I,II and III. Across sublaminae, contralateral ON axons showed greatest number of boutons in the middle third of lamina A. OFF axons had more uniform bouton distribution over the ventral 2/3 of lamina A. Sublaminar variation of ON and OFF axon terminations in lamina A1 was less clearly defined. Correlating terminal distributions with conduction velocity indicated that terminations of slower axons tended to be concentrated in the middle of lamina A or A1 while terminations of faster axons were more evenly distributed. Finally, axons terminating within the central 10 degrees of visual field had significantly lower bouton densities than those located more peripherally.

We interpret these data as providing evidence for subtle yet clear morphological variation in LGN terminations associated with specific physiological features of a class of retinal ganglion cell. Further, inter- and intralaminar morphological distinctions in terminations may provide functional distinction among cells in the A laminae as well.

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20.12 MORPHOMETRIC AND ELECTRICAL PROPERTIES OF NEURONS IN THE LATERAL GENICULATE NUCLEUS OF THE CAT. S.A. Bloomfield* and S.M. Sherman, Dept. of Neurobiology & Behavior, SUNY at Stony Brook, NY 11794.

Previous reports from our laboratory have described strong structure/function correlates of X- and Y-cells in the cat's LGN. We now wish to extend this analysis to include biophysical properties. The dendritic arbors of geniculate neurons were modelled as "equivalent cylinders" according to Rall's formulation. This mandates that dendritic branching patterns obey the "3/2 power rule": $D^{3/2}=\Sigma d^{3/2}$, where D=diameter of mother dendrite and d=diameter of each daughter dendrite. Measurements made, using the light microscope, at 75 branch points of physiologically-identified, HRP-stained X- and Y-cells revealed a strong 3/2 power relationship. Since diameters of distal branches approached the limits of resolution of the light microscope, 21 additional measurements were made with the electron microscope, which further support our conclusion that LGN neurons can be modelled as equivalent cylinders.

When the dendritic tree is equivalent to cylinders of the same electrotonic length, then $R_N=R_m/A_N(L/\tanh L)$, where R_N=input resistance, R_m=specific membrane resistance, A_N=total soma-dendritic membrane surface area, and L=electrotonic length. Although determination of A_N is usually a tedious task, we found a strong linear relationship ($r=+0.99$) between the surface area of a single dendritic branch and the diameter of the first-order dendrite. Hence, A_N for any neuron can be determined by measuring the diameters of the first-order dendrites and sequentially applying the linear algorithm that we derived. Interestingly, this algorithm is applicable to both X- and Y-cells. Using this method, we find that geniculate neurons display A_N values of 10,000-25,000 μm^2. R_N values of geniculate neurons were measured using constant current pulses. Because Y-cells clearly have larger A_N values than do X-cells, we expected R_N values to be lower for Y- than for X-cells. However, R_N values ranged from 15-25 MΩ, with no obvious differences between X- and Y-cells. Perhaps more data will reveal such a difference or perhaps the R_m values are higher for X- than for Y-cells. Given typical electrotonic lengths of 0.5 to 1.5, R_m values for LGN neurons range from 1,000-3,000 $\Omega\text{-cm}^2$. Presently, we are analyzing voltage transients to directly measure electrotonic lengths and time constants of geniculate neurons.

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20.13 BINOCULAR INTERACTIONS IN THE LATERAL GENICULATE NUCLEUS OF THE CAT. J. -T. Xue*, A. S. Ramoa*, T. Carney* and R. Freeman Neurobiology Group, University of California, Berkeley, CA 94720

Previous studies have demonstrated inhibitory, and on occasion excitatory, binocular interactions in LGNd neurons. Moreover, the scatter of binocular receptive fields was similar to that observed for disparity selective neurons in the visual cortex (Sanderson, Bishop and Darian-Smith; 1971). In spite of these similarities, LGN neurons have yet to be tested for disparity selectivity. We utilized phase shifting of dichoptically presented sinusoidal gratings to investigate binocular interactions in the LGN. This technique is highly effective for the study of binocular interactions in the visual cortex.

Extracellular recordings were made from neurons in LGN laminae A, A1 and B. Optimal parameters were determined for stimuli presented to the dominant receptive field. Selectivity was determined by shifting the spatial phase, in 30 degree steps, of the grating viewed by one eye with respect to the grating viewed by the other eye. Consequently, the drifting gratings presented to each eye were identical except for their relative phase. While none of the LGN cells were disparity selective, binocular interactions occured for 91% of LGN cells. Eighty two percent of these cells exhibited a purely inhibitory effect from the nondominant receptive field stimulation. LGN cells usually exhibited two types of inhibitiory effects; 1)inhibition of the spontaneous rate, and 2) inhibition of the response evoked from the dominant receptive field. With respect to the second type of inhibitory effect, A1 laminae X-cells were more strongly inhibited than Y-cells and A & B laminae X-cells ($p<0.05$).

In contrast to LGN neurons, preliminary data indicate that perigeniculate neurons are typically selective for the relative spatial phase of dichoptically presented gratings.

In conclusion, while binocular spatial phase selectivity is absent, inhibitory binocular interactions are common to both X and Y cells in the LGN.

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20.14 INPUT AND OUTPUT ORGANIZATION OF A LOCAL CIRCUIT NEURON IN THE CAT'S LATERAL GENICULATE NUCLEUS. J.E. Hamos, S.C. Van Horn*, D. Raczkowski, D.J. Uhlrich*, and S.M. Sherman, Dept. of Neurobiology and Behavior, SUNY at Stony Brook, NY 11794.

The present study describes the synaptic organization of a local circuit neuron in the lateral geniculate nucleus. We iontophoresed horseradish peroxidase (HRP) into a geniculate neuron which had been classified as an X-cell using a series of physiological tests. Subsequently, we processed the LGN for HRP histochemistry and electron microscopy. The injected neuron has morphological features characteristic of class 3 neurons (Guillery, J. Comp. Neurol. 128:21,1966). Numerous thin, varicose dendrites radiate from the small cell body. These dendrites emit many multilobed appendages or terminal processes, giving the dendrites an axoniform appearance. The overall dendritic arborization spans the entire depth of lamina A and extends roughly 100μm in both its medio-lateral and rostral-caudal axes. This neuron could not be confirmed as a relay cell.

With the electron microscope, we reconstructed synaptic features by serially thin-sectioning two 50μm blocks containing the majority of the injected neuron's dendritic arborization. Both the larger dendritic shafts and the swellings along the neuron's dendrites and at terminal processes of dendritic appendages are contacted by all varieties of terminals typical of the lateral geniculate nucleus. The swellings, in particular, are commonly contacted by retinal terminals. Furthermore, the labeled dendritic swellings are, themselves, synaptic terminals which are filled with a pleomorphic population of vesicles and make symmetric contacts in either of two fashions. Many contact the proximal and medium-sized dendrites and cell bodies of target neurons without any clearcut relationship to nearby synapses. Other labeled swellings synapse in a more complicated fashion among clusters of appendages that extend from postsynaptic dendrites of other cells. Within these clusters, labeled dendritic swellings are both presynaptic to unlabeled dendritic appendages and shafts and also postsynaptic to retinal terminals. These latter, more complex arrangements have been termed triadic relationships, which we have shown to be associated with X-cells (Wilson et al., Proc. R. Soc. B, in press). Therefore, the labeled X-cell has numerous presynaptic dendrites that contribute F terminals (pleomorphic vesicles and symmetric contacts) to triadic relationships as well as other F terminals that end more simply on postsynaptic targets.

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20.15 **A COMPARISON OF VISUAL RESPONSE PROPERTIES IN THE STRIATE-RECIPIENT AND TECTO-RECIPIENT ZONES OF THE CAT'S LATERAL POSTERIOR NUCLEUS**

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The lateral posterior (LP) nucleus of the cat's thalamus contains at least two visual zones. The medial zone (LPm) receives a major ascending input from cells situated in the superficial layers of the superior colliculus, while the lateral region (LP1) is characterized by dense descending projections from striate cortex. We have previously documented the visual response characteristics of LPm neurons (Chalupa et al 83). In the present study, identical methods were employed to investigate the visual receptive field properties of single cells in the striate-recipient region. In agreement with previous studies (see Raczkowski and Rosenquist, 81) both the LPm and LP1 were found to be retinotopically organized. Three distinct functional properties differentiated the sample of neurons recorded in the striate-recipient zone from those in LPm. First, within 40 degrees of the area centralis representation, the average receptive field area in the LP1 was less than one-half that of LPm cells. Second, nearly twice as many cells in LP1 (about 45% of the sample) than in LPm were found to be orientation selective. Third, the internal organization of receptive fields was more complex in the striate-recipient zone than in the LPm, in that, well defined subregions yielding responses of opposite polarity were typical of LP1 neurons.

Cells within the two visual zones of LP also showed functional similarities. For instance, the degree of binocularity was the same and included the presence of a substantial proportion of cells which required binocular activation to yield reliable responses. In addition, approximately half of the cells in the LP1 were directionally selective and the distribution of the preferred directions was similar to cells in the LPm. Finally, the responses of most cells were markedly attenuated when the size of the stimulus exceeded the activating region of the receptive field.

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21.1 TIME COURSE OF VISUAL SUPPRESSION DURING CONVERGENCE. K.A. Manning*, J.P. Kelly* & L.A. Riggs. Hunter Laboratory of Psychology, Brown University, Providence, R.I. USA 02912.

Visual suppression has long been known to accompany saccades. More recently, convergence and divergence have also been shown to involve a suppression of vision (Soc. Neurosci. Abstr. 9:67, 1983). The present experiments have measured the time course of the change in visual sensitivity accompanying convergent eye movements.

Three human subjects executed convergent movements (6 or 8 deg rotation in each eye) by switching fixation from a far to a near point in a brightly illuminated Ganzfeld. The time of the eye movement in relation to the EOG was calibrated by simultaneous photographic recording of the moving eye and the EOG trace. Sensitivity to a 20 msec, full-field decrement in illumination was measured with a temporal forced-choice procedure, 1) during fixation of the far point, and 2) before, during and after convergence. We found that the amplitude and the time course of the change in sensitivity during convergence, relative to sensitivity in the fixation condition, were similar for all subjects. Sensitivity decreased when stimuli were delivered near the start of the eye movement. The peak loss in sensitivity (.41 to .44 log unit) occurred when stimuli were presented 20 to 100 msec after eye movement initiation. The loss in sensitivity then decreased, first rapidly, and then more slowly and variably, as the stimuli were delivered increasingly later in the 400-500 msec course of the eye movement. The loss in visual sensitivity during convergence persisted, 1) when the subject wore full-field, "white-out", diffusing goggles (Invest. Ophthalmol. 23:138, 1982), and 2) when changes in pupil diameter and in accommodation were eliminated. The magnitude and time course of convergence-related suppression resemble those of saccadic and blink-related suppression measured under comparable conditions.

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21.2 SACCADIC EYE MOVEMENTS ARE PROGRAMMED WITHOUT FEEDBACK ABOUT SMOOTH CHANGES IN EYE POSITION A. McKenzie* and S.G. Lisberger (SPON: M.P. Stryker) Dept. Physiology and Div. Neurobiology, Univ. California, San Francisco, CA 94143.

The "local feedback model" holds that saccades are programmed to correct the error between brain signals representing current and desired eye position in space. Work by Mays and Sparks suggested that the signal representing current eye position is updated whenever a saccade occurs, even if it intervenes between a flashed target and the flash-directed saccade. We now report that this signal is not updated during smooth changes of eye position in space.

Monkeys tracked a spot moving at 30°/sec along the horizontal meridian. At an unexpected time, the tracking target was extinguished and a second target flashed for 10 ms at one of 20 positions (0 to 12.5° up, ±12.5° horizontal). In the interval between the flash and the saccade (about 200 ms), there was a smooth eye movement of 4-5°, for which the saccadic system would have to compensate to point the eyes to the position of the flashed target in space.

During pursuit, saccades were made solely on the basis of the retinal error seen during the 10 msec flash, without accounting for the eye movement that had occurred after the flash. Thus, saccades were consistently in error with respect to the spatial location of the flash. For leftward pursuit the two monkeys made 97 and 83% of the error predicted if saccades were based only on retinal information; for rightward pursuit they made 95 and 77% of this error. In further experiments, we obtained smooth eye movement in space by having the monkey track a spot that moved with him during horizontal head rotation at 30°/s (cancellation of the vestibulo-ocular reflex). Again, flashed targets elicited saccades that were based solely on the retinal error seen at the flash.

In a previous experiment, one monkey had been trained on a tracking task (with head fixed) in which the flashed target was relit in its original position. Even with this feedback, saccades were initially based on retinal information, but were gradually directed towards the spatial position of the target.

We conclude that the eye position signal used in programming saccades does not receive feedback about smooth changes in eye position in space. This suggests that "local feedback" does not come from the brainstem oculomotor integrator, but instead may reside within structures that are involved only in programming saccadic eye movements.

(Supported by EY03878, and the McKnight Foundation)

21.3 SMOOTH-PURSUIT EYE MOVEMENT DEFICITS WITH PHARMACOLOGICAL LESIONS IN MONKEY DORSOLATERAL PONTINE NUCLEUS. D.A. Suzuki, J. May and E. Keller. Jules Stein Eye Inst., UCLA Sch. Med., Los Angeles, CA 90024 and Smith-Kettlewell Inst. Visual Sci., San Francisco, CA 94115.

Anatomical observations and physiological recordings indicate that the dorsolateral pontine nucleus (DLPN) is a major pontine link between cortical and cerebellar areas that may be involved with the regulation of smooth-pursuit eye movements (SPEMs). Cortical areas of interest include the middle temporal and posterior parietal cortices. The cortico-ponto-cerebellar pathway via the DLPN may be responsible for some of the sensorioculomotor signals reaching the flocculus and posterior vermis, two cerebellar areas of prominance in SPEM control. In this study, we sought to determine if the DLPN is necessary for normal SPEMs. Transient lesions caused by injections of lidocaine and permanent cellular lesions induced by ibotenic acid resulted in deficits in SPEM control.

Lidocaine was injected in three M. radiata into a region determined to be DLPN on the basis of neural responses to visuo-oculomotor stimuli. 0.5-10 ul were administered at rates of 0.05-0.4 ul per minute. In one monkey, 10 ug of ibotenic acid were administered as 1.3 ul over 15 minutes.

In each of the monkeys, direction specific deficits were observed in post-injection tests of SPEMs. The directions of SPEM that were affected were identical to the directional preferences of visual and/or SPEM-related units recorded in the same locations within DLPN. With 10 ul injections of lidocaine (2%), sinusoidal (0.4Hz±10deg) SPEM gain decreased from 0.92 pre- to 0.50 post-injection. Thirty minutes after the injection, partial recovery of gain to 0.83 was observed. A 0.5 ul injection (4%) primarily resulted in deficits in the initiation of pursuit for targets with retinal images that were eccentric from the fovea. Since the observed deficits exhibited the same directional preferences as for units recorded in the area, suppression of activity in fibers of passage is not thought to be the basis for the observations. Similar results obtained with ibotenic acid, which is specifically toxic to cell soma, support our conclusions that 1) the DLPN is part of the neural substrate for the SPEM control system and that 2) the DLPN processes information that is utilized in maintaining pursuit and in initiating tracking of eccentric targets.

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21.4 VISUO-MOTOR FEEDBACK CONTROL IS MEDIATED BY THE LATERAL CEREBELLUM IN TRAINED MONKEYS. R.C. Miall*, D.J. Weir*, R.I. Kitney* and J.F. Stein* (SPON: M. Brown). Univ. Lab. Physiology, Oxford and Dept. Elec. Eng., Imperial College, London.

We have shown by spectral analysis that a frequency component of approx. 1.3 Hz is found both in records of limb movements made by monkeys performing a manual tracking task, and in recordings made from lateral cerebellar neurones (Kitney R.I., Miall R.C., Riddell P.M., Stein J.F., J. Theor. Biol., 107:367-385). We suggested that this frequency resulted from visual feedback control operating intermittently to correct positional errors, and that the corrections were mediated by the lateral cerebellum. However, our support for these ideas was only indirect.

We have trained Rhesus monkeys to use a joystick to track periodic visual targets; a small monitor spot provided them with visual feedback of their position. Under normal conditions they made intermittent corrective movements at about 1.3 c.p.s. We then introduced additional delays in the feedback of monitor spot position. As predicted by our hypothesis, we were able to shift the average frequency of corrections from 1.3 to below 0.5 c.p.s.

We then interrupted the normal operation of the lateral cerebellum by temporarily cooling dentate and interpositus nuclei. This reduced the frequency of positional corrections and the movements became more erratic. We suggest therefore that cooling of the cerebellum increases the time needed for calculation of a corrective movement and reduces normal fine tuning of that movement.

These results support two conclusions: First the intermittent positional corrections seen in both human and primate motor control are not the result of asynchronous sampling, but are neccessitated by delays in the visuo-motor feedback control system. Second, this feedback loop probably includes the lateral cerebellum, since neurones there show the frequency which is characteristic of its operation, and when they are inactivated accurate visuo-motor control is lost.

21.5 A QUANTITATIVE STUDY OF CONTRALATERAL INATTENTION IN MONKEY FOLLOWING LESIONS OF POSTERIOR PARIETAL, PRESTRIATE, AND PREFRONTAL CORTEX. J.C. Lynch and J.W. McLaren. Dept. of Anatomy, U. Miss. Med. Center, Jackson, MS 39216 and Dept. of Ophthalmology, Mayo Clinic, Rochester, MN 55905

A major symptom of posterior parietal lobe damage in humans is profound neglect of sensory stimuli contralateral to the side of the lesion. Some investigators have reported similar symptoms following posterior parietal association cortex (PPAC) lesions in monkeys, while others have failed to observe such neglect. In order to study visual inattention quantitatively following PPAC lesions, 5 rhesus monkeys were trained on 2 visuomotor tasks. In the first, each monkey visually fixated a red LED (0.2° diam.) in the center of a horizontal array with additional LEDs 8°, 16°, and 24° on either side of the center. When the center target came on, the monkey pulled a lever and watched the target until it either dimmed, or it went out and a second LED came on and then dimmed. The monkey had to maintain fixation in order to see the dimming, whereupon it could obtain a liquid reward by quickly releasing the lever. The second task was similar except that on an average of 1 out of 7 trials 2 LEDs went on simultaneously and symmetrically either 8°, 16°, or 24° on either side of the central target when the central target went off. Both lights dimmed after an appropriate delay. After each monkey was performing these tasks reliably, a unilateral cortical lesion was made either limited to the inferior parietal lobule (IPL) or including both IPL and dorsal prestriate cortex (IPL-PS). After testing, most monkeys then received a symmetrical lesion on the other side and were tested again. One monkey also had a frontal eye field (FEF) lesion after its bilateral IPL-PS lesions. After unilateral IPL or IPL-PS lesions, no monkey exhibited qualitative signs of unilateral neglect or ignored the small target light when it appeared in the visual field contralateral to the lesions, although saccade latencies were slightly increased. However, in the double simultaneous stimulation paradigm, all monkeys exhibited visual extinction which lasted for varying lengths of time. The extinction was as strong for targets 8° from the center as for targets 16° or 24° from the center. In most monkeys the extinction was reversed after the second lesion. The monkey which had a FEF lesion after IPL-PS lesions showed striking qualitative neglect of visual, somatosensory, and auditory stimuli and also ignored the single LED target when it appeared in the visual field contralateral to the FEF lesion. (Supported by NIH grants EY02640 and EY04159)

21.6 EFFECTS OF UNILATERAL SUPERIOR TEMPORAL SULCUS LESIONS ON VISUAL ORIENTATION IN MONKEYS. K.E. Luh*[1], C.M. Butter[1], and H.A. Buchtel[1,2]. [1]Dept. of Psychology, Univ. of Michigan, Ann Arbor, MI 48109 and [2]Dept. of Psychiatry, Univ. of Michigan and VAMC, Ann Arbor, MI 48105.

Unilateral damage to the frontal eye field or posterior parietal cortex produces transient contralateral hemi-spatial neglect in primates. The superior temporal sulcus (STS) has both direct and indirect connections with these cortical areas and also possesses similar electrophysiological response characteristics to sensory stimuli. Petrides and Iversen have reported that bilateral STS lesions cause inattention to visual, auditory, and tactile stimuli (Brain Res., 161:63, 1979), thus it appears that the STS is also involved in orientation behavior. To further investigate this we studied the effects of unilateral STS lesions on orientation to visual stimuli in monkeys.

Three monkeys (M. fascicularis) were trained to orient to eight small lamps placed in a horizontal row 38 cm from the monkey's head. During each test session stimuli were presented unilaterally on some trials and bilaterally on others. If the animal oriented correctly, it was rewarded with a piece of apple. Pre-operatively, all monkeys responded on nearly all trials and showed no left-right biases on bilateral trials. Paw preference was slight or non-existent.

After pretraining to stable performance, two of the monkeys received unilateral STS lesions. Each animal manifested a pronounced but transient contralateral hemi-spatial neglect to both visual and tactile stimuli, and a marked preference for the ipsilateral paw. Formal retesting began shortly after surgery and the monkeys responded significantly less frequently to single stimuli contralateral to their lesions for approximately four weeks; their response rates to ipsilateral stimuli were not altered. When stimuli were presented bilaterally the monkeys oriented predominantly to stimuli ipsilateral to the lesion. This bias persisted at least seven weeks, although there was gradual improvement toward baseline. The third monkey received a unilateral control lesion anterior to the frontal eye field that was of equivalent size to the STS lesions. Visual orientation performance was unaffected by this lesion.

These data suggest a role for the STS in orientation behavior, perhaps by a contribution from the STS to a system that includes posterior parietal and frontal mechanisms.

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21.7 COLUMNAR ORGANIZATION OF CALLOSAL CONNECTIVITY IN THE MACAQUE FRONTAL EYE FIELDS AND ITS RELATION TO ELICITED EYE MOVEMENTS. C. J. Bruce and P. S. Goldman-Rakic. Sec. Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

We traced the callosal connections of the frontal eye fields (FEF), using horseradish peroxidase (HRP) or tritiated amino acids, and, in the same monkeys, examined the relationship between callosal connectivity and eye movements elicited by FEF microstimulation. Multiple injections of tracer were made into the anterior bank of the arcuate sulcus. Tracers spread to the fundus of the sulcus, saturating the low-threshold FEF as defined physiologically (Stanton et al., Soc. Neurosci. Abs. 8:293, 1982). Terminal labeling with either tracer showed that the FEF were callosally connected. Like prefrontal cortex immediately anterior, the callosal innervation was columnar, having bands of dense terminal label interspersed with label-free bands. Callosal bands were .4 to 1.3mm wide; most sections had 1 or 2 bands at different depths of the arcuate sulcus. HRP-labeled callosal cells of origin were also unevenly distributed, and dense concentrations of labeled cells were congruent with regions of heavy terminal labeling. Most callosally projecting cells were in layer III, but layer V also had labeled cells. Some especially large pyramidal cells were labeled in both III and V; such large pyramids are cytoarchitectonic features of the FEF.

Electrical stimulation of the FEF elicits saccadic eye movements. We hypothesized that saccade direction is related to callosal connectivity; vertical saccades are thought to involve the FEF bilaterally, whereas each hemisphere directs horizontal saccades into the contralateral field. Hence, prior to injecting tracers in the right FEF, the left FEF was explored with microstimulation. As in previous studies, direction of electrically evoked saccades varied regularly, often with horizontal, oblique, and vertical saccades being evoked in a single electrode penetration down the anterior bank of the arcuate sulcus. Electrode sites evoking horizontal or vertical saccades (within 30°) were electrolytically marked. Subsequent analysis revealed that the organization of saccade directions paralleled the anatomical columns; in particular, marked vertical saccade sites were in callosal bands, whereas horizontal sites were in acallosal zones. This organization emphasizes interhemispheric coordination for vertical saccades. Supported by USPHS and H.F. Guggenheim Foundation grants.

21.8 UNILATERAL FRONTAL EYE FIELD LESIONS DEGRADE SACCADIC PERFORMANCE IN THE RHESUS MONKEY. S.-Y. Deng*, M.A. Segraves, L.G. Ungerleider, M. Mishkin, and M.E. Goldberg, Lab. of Sensorimotor Research, National Eye Institute, and Lab. of Neuropsychology, NIMH, NIH, Bethesda, MD 20205; and Dept. of Neurology, Georgetown University School of Medicine, Washington, DC 20007.

Although neuronal activity preceding saccades has been described in the monkey frontal eye fields (FEF), no clear oculomotor deficit has been demonstrated in monkeys with lesions in this area. We analyzed the performance of monkeys trained to do a number of oculomotor tasks after they had received unilateral surgical FEF lesions.

The monkeys learned to fixate spots of light and make visually guided saccades. Having learned to make saccades into the field ipsilateral to the lesion, the monkeys were immediately able to make saccades into the contralateral field. These saccades were of normal latency, velocity, and accuracy as judged by comparison with those into the ipsilateral field.

The monkeys were then trained to make saccades to remembered targets. They had to fixate a spot of light while a second target flashed in the periphery for 300 msec; 100 msec later the monkeys had to make a saccade to where the stimulus had been and they were rewarded for making a saccade of appropriate dimensions. Although the monkeys learned the task easily when the stimulus was in the ipsilateral field, they had great difficulty performing the same task for stimuli in the contralateral field. At first they did not make any saccades at all. When they began to make saccades into the contralateral field the saccades were usually less accurate and slower than those of comparable distance into the ipsilateral field. They still failed to make saccades in many trials. They also frequently made the saccade during the interval in which the fixation point was still present. The deficits could be exaggerated by extending the interval between target presentation and fixation point disappearance. The deficits lasted for at least ten months.

These results are consistent with a role of the FEF in the generation of memory-guided saccades, although this area is clearly not crucial for the generation of visually guided saccades. The diminution of the peak velocity of memory-guided saccades indicates that the FEF contribute not only to the targeting and triggering of such saccades, but also to their ongoing dynamic programming.

21.9 THE EFFECT OF SUPERIOR COLLICULUS AND FRONTAL EYE FIELD LESIONS ON SACCADIC LATENCY IN THE MONKEY. P.H. Schiller*, J.H. Sandell, and J.H.R. Maunsell (SPON: M.P. Ogren). Department of Psychology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

Current evidence suggests that visually guided eye movements are produced via two parallel channels, one involving the superior colliculus (SC) and the other the frontal eye fields (FEF): ablation of the SC abolishes electrically triggered saccades from visual cortex but not from FEF (Schiller, Brain Res., 122: 154-6, 1977, Keating, NS Abstr. #220.8, 1983) and visually guided saccades are eliminated only when both the SC and the FEF are ablated (Schiller, True and Conway, J. Neurophysiol., 44: 1175-89, 1980).

It has recently been discovered that the latency distribution of saccades to individually presented visual targets is bimodal, with the fast peak at approximately 100ms (Fischer and Boch, Brain Res., 260: 21-6, 1983). We examined the hypothesis that the rapid (express) saccades forming the first peak in the distribution are generated by the collicular system. We ablated either the SC or the FEF in rhesus monkeys fitted with scleral search coils that had been trained to fixate and to saccade to visual targets presented at random locations. Following unilateral SC lesions monkeys no longer made express saccades to stimuli presented in the contralateral visual field and the saccadic latency distribution became unimodal. The latency distribution for ipsilateral stimuli was unaffected. FEF lesions did not produce any detectable long-term deficits on this task.

Our results, taken together with earlier findings showing a lack of color-opponent input to the SC (Schiller, Malpeli and Schein, J. Neurophysiol., 42: 1124-33, 1979) suggest that the SC is involved in the generation of rapid, reflex-like saccades which are mediated by the broad-band system.

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21.10 **TECTOSPINAL NEURON DISCHARGES IN THE ALERT HEAD-FREE CAT.** D. Munoz, D. Guitton and M. Volle*. Montreal Neurological Institute, McGill Univ. Montreal, Canada H3A 2B4.

The deeper layers of the caudal superior colliculus (SC) are implicated in the coding of gaze shifts that require coordinated eye-head movements. Tectospinal neurons (TSNs) project to both the spinal cord and many brainstem centers involved in eye and head motor control (Grantyn & Grantyn, 1982). **Methods:** 22 TSNs were recorded in the caudal SC of alert head-free and head-fixed cats, trained to orient to a spot of light (LED) in return for a food reward. Stimulating microwires were implanted into the spinal cord at C1 to antidromically identify TSNs. Collision tests were routinely performed. Eye and head movements were measured by the search coil in magnetic field technique. EMG activity was recorded from biventer cervicus and splenius neck muscles. **Results:** Most TSNs exhibited both visual and motor related discharges. The visual responses were found to be directionally selective when objects were hand-moved across the receptive field. A very strong discharge was evoked by movement away from the area centralis. Movement in the opposite direction evoked no discharge. There was only a weak discharge in response to the onset of an optimally located, stationary LED. The most vigorous motor related responses were obtained not when the cat oriented to the LED but rather when its orienting behavior was in relation to a morsel of food. When food was positioned anywhere in front of the cat and the head-free cat looked away, a TSN discharged tonically for a family of gaze errors that defined a gaze error field (GEF) with the cell's preferred error at the field's center. The same GEF was observed head-free or fixed. The line between the center of the GEF and the morsel of food defined the optimal gaze error vector (GEV) for that cell. When the cat looked at the stationary food with saccadic gaze shifts (head-free or fixed) of amplitude and direction similar to the GEV, the TSNs occasionally showed a phasic discharge rate. This phasic component was much more consistent when the animal oriented to a piece of food moving parallel to the GEV. In this condition high frequency bursts of spikes were well correlated to saccadic gaze shifts (head-free or fixed) similar to the GEV and to the accompanying bursts of EMG activity recorded from the neck. Stimulation of the SC at the site of a TSN generated head-free saccadic gaze shifts of amplitude and direction equal to the GEV. **Conclusions:** (1) Surprisingly, TSN responses were stimulus specific: they discharged in relation to movements towards food but not to the same movements towards a LED. (2) With head-free, TSN discharges were not linked preferentially to either eye or head movements alone. Rather, their discharge was related to the sum of eye-re-head plus head-re-body; i.e. to movements of the visual axis relative to the cat's body. Supported by the MRC of Canada.

21.11 THE AFFERENT CONNECTIONS OF THE ANTERIOR PRETECTAL NUCLEUS. V. Arango* and F. Scalia, Department of Anatomy and Cell Biology, Downstate Medical Center, Brooklyn, N.Y. 11203.

The anterior pretectal nucleus (PA), a large ovoid cell-mass, is readily delimited in the rat pretectal region. It is identified with equal clarity by its overall dense staining for AChE. These features, and our demonstration (Arango and Scalia, Anat. Rec., 205:11A, 1983) that the entire nucleus projects to the parvocellular red nucleus (Rp) and zona incerta (ZI) emphasize its unity, although it does contain two divisions, a dorsal pars compacta (PAc) and a ventral pars reticulata (PAr). In a study of their efferent connections (above), we showed that PAc projects to LD and LP in the dorsal thalamus, whereas PAr projects to thalamic nuclei VL and CL, and to n. reticularis tegmenti pontis and supra-peduncular pontine grey. These data and the demonstration (Takahashi, Ph.D. Thesis, 1981) that area 17 projects to PAc (and LD, LP), while area 4 projects to PAr, supported differential roles for these subdivisions in visual vs. somatic sensorimotor systems. Further study of the afferent connections of PA is reported here.

Free HRP was injected into either PAc or PAr in Long Evans hooded rats. Brain sections were stained for HRP with TMB or Co-Ni intensified DAB in alternate series. After small injections confined to PAc, HRP-labeled cells were found ipsilaterally in LGv, area 17 and superficial superior colliculus, and bilaterally in n. parabigeminalis, all visual system structures. Cells in ZI and Rp were also well-labeled. When PAr was injected, labeled cells again appeared in ZI and Rp. In addition, many cells were labeled contralaterally, in the dorsal column nn., interpolar and oral spinal trigeminal n. and dentate n., and ipsilaterally in n. paragigantocellularis dorsalis, n. prepositus hypoglossi and a related perigenual (N. VII) cell cluster. A few cells appeared in n. raphe obscurus and ipsilateral n. gigantocellularis.

These data give further support to the functional subdivision of PA into a dorsal, vision-related area (PAc) and a ventral zone (PAr) connected with somatic sensori-motor and cerebellar systems. However, the common structural features noted above suggest further exploration for a possible role for PA in integration across these systems.

21.12 LATERALITY OF TECTAL EFFERENT PROJECTIONS IN RANA PIPIENS. T. Masino, S.K. Kostyk, and P. Grobstein. Dept. Pharm. and Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

Loci at all locations within binocular visual field, both to the right and the left of the mid-sagittal plane, are mapped in each tectal lobe in the frog. Each tectal lobe has also been shown to be capable of triggering orienting turns in both directions. Recent work suggests that premotor circuitry for orienting turns may be lateralized, with that for turns in one direction on one side of the brain and that for turns in the other on the other. We were interested in whether there are corresponding differences in the laterality of projections of tectal regions representing visual fields to the left and right of the mid-sagittal plane.

A unilateral lesion of the ventral white tracts in the caudal midbrain abolishes orienting turns in one direction. This finding might be accounted for by interruption of the projections from the regions of each tectal lobe which represent the same visual hemifield. We have investigated this possibility by placing HRP in unilateral lesions of the caudal midbrain ventral white tracts and studying the resulting patterns of retrograde labeling of cells in both tectal lobes. The labeling patterns did not bear any clear relation to the tectal regions which map one hemifield. Labeled cells were found distributed throughout both tectal lobes.

A more comprehensive investigation of possible differences in the laterality of tectal efferent projections was made by studying patterns of anterograde labeling following small injections of HRP at loci within tectal regions representing left and right visual fields. We did not find differences either in the terminal zones innervated or in decussation patterns at any level of the brain. We are also analyzing this and additional material to determine whether there are differences in the terminal zones innervated by tectal regions representing rostral as opposed to caudal and ventral as opposed to dorsal visual field. To date, no clear differences have been noted.

Our observations make it unlikely that the triggering of a right as opposed to a left turn can be accounted for in terms of significant differences in the structures to which differing tectal regions project. Our observations also suggest that in general the targets of efferent projections may not vary significantly for different tectal loci. This has implications for understanding not only the control of turn direction but of other aspects of frog orienting behavior as well.

21.13 FOREBRAIN INVOLVEMENT IN PREY ORIENTING BEHAVIOR IN THE FROG. P. Patton and P. Grobstein. Dept. Pharm. Physiol. Sci., Univ. of Chicago, Chicago, Illinois 60637.

Unilateral forebrain lesions in the cat result in loss of orienting responses to stimuli in the contralateral visual hemifield. The deficit is not due to removal of circuitry essential for orienting since it can be alleviated by a subsequent midbrain lesion (Sprague, Science 153:1544, 1966). A similar modulation of midbrain orienting circuitry by the forebrain may also exist in the toad (Ewert, Brain, Behav. Evol. 3:36, 1970). We have been studying forebrain involvement in visual orienting behavior in the frog, Rana pipiens.

Lesions intended to remove the entire telencephalic lobe on one side of the brain were made by suction aspiration. Frogs were subsequently observed in a standard perimetry test. The unilateral forebrain lesions produced contralateral visual deficits. Response frequencies were however dramatically lower not throughout the entire contralateral hemifield, as in the cat, but only in the monocular part. The deficit region is like that following unilateral tectal lesions in frogs.

We are currently studying the behavior of unilaterally forebrain lesioned frogs after a subsequent lesion of the contralateral tectal lobe, a lesion which alleviates forebrain deficits in cats. Testing has been completed on one frog. Response frequency for stimuli in the area affected by the forebrain lesion significantly increased following the tectal lesion. While preliminary, the result suggests that orienting deficits resulting from forebrain lesions in the frog can be alleviated by subsequent midbrain lesions. An additional reason for believing that the forebrain deficits in the frog result from disturbances of modulating circuitry, rather than destruction of essential circuitry, is that the forebrain lesions, while dramatically reducing the frequency of responses in the monocular field, did not abolish them entirely.

Forebrain modulation of midbrain orienting circuitry appears not to be specific to vertebrates having a well-developed cortex. A consideration of the similarities and differences in brain organization between different vertebrate classes might be helpful in identifying the particular neural structures involved in such modulation. The differences in deficit extent following unilateral forebrain lesions in cats and frogs may also be significant in this regard. In both animals, the entire binocular field, including loci on both sides of the midline, is represented in both tectal lobes. The differing deficits may indicate that the descending modulating pathways are bilateral in the case of the cat but unilateral in the case of the frog.

21.14 FROG PREY ORIENTING BEHAVIOR: EFFECTS OF CEREBELLECTOMY. A. Reyes* and P. Grobstein. Dept. Pharm. and Physiol. Sci., University of Chicago, Chicago, Illinois 60637.

In frog prey orienting behavior, an appropriate visual stimulus at a given location triggers a directed ballistic movement. The triggered movement varies with stimulus location in all three spatial dimensions. The behavior depends on an ability to generate a variety of motor patterns and to select the appropriate pattern for a stimulus at a given location. The latter cannot be accounted for solely in terms of the retinal locus activated by the stimulus but requires combining this information with additional information, including information about stimulus distance and body posture.

We have studied the behavior of frogs following aspiration lesions which subsequent histology showed to have completely removed cerebellar cortex. Frogs displayed some clumsiness immediately after the lesion; this disappeared within two to three weeks. At this time we systematically studied orienting behavior. In a standard perimetry test, lesioned frogs responded to stimuli at increasing angles on the horizontal with increasing turn amplitudes. The amplitudes were normal except for a slight undershooting for the most eccentric stimuli. We repeated the perimetry test incorporating an initial stimulus presentation which caused the animals to adopt an upward posture prior to presentation of the stimulus which triggered a turn. Movement trajectories seemed somewhat abnormal but turn angles were normal except, again, for slight undershooting at the most eccentric positions. Lesioned animals also varied their output appropriately for stimuli at increasing distances along the mid-sagittal plane (in binocular field) and along the frontal plane (in monocular field). In the latter case, lesioned animals, as in other tests, displayed some minor abnormalities in turn amplitude, but only for stimulus locations requiring the most complex movements.

Our observations revealed relatively little effect of cerebellectomy on frog orienting behavior. The minor abnormalities observed seem most readily interpretable as disturbances in coordination of body parts during movement. Neither the ability to generate motor outputs nor the ability to select the appropriate motor output for a stimulus at a given location appears to depend strongly on the cerebellum. The insensitivity to cerebellectomy of the sensorimotor transformation underlying frog orienting behavior may relate to a relative lack of dependence of this transformation on reafferent processing during the course of the movements.

21.15 THE RELATIONSHIP BETWEEN THE OPTIC NERVE FIBERS AND TECTAL EFFERENT CELLS IN THE OPTIC TECTUM OF RANA PIPIENS T.E.Hughes*, D.Ingle, W.C.Hall. Anatomy Dept., Duke Univ., Durham, N.C. and the Rowland Inst., Cambridge, MA.

In all vertebrates studied, a population of cells in the deep tectum gives rise to a pathway, the predorsal bundle, that crosses the midline and descends to paramedian brainstem motor areas. However, the morphology and afferent connections of these cells may vary among species. For example, the dendrites of predorsal bundle cells in many mammals are restricted primarily to the layer in which their somas reside, and therefore have little overlap with the more superficial terminal field of the retina. On the other hand, Golgi descriptions of the tectum of many nonmammalian vertebrates reveal that the dendrites of the cells in the deep tectum often extend into the superficial fiber layers, including those in which the optic nerve terminates. The present experiments were designed to determine whether the predorsal bundle cells in the frog, Rana pipiens, are included among those which have dendrites that extend into these superficial layers and, if so, whether they receive monosynaptic input from the retina.

Small injections of horseradish peroxidase were made into the predorsal bundle of R. pipiens to retrogradely fill the cells of origin in the tectum. In the frog, the retrograde filling reveals the extent and laminar distribution of the dendritic fields. The somas of the predorsal bundle cells are found in the upper half of layer six. Three or four large dendrites ascend through the superficial layers, and then turn to course parallel to the surface. Using electron microscopy, we studied the synaptic relationships of the labeled dendrites of the predorsal bundle cells as they pass through the superficial neuropil. In layer seven the dendrites receive few synapses, in layer eight and above they receive many.

Using either the anterograde transport of horseradish peroxidase following injections into the optic nerve or anterograde degeneration we have studied the retinal terminals in the ranid tectum with the electron microscope. A variety of morphologically distinct retinal terminals can be identified above layer six. These terminals synapse upon dendritic processes of different sizes and various orientations.

By combining retrograde labeling with anterograde labeling in single animals, it should be possible to determine whether the optic axons synapse directly upon the apical dendrites of the predorsal bundle cells.

21.16 ORIGINS OF AN EYE MOVEMENT-RELATED COROLLARY DISCHARGE AND PHOTIC RESPONSES OF THE GOLDFISH TORUS LONGITUDINALIS. D.P.M. Northmore, Institute for Neuroscience, University of Delaware, Newark, DE 19711. (SPON: L.C. Skeen).

In cyprinid fishes it has been shown that the cerebellum, specifically the valvula, provides synaptic input to a midbrain structure, the torus longitudinalis (TL). The TL, which is unique to teleosts, is an elongated nucleus running the length of the medial junction of the tectal lobes. It is the sole source of unmyelinated fibers in the most superficial layer of the optic tectum. Because these fibers make synaptic contact with tectal cells that also receive direct retinal input, the system is potentially capable of integrating motor information with visual input.

Multiunit activity was recorded from the TL in unanesthetized goldfish while spontaneous eye movements were monitored with a corneal search coil. Saccades, but not slow, drifting eye movements, were accompanied by bursts of activity that started at the beginning of the saccade, and peaked after the eye had come to rest. Normally, the amplitude of the bursts after integration was proportional to the saccade amplitude, but independent of saccade direction or eye position. A visual or proprioceptive origin of the bursts can be ruled out because they occur in darkness, are not evoked by passive eye movement, and persist after total paralysis with Flaxedil.

Also recorded in TL was a sustained discharge to dimming in the contralateral visual field, 10-30 deg below the horizontal meridian. Its diffuse receptive fields mapped naso-temporally on the rostro-caudal axis of TL. The photic responses were recorded more superficially in TL than the saccadic.

Lesions of one tectum abolished the photic responses in the adjacent TL, but only attenuated the saccadic bursts. Lesioning the valvula, but not other parts of the cerebellum abolished saccadic bursts without affecting the photic response. After small lesions in the TL itself, saccadic responses were diminished, and sometimes depended upon the direction of eye movement, and the eye-in-orbit position. These results show that the visuotopic photic response arrives from the adjacent tectum, and that the saccadic activity originates in the valvula of the cerebellum. The latter is apparently capable of transmitting to tectum, via TL, information about saccade timing and direction, and possibly eye position. (Supported by EY 02697).

22.1 TOPOGRAPHIC DISTRIBUTION OF MONOAMINERGIC NEURONS IN THE RAT MEDULLA USING QUANTITATIVE THREE-DIMENSIONAL COMPUTER RECONSTRUCTION M. Kalia, D.J. Woodward, W. K. Smith, K. Fuxe, and M. Goldstein . Dept. Pharm., Thomas Jefferson Univ., Phila. PA 19107, Dept. Cell Biol., UTHSC at Dallas, TX 75235 and Karolinska Inst., Stockholm 104 01.

We examined serial 40 um vibratome, immunoperoxidase stained sections of the medulla using tyrosine hydroxylase (TH), dopamine beta hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT) antisera followed by Nissl staining to locate catecholaminergic (CA) neurons in cytoarchitectonic regions followed by a 3 dimensional (3D) computer reconstruction of these cell groups to determine their spatial organization. Overlay drawings of low and high power photomicrographs showing cell bodies and nuclear boundaries were entered into a digital computer storage system. Every nth section in the series was plotted to yield an accurate representation of regional densities of cells and location of nuclei. Graphics software allowed 2D plot of individual sections as well as 3D plots of groups of sections. Data files were scanned in a number of ways to obtain total cell counts of TH, DBH and PNMT immunoreactive cells within a designated area or cell counts of only one type of immunoreactive cell. This combination of data manipulation produced the following results:1) A1 group is a homogeneous population of noradrenergic (NA) neurons at levels caudal to the obex and at the obex is mixed with adrenergic (A) cells with dimensions of 1.3 X 2.7 mm, extending from -2.5 to +0.2. Part of this cell group lies in the lateral reticular nucleus. 2) A2 group is not purely NA as previously suspected. It is a very mixed cell group containing mainly dopaminergic neurons in the ap, PVR and dmnX, mainly NA neurons in the mnTS, mainly A neurons in the ds and dnTS, and a mixture of all three CA neurons in the other subnuclei of the nTS. The dimensions of this group are 0.4 X 3 mm extending from -2.7 to +0.3. 3) C1 group is a homogenous population of A cells extending from +1 to +2.5 with dimensions of 1.5 X 1.5 mm and consisting of scattered neurons some of which occupy the gigantocellular reticular nucleus 4) C2 group is a homogenous population of A neurons extending from +1 to +3 with dimensions of 2.5 X 3 mm. Thus, accurate visual imaging and quantitation of the spatial organization of medullary CA neurons within the classical anatomical framework of cytoarchitecture has provided new data about this region of the CNS. Supp: USPHS Grants HL 30991, NIAAA 390, DA 2338, Biol. Humanics Found., Swedish Med. Res. Found. 14X04246-10B.

22.2 ESTROGENIC MODULATION OF α_2-NORADRENERGIC RECEPTOR BINDING IN SEVERAL BRAIN AREAS ASSESSED BY TRITIUM-SENSITIVE FILM AUTORADIOGRAPHY. A.E. Johnson*, B. Nock*, H.H. Feder*, and B.S. McEwen. (SPON: E. Satinoff). Institute of Animal Behavior, Rutgers University, Newark, N.J. 07102 and Rockefeller University, New York, N.Y. 10021.

Noradrenergic (NA) transmission appears to play an important role in ovarian steroid regulation of anterior pituitary function and lordosis in female rodents. One way in which ovarian hormones might affect the function of the NA system is by altering the number of NA receptors. We used quantitative autoradiography to examine the influence of estrogen on α_2-receptor density in several areas of the guinea pig brain.

Adult guinea pigs were ovariectomized and treated one week later with either 10μg estradiol-17β benzoate (E) or oil for two consecutive days. Two days after the second injection, animals were decapitated, brains were rapidly removed, frozen on dry ice, and stored at -60°C. Brain sections (32μM) were cut and thaw-mounted onto subbed slides. α_2-Receptors were labelled using 1.0nM (3H)p-aminoclonidine (PAC) ± 100μM (-)norepinephrine. Tissues were exposed to tritium-sensitive LKB Ultrofilm for 12 weeks. Autoradiograms were analyzed using a computer assisted densitometer that converted optical density to fmol/mg protein using a standard curve derived by coexposing tritiated brain mash standards in each film cassette.

We found that E increased PAC binding in several E concentrating regions of the preoptic area (POA) including the median and periventricular nuclei, suprachiasmatic area, and medial and lateral POA. In contrast, E treatment decreased PAC binding in the ventromedial nucleus of the hypothalamus but had no effect in other E concentrating areas of the hypothalamus, amygdala, or hippocampus. E did not affect PAC binding in areas which do not concentrate E such as the caudate nucleus and several thalamic nuclei.

These findings indicate that E can alter the number of α_2-receptors in specific brain areas known to contain E concentrating cells and suggest that the effects of E on anterior pituitary function and female sexual receptivity may be mediated in part by α_2-adrenergic receptors. Supported by NIH-HD-04467, NIMH-29006, NS07080 (USPHS) and RF81062 (Rockefeller Foundation).

22.3 ULTRASTRUCTURAL ANALYSIS OF TYROSINE HYDROXYLASE-CONTAINING NEURONAL CONNECTIVITY IN THE VENTRAL PERIVENTRICULAR HYPOTHALAMUS OF THE MACAQUE. K.K. Thind and P.C. Goldsmith. Dept. of Ob/Gyn & Repro. Sci. and the Repro. Endocr. Ctr., Univ. of Calif., San Francisco, CA 94143.

Tyrosine hydroxylase (TH) immunopositive contacts involving periventricular dopamine (DA) neurons were examined in the adult female macaque brain. Following perfusion with a buffered aldehyde mixture, coronal vibratome sections (40 um) from the arcuate, ventral anterior periventricular, and suprachiasmatic nuclear regions were immunostained with 1:400 rabbit anti-TH antiserum (#16, provided by Dr. A.W. Tank, Univ. of Colo.), using the PAP technique or colloidal gold (15 nm) labelling. Thin sections were examined by electron microscopy. Electron micrographs of TH-immunopositive (TH+) elements were analyzed for the presence of membrane appositions (MAP's) and synapses.

In a survey of 280 TH+ PROFILES, 68% engaged in MAP's or synapses. Of these, 24% had distinct postsynaptic densities (PSD's), and 13% of these showed multiple PSD's. Analysis of 270 TH+ CONTACTS gave 70% MAP's and 30% PSD's. Axons (AXO), dendrites (DEN), and somata (SOM) occurred in pre/post-contact arrangements with the following frequencies:

Contact pre/post	Percent occurrence of contact type MAP's	PSD's
AXO/DEN*	74	95
DEN*/DEN*	20	4
AXO*/DEN*	4	1
AXO/SOM*, DEN*/DEN, DEN*/SOM and DEN/SOM*	2	-

* = TH+ component.

Almost all AXO/DEN* synapses were clearly asymmetrical. All presynaptic axons contained varying numbers of small, clear vesicles (45 nm), frequently accumulated at the contact site; 3 also contained scattered dense core vesicles (100 nm). Although the origin of these presynaptic axons is unknown, the prevalence of MAP's, and the presence of synapses exclusively on TH+ dendrites, indicates that DA neurons receive extensive afferent inputs. Taken together, these results suggest that periventricular DA neurons may perform an important role in local integration as well as in the final common pathway of the neuroendocrine system in primates.

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22.4 CATECHOLAMINERGIC PATHWAYS OF THE DOG BRAIN STEM. C.L. Chernicky, K.L. Barnes, C.M. Ferrario and J.P. Conomy. Research Division and Department of Neurology, Cleveland Clinic Foundation, Cleveland, OH 44106.

There is abundant evidence for the participation of catecholamine containing neuronal systems in the control of cardiovascular function. Because these systems have not been well characterized in the dog, the present study examined catecholaminergic pathways in the brain stems of 4 mongrel dogs (5-8 kg) with the glyoxylic acid (GA) histofluorescence technique. The animals were anesthetized (pentobarbital, 35 mg/kg, i.v.) and perfused via the ascending aorta with 0.9% saline followed by ice cold 2% GA in phosphate buffer, pH 7.0. The brain stems were removed, blocked and rapidly frozen onto cryostat chucks. Serial sections from C2 through the superior colliculus were cut at 25 μm; every 5th section was processed for histofluorescence with the GA method of de la Torre. Adjacent sections stained with cresyl violet were used for the identification of structures. The sections were examined with a Zeiss fluorescence microscope and mapped using an X-Y plotter interfaced to the microscope.

Three principal regions containing catecholamines (CA) were identified within the dog brain stem and pons: 1) a ventrolateral region corresponding to A1 in the caudal portion and to the A5 cell group rostrally; 2) a dorsomedial region (A2) and 3) a more rostral dorsolateral area consisting of the locus coeruleus (A6) and the locus subcoeruleus (A6sc). The A1 region extended from the pyramidal decussation to the level of the rostral inferior olive. Fluorescent cells and fibers were scattered diffusely throughout the ventrolateral medulla dorsal and lateral to the lateral reticular nucleus and inferior olive. The A5 region extended from the rostral inferior olive to the rostral superior olive. The A5 cells were situated dorsal and lateral to the facial nucleus, with a number of cells located just medial to the exiting facial nerve fibers. The A6 and A6sc cell groups began just caudal to the facial nerve and extended rostrally to the posterior end of the inferior colliculus. This study provides the first anatomical mapping of CA in the A1 and A2 cell groups of the lower brain stem of the dog and confirms the work of Ishikawa et al (Brain Res 86: 1-16, 1975) for the A5 and A6 regions in this species. (Supported in part by grants from NHLBI, HL-6835 and the Reinberger Foundation).

22.5 IMMUNOHISTOCHEMICAL LOCALIZATION OF DOPAMINE AND NORADRENALINE IN THE CENTRAL NERVOUS SYSTEM. H.W.M.Steinbusch, R.Dirks*, J.G.J.M.Bol*, J.de Vente* and F.Rerkenbosch* (SPON:Tj.B. van Wimersma Greidanus). Dept.Pharmacology, Free University, 1081 BT Amsterdam, The Netherlands.

The localization of dopamine (DA) and noradrenaline (NA)-containing cell bodies, dendrites and varicose fibers in the CNS has been studied with the unlabeled PAP method using highly sensitive and well-characterized antibodies to DA and NA themselves. The antibodies were raised against conjugate-complexes, using thyroglobulin as carrier-protein and either carbodiimide or a mixture of formaldehyde (For)/glutaraldehyde (Glu) as coupling reagents. Optimal coupling conditions were tested by using radio-active labeled DA or NA. Fixation for immunohistochemical purposes was critical, small amounts of Glu need to be included in the buffered For. The DA antibody shows no cross-reactivity to NA but a small degree to serotonin. The NA antibody cross-reacts to some extent with DA but not to other monoamines. The specificity is currently under investigation using solid-phase absorption tests, pharmacological pretreatments and gelatine models, to which various monoamines are incorporated. DA-immunoreactive ($_i$) cell bodies were demonstrated in known areas as the substantia nigra, the nucleus arcuatus but also in the zona incerta. No staining of DA$_i$-perikarya was observed in the locus coeruleus. DA$_i$-varicose fibers were distributed throughout the entire CNS. A dense innervation of DA$_i$-fibers were seen e.g. in the nucleus raphe dorsalis, the locus coeruleus and the cortex. NA$_i$ cell bodies were confined to the locus coeruleus and subcoeruleus and areas ventrolaterally in the rhombencephalic and mesencephalic reticular formation. NA$_i$-varicose fibers were widespreaded in the CNS. A particular dense NA-innervation was found e.g. in the caudal part of the nucleus raphe dorsalis in the habenula and in the nucleus paraventricularis hypothalami.

The two antisera enable the visualization of very fine varicose-fibers in almost all parts of the brain. With regard to their specificity it should be mentioned that the localization of their cell bodies resemble the one seen with the corresponding biosynthetic enzymes, i.e. TH for DA and DBH for NA. The major advantage of the obtained antibodies is lying in the fact that they directly visualized the transmitters themselves and can be used on tissue fixed in such a manner that it can easily be used for the visualization of neuropeptides as well. First results will be presented to show their application in various species.

22.6 DISTRIBUTION OF HISTAMINE-, IN RELATION TO DOPAMINE- AND NORADRENALINE-IMMUNOREACTIVE CELL BODIES IN THE CENTRAL NERVOUS SYSTEM OF THE RAT. R.Dirks*, H.W.M.Steinbusch, J.G.J.M.Bol* and A.H.Mulder* (SPON:H.W.M. Steinbusch). Dept.Pharmacology, Free University, 1081 BT Amsterdam, The Netherlands.

An immunohistochemical study was carried out to establish the anatomical interrelationships between histaminergic, dopaminergic and noradrenergic cell bodies throughout the rat CNS. The distribution of histamine (HIS)-, dopamine (DA)- and noradrenaline (NA)-immunoreactive ($_i$) cell bodies was mapped with the indirect PAP-method using well-characterized antibodies to HIS, DA and NA. HIS$_i$-cell bodies were strictly confined to the caudoventral part of the hypothalamus, in the region of the recessus mamillaris (rm). Their localization resembles the one observed immunohistochemically with an antibody to histidine-decarboxylase. Rostrally HIS$_i$-perikarya were visualized at the dorsal tip of the rm. The majority of the HIS$_i$-cell bodies were found in the region of the nuclei premammillaris ventralis and mammillaris prelateralis. In the latter nucleus the cells are densely packed. Caudally, a few HIS$_i$- perikarya were detected at the basal corner, just ventromedially to the crus cerebri. Most of the HIS$_i$-cell bodies appear to be bipolar. DA$_i$-cell bodies were detected in all the regions, previously shown immunohistochemically to contain tyrosine hydroxylase, but lack dopamine-beta-hydroxylase (DBH), such as the substantia nigra (SN), the periventricular gray of the thalamus or the zona incerta. NA$_i$-cell bodies were demonstrated in the same nuclei and areas that were found previously to be TH-positive, DBH-positive, and PNMT-negative such as the locus coeruleus (LC) and the ventrolateral corner of the formatio reticularis.

It was observed that the caudal histaminergic cell group, situated in the vicinity of the area tegmentalis ventralis is partly intermingled with dopaminergic cells. The arrangement of the histaminergic cells, ciz. a compact cell group with a widespread distribution of fibers, resembles that of the DAergic and NAergic system in the SN and the LC, respectively.

22.7 DENSE SEROTONERGIC INNERVATION OF SELECT CORTICAL NEURONS IN CAT NEOCORTEX. I. Törk* and K. A. Mulligan* (SPON: C. Straznicky). School of Anatomy, University of New South Wales, Kensington, NSW 2033, Sydney, Australia.

Recent immunohistochemical studies have demonstrated the existence of a dense serotonergic innervation of the neocortex in the rat, monkey and cat. Although the density and disposition of the immunoreactive fibers was found to vary between different cortical areas and layers, there is general agreement that cortical layers I-III receive the highest level of serotonin input. In this paper we report on the existence of basket-like formations of serotonergic fibers which surround the somata and dendrites of some cortical neurons in these layers.

Cats were perfused intracardially with 4% paraformaldehyde solution and 50 um thick Vibratome sections were cut from the somatic sensory, visual, auditory and motor cortical areas of the neocortex. The serotonergic fibers were demonstrated using a monoclonal antibody against serotonin (5-HT) and a biotin-avidin-peroxidase reagent. In neutral red and cresyl violet counterstained preparations we could observe the relationship of the 5-HT immunoreactive fibers to some cortical neurons. Numerous varicosities, up to 2 um in diameter, studded the surface of the somata and dendrites of these neurons. Since most varicosities belonged to just a few intertwined axons which made repeated contacts with the same cell, a serotonergic 'basket' was formed around the neuron. In many cases the density of the basket was such that the major branches of the dendritic tree of the neuron inside it could be recognised. Camera lucida drawings of the 5-HT baskets were compared with images of Golgi impregnated neurons of the feline cortex. In layer I the serotonergic baskets correlated well with the morphological features of the horizontal cells. In layers II-III the target cells appeared to be non-pyramidal neurons; the most common target cells were oriented normal to the pial surface with dendrites extending both superficially and deeply, resembling the morphology of bitufted and bipolar neurons. The monoaminergic innervation of the cortex is often described as diffuse and non-specific: the present results provide new morphological evidence for the existence of a direct and intensive interaction between serotonergic fibers and certain cortical neurons.

22.8 SYNAPTIC RELATIONSHIPS OF DENDRITES IN THE SUBSTANTIA NIGRA AND VENTRAL TEGMENTAL AREA OF PRIMATES. D.L. Felten, Dept. of Anatomy, Univ. of Rochester Sch. Med., Rochester, NY 14642

Neurons of the substantia nigra (SN) and ventral tegmental area (VTA) of the young adult rhesus monkey and baboon brain were examined with electron microscopy for synaptic interactions among local dendrites and for relationships of afferent terminals to the neurons in these areas. The medium-sized neurons in the SN and VTA were identified readily by their perikaryal cytoplasmic characteristics, by the presence of neuromelanin pigment granules and cytoplasmic dense bodies, and in selected specimens by x-ray analytical EM identification of chromium-tagged dopamine. The somatic limiting membranes of the main SN and VTA cells were invested mainly with glial processes, and contained only sparse axo-somatic synapses. The distal primary, secondary, and smaller dendrites were covered densely by axo-dendritic synapses, including abundant terminals with pleomorphic vesicles or clear round vesicles of varying size, and fewer terminals with flattened vesicles or dense-core vesicles. Considerably more clear round-vesicle synapses were noted in the lateral VTA than in the SN. Most of the pleomorphic synapses were symmetrical; most of the other types were asymmetrical. The dendrites of SN and VTA neurons formed small clusters or bundles oriented mainly vertically in these regions. In pars compacta and pars reticulata of SN, and in the lateral portion of VTA, numerous dendro-dendritic appositions were found, more abundant in pars compacta than in the other regions. Scattered vesicles were found in dendrites at the site of some appositions, but prominent membrane specializations were infrequent. Small dendrites clustered around synapsing axon terminals, and abutted each other. In view of the substantial electrophysiological evidence of dendro-dendritic transmission in the rat substantia nigra, the present findings offer anatomical support for similar channels in primates. The topographic distribution of afferent synapses suggests that interactions with and among smaller dendrites is key for regulating the excitability of the neurons of SN and VTA. In this model, no single input would have predominance for influencing events at the axon hillock, necessitating the integrated input of many afferents, and the dendro-dendritic interactions themselves, to regulate the excitability of these neurons and their efferent output to target structures. Supported by a MacArthur Foundation Fellowship.

22.9 ORGANIZATION OF LATERAL HABENULAR AFFERENTS TO THE DORSAL RAPHE NUCLEUS. M.R. Park. Dept. Anatomy, Univ. Tennessee Center for the Health Sciences, Memphis, TN 38163.

Knowledge of the spatial organization of the projection from the lateral habenula to the dorsal raphe is of interest in helping to interpret the various results obtained from physiological, biochemical, and neuroanatomical experiments. If lateral habenula projection fibers reach and terminate within the dorsal raphe nucleus, then this would support the physiological data from this laboratory that this is a monosynaptic pathway. The projection has been examined at the light microscopic level, making use of both anterograde and retrograde tract tracing techniques, principally wheat germ agglutinin-horseradish peroxidase conjugate (WGA-HRP) processed with the tetramethyl benzidine (TMB) reaction. Pressure injections of 0.2-2% WGA-HRP, in saline and volumes of 30-150nl, were made in the lateral habenula of male Long-Evans rats. Postinjection survival times were 1-4 days.

Many of the present observations, based upon the anterograde transport of WGA-HRP, confirm those of Herkenham & Nauta (1979). Lateral habenula injections produced dense anterograde labeling along the U-shaped trajectory, seen in sagittal section, of its projection to dorsal raphe. Fibers in the central 3/5 of the fasciculus retroflexus, not part of the lateral habenular efferent system, are not labeled by spread of even 2% WGA-HRP ventrally into the fasciculus. Labeled fibers turn first caudal, then dorsal, parallel to the decussation of the superior cerebellar peduncle. Dense fiber labeling continues to the level of the medial longitudinal fasciculus (MLF), rostral to and distinct from the field of retrogradely labeled median raphe neurons. Dorsal to the MLF, the density of labeling diminishes considerably. Still, labeled fibers and terminals can be seen sparsely distributed throughout the caudal half of the nucleus. The fibers coalesce to form an oblique band of terminal labeling lying along the border of dorsal raphe with the dorsal tegmental nucleus, dorsal to the caudal portion of dorsal raphe. The rostral half of dorsal raphe is devoid of anterograde label. These findings contribute to the notion that lateral habenula-dorsal raphe fibers terminate as a shell arranged along the boundaries of the dorsal raphe nucleus. Dorsal raphe neurons with dendrites long enough to reach either the marginal shell or the rostral half of the nucleus could receive monosynaptic input from lateral habenula.

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22.10 THREE-DIMENSIONAL COMPUTER RECONSTRUCTION OF THE LOCUS COERULEUS: AGING AND DISEASE. B. Walker, W.K. Smith, D.J. Woodward, and D.C. German. Depts. of Physiol., Psychiat., & Cell Biol., Univ. of TX Health Sci Center, Dallas, TX 75235.

The nucleus locus coeruleus (LC) is the largest collection of norepinephrine (NE) containing neurons within the human brain. It is found in the dorsolateral pontine tegmentum. The LC is known to project extensively throughout the entire cerebral cortex, cerebellar cortex and spinal cord. Although its exact function remains unclear, it has been implicated in anxiety and fear as well as selective attention. Cell loss within the nucleus has been documented in aging, Parkinson's and Alzheimer's disease. The cells of the LC are primarily medium sized round, oval or multipolar cells with eccentric nuclei. The majority of these cells contain neuromelanin pigment which varies from a few granules to a mass which fills the entire cell. This pigment, a by-product of NE synthesis, begins to accumulate at about four years of age.

The purpose of the present experiment is to evaluate regional variations in cell loss in the LC seen with aging and disease using three-dimensional computer reconstruction. The brains are fixed in 10% neutral buffered formalin and cut in 50 μm sections, perpendicular to the long axis of the brainstem. Each pigmented cell is counted and every sixteenth section is entered into the computer. Comparisons are being made among varying section thicknesses and stains (cresyl violet, Schmorl's ferricyanide and tyrosine hydroxylase) across different age groups. Tyrosine hydroxylase immunohistochemistry is being used to examine the reliability of using neuromelanin as a NE cell marker. To date, six normal brains have been reconstructed. The ages range from 5 to 104 years. The 5 year old brain contained an estimated 48,800 total pigmented cells within the locus coeruleus, while the 104 year old brain had only 28,900 total pigmented cells. This represents a 40% diminution in cell number in the older brain. Although this appears to be a diffuse loss, brains from different age groups within these two extremes are being examined for any regional patterns of loss. Brains from patients with Alzheimer's and Parkinson's disease are also being examined for patterns of regional cell losses. Research supported by the Biological Humanics Foundation, Dallas Area Parkinsonism Society and grant NS-20030.

22.11 MESOTELENCEPHALIC PROJECTIONS IN MICE WITH DIFFERING NUMBERS OF MIDBRAIN DA NEURONS: AN HRP STUDY. L.A. Mattiace*, K. McDermott and D.C. German. Depts. of Physiol. & Psychiat., Univ. of Texas Health Science Center, Dallas, Texas 75235.

Mice with a larger number of midbrain DA cells are more sensitive to DA-mediated behaviors (i.e. locomotion, exploration, and catelepsy) than mice with fewer DA cells. Baker et al. (Proc. Natl. Acad. Sci., USA, 77: 4369, 1980) have reported a 20% difference in the number of tyrosine hydroxylase (TH)-containing neurons in the midbrain between BALB/c and CBA mice. Specifically, the BALB/c has more DA cells than the CBA in subregions of the substantia nigra and in the ventral tegmental area (German et al., Neurosci. Abs., 1150, 1983).

Using genetically different mouse strains, we attempted to determine: (1) whether there is a topographical organization of mesotelencephalic projections in the mouse that is similar to that found in the rat; (2) the location of cells in the midbrain which project to certain forebrain sites (i.e. striatum, nucleus accumbens, medial frontal cortex); and (3) whether there is a quantitative difference between the projections among the two mouse strains (i.e. do comparable injection sites label the same number of cells, in the same midbrain region, across mouse strains?). One percent HRP-WGA, a sensitive retrograde and orthograde labeling marker, was iontophoretically injected into target sites in both mouse strains. Mice were sacrificed 24 hours post-injection and processed by the TMB method (Mesulam, 1982). To date, caudate injections have been made in six BALB/c and two CBA mice. Computer reconstructions of labeled midbrain neurons in comparably injected BALB/c and CBA mice (injection site = approximately 1 X 1 mm, length X width) indicate that: (1) labeled cells were observed from the rostral to the caudal extent of the midbrain DA cellular complex in both strains; (2) there were 18% more labeled cells in the CBA than in the BALB/c (285 vs. 242 labeled cells); and (3) in the six BALB/c mice, a topographical organization was observed such that lateral injection sites resulted in the labeling of lateral midbrain neurons, whereas medial injection sites resulted in the labeling of medial midbrain neurons. These preliminary data suggest that although BALB/c mice have more DA cells than CBA mice, the CBA mouse may have a larger mesostriatal system than the BALB/c mouse. Supported by MH-30546, NS-20030 & Biological Humanics Foundation.

23.1 ANTICATECHOLAMINERGIC DRUGS REDUCE SEROTONIN (5HT) UPTAKE IN PLATELETS. E.E. Codd* and R.F. Walker* (SPON:L.L. Boyarsky) Sanders-Brown Research Center on Aging, U. Kentucky Medical Center, Lexington, KY 40536

The catecholamine hypothesis of affective illness states that norepinephrine metabolism is reduced in depression. The uptake of 5HT into platelets from depressed patients is also reduced. The purpose of this study was to determine if drug-induced depression of catecholamine levels is associated with reduced platelet 5HT uptake in rats thus testing the validity of using 5HT uptake as a physiological marker for depression. Male rats received two injections of 6-hydroxydopamine (10mg/kg) and α-methyl-p-tyrosine (200mg/kg). After 7 days they were decapitated and trunk blood was collected in 0.1 volume citrate-dextrose anticoagulant. Brains and spleen were removed, frozen and stored at -80C for subsequent radioenzymatic analysis of catecholamine content. Platelet rich plasma (PRP) was prepared by centrifugation of blood at 200xgx15min. Two consecutive centrifugations differentially segregated older and younger platelets, respectively. V_{max} and K_m for 5HT uptake was determined by incubating platelets from control and drug-treated rats with ^{3}H-5HT (0.1-2.0uM) for 30 seconds. Eadie-Hofstee analyses of the data showed that V_{max} was reduced in both pools of platelets from rats receiving anticatecholamine neuroleptics. However K_m was not affected by treatment. Catecholamine content of brain and spleen was reduced, while free 5HT was elevated in plasma from drug treated rats when compared with their controls. Coincubation of platelets with norepinephrine (10^{-7}M) increased 5HT uptake only in drug treated animals. The findings of this study suggest that reduced uptake of 5HT into platelets from depressed psychiatric patients is related to catecholamine deficits implied from pharmacologic studies. The results also suggest that platelet adrenoreceptors regulate the uptake 5HT into these structures. Perhaps the CNS transmits regulatory signals to platelets via sympathetic innervation of the vasculature. Support by NIH AG02867.

23.2

WITHDRAWN

23.3 CEREBROSPINAL FLUID NOREPINEPHRINE IS UNAFFECTED BY PERIPHERAL SYMPATHECTOMY E.R.Peskind*,M.A.Raskind*,C.W.Wilkinson*,J.B.Halter*(SPON:S. W.Bledsoe). Geriatric Research, Education and Clinical Center, VA Medical Center, Seattle WA 98108.

Norepinephrine (NE) does not cross the blood-brain barrier, yet a close correlation exists between cerebrospinal fluid (CSF) and plasma NE levels. It has been speculated that peripheral sympathetic fibers innervating the cerebral and spinal cord vasculature may enter the subarachnoid space and significantly contribute to CSF NE levels.

We investigated the possible contribution of peripheral sources of NE to CSF NE by measuring plasma and CSF NE in rats in each of three groups: 1)chemical sympathectomy (SYMPX) by neonatal injections of guanethidine, 2)chemical SYMPX plus adrenal medullectomy (ADR MEDX), and 3)saline-injected sham-operated controls. At 12 weeks of age, CSF was collected by cisternal puncture. Plasma, heart, adrenal, hypothalamus, and cerebral cortex samples were collected following decapitation. CSF and plasma NE levels were determined by a sensitive radioenzymatic assay. Tissue levels were determined by high performance liquid chromatography. Results were as follows:

NOREPINEPHRINE LEVELS ($\bar{X}$±SEM; ONE-WAY ANOVA)

	CSF (pg/ml)	plasma (pg/ml)	heart (ng/g)	hypothal (ng/g)
SYMPX (N=13)	1410±56	1425±128	21±6	1456±70
SYMPX/ADR MEDX (N=12)	1373±55	518±55	34±12	1288±66
CONTROLS (N=16)	1317±55	3119±302	917±11	1473±63
F-statistic (df=2,38)	0.74	37.77	523.02	2.23
p	NS	<.001	<.001	NS

Adrenal NE levels were significantly lower (F=147.26, p<.001) in SYMPX/ADR MEDX rats (4±2) than in either SYMPX (279±15) or CONTROL (210±11) rats. Cerebral cortex NE levels in SYMPX (238±8), SYMPX/ADR MEDX (238±10) and control rats (243±7) did not differ significantly (F=0.13,NS).

Chemical SYMPX effectively reduced heart and plasma NE levels. ADR MEDX lowered adrenal NE levels and further reduced plasma levels. Despite the marked reduction of peripheral tissue and plasma NE by SYMPX and ADR MEDX, CSF NE was clearly unaffected; hypothalamic and cerebral cortical NE levels were also unaffected by these treatments. These data establish the independence of NE in central and peripheral compartments and support the validity of CSF NE as a measure of central noradrenergic activity.

23.4 EFFECT OF ACUTE RESTRAINT STRESS ON DOPAMINE AND SEROTONIN TURNOVER IN NIGROSTRIATAL AND MESOLIMBIC DOPAMINERGIC SYSTEMS. J. Culman*, G.C.Chiueh, M.Koulu* and I.J.Kopin (SPON: C.M.Woodbury). Lab.of Clinical Science, NIMH and NINCDS, Bethesda, MD 20205.

Using HPLC and electrochemical detection to measure amine metabolites and accumulation of dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5-HTP) in *micropunches* of brain tissue after administration of m-hydroxybenzylhydrazine to inhibit decarboxylation, turnover rates of dopamine (DA) and serotonin (5-HT) were studied in the nigrostriatal and mesolimbic systems during acute immobilization (IMO) stress.

In the ventrotegmental area (A10 area), acute IMO stress caused an increase of homovanillic acid (HVA) and 5-hydroxyindolacetic acid (HIAA) concentrations as well as an increase in the accumulation of DOPA and 5-HTP after decarboxylase inhibition. In the substantia nigra, however, only the HIAA level was increased. IMO stress increased the concentrations of dihydroxyphenylacetic acid, HVA and HIAA in the caudate nucleus and nucleus accumbens without changing the concentration of DA. In both areas acute stress increased the accumulation of 5-HTP after decarboxylase inhibition but DOPA accumulation was enhanced only in the caudate nucleus. In the dorsal raphe nucleus, where 5-HT cell bodies are located, acute IMO markedly accelerated 5-HT synthesis and degradation as documented by increased 5-HTP and 5-HT levels and 100 % increase of HIAA level after 2 h of IMO.

The present results demonstrate the increased turnover of DA and 5-HT in the caudate and accumbens nuclei, presumably due to an increase in the release of these monoamines from the nerve terminals during acute stress. The stress-increased DA turnover in the A10 area supports previous findings of mesolimbic activation during stress. Enhanced HIAA levels in all examined areas and increased 5-HTP accumulation after decarboxylase inhibition in nucleus accumbens, caudate nucleus and A10 area may suggest a role for 5-HT in the regulation of the activity of mesolimbic and nigrostriatal dopaminergic systems.

23.5 COMPARISON OF MORPHINE-INDUCED EFFECTS ON DOPAMINE AND NON-DOPAMINE NEURONS IN THE RAT VENTRAL TEGMENTAL AREA.
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We have previously reported (Gysling and Wang, Brain Res. 277, 1983, 119-127) that intravenous administration of morphine (MOR) produced a marked increase in spontaneous firing of dopamine (DA)-containing neurons in the rat ventral tegmental area (VTA or A10). Naloxone (NAL) reversed this MOR-induced activation of DA activity. Microiontophoretic application of MOR or enkephalin analogues also significantly increased the spontaneous activity of A10 DA cells; however, this effect was not consistently blocked by either iontophoretic or intravenous NAL. By contrast, both intravenous and iontophoretically administered MOR markedly suppressed the firing rate of non-DA cells found in the vicinity of A10 DA cells, and this effect was completely reversed by NAL. To explore the possibility that MOR-induced activation of A10 DA cells could be mediated indirectly by non-DA cells, the cumulative log dose-response curves of MOR on both DA and non-DA in the VTA were compared.

In chloral hydrate anesthetized rats, intravenous MOR increased the firing rate of 11 of 17 A10 DA cells. Among these 11 DA cells, 3 cells began to increase their firing rate at cumulative dose of 3 to 7 µg/kg. At the cumulative doses of 0.5 mg/kg the averaged increase of firing of A10 DA neurons was 20%. Six of 17 A10 DA cells which decreased their discharge rate during MOR administration showed signs of depolarization inactivation, i.e. decreased amplitude of action potentials and increased bursting firing pattern. In general, MOR significantly decreased the firing rate of non-DA cells in the VTA. The ID50 was 0.511 mg/kg. However, in many cases the amplitude of action potential of these non-DA cells was also reduced, indicating that reduction of the spontaneous firing rate by MOR could be the result of depolarization inactivation. Indeed, iontophoretic application of MOR in some cases induced excitation followed by depolarization inactivation which could be reversed by concurrent iontophoresis of GABA or NAL. Therefore, it appears that MOR can activate both DA and non-DA cells in the VTA. Experiments are presently in progress to test more directly whether the MOR-induced excitation of DA firing activity is indirectly mediated by non-DA neurons in the VTA. (Supported by USPHS grants MH-34424, MH-38794, and a research scientist development award type II MH-00378.)

23.6 PREVENTION OF THE DOPAMINERGIC NEUROTOXICITY OF 1-METHYL-4-PHENYL-1,2,5,6-TETRAHYDROPYRIDINE (MPTP) IN MICE BY MONOAMINE OXIDASE INHIBITION.
R.E. Heikkila, A. Hess and R.C. Duvoisin. Department of Neurology and Anatomy, UMDNJ, Rutgers Medical School, Piscataway, NJ 08854.

MPTP causes a severe degeneration of the dopaminergic nigrostriatal pathway in several animal species including humans, monkeys and mice. Changes observed after MPTP administration include marked decrements in the neostriatal content of dopamine and its major metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Other changes include a greatly diminished capacity of neostriatal synaptosomal preparations to take up ^{3}H-dopamine as well as a marked loss of nerve cells in the zona compacta of the substantia nigra. In contrast, there are no pronounced losses of serotonin and several amino acids in the neostriatum, or of dopamine and its metabolites in other brain areas in MPTP-treated animals. It has been suggested that the oxidative metabolism of MPTP to a pyridinium metabolite is a critical feature in the neurotoxic process (Markey et al., 1984). It has furthermore been discovered in rat brain preparations in vitro, that the monoamine oxidase inhibitor (MAOI) pargyline was capable of preventing the formation of the pyridinium metabolite from MPTP (Castagnoli et al., 1984). This observation suggested that monoamine oxidase (MAO) might be responsible for the oxidative metabolism of MPTP in vivo.

We now report that pargyline and two other MAOI, nialamide and tranylcypromine, administered to mice prior to MPTP, protected against the dopaminergic neurotoxicity of MPTP. Mice treated with these MAOI prior to MPTP, exhibited no decrement in their neostriatal content of dopamine. All of these drugs are inhibitors of both MAO-A and MAO-B. In other experiments, deprenil a relatively specific inhibitor of MAO-B was completely protective against the dopaminergic neurotoxicity of MPTP, while clorgyline, a relatively specific inhibitor of MAO-A, afforded no protection or was marginally protective. Our data are consistent with the premise that MAO and specifically MAO-B, play a crucial role in MPTP-induced degeneration of the neostriatal dopaminergic neuronal system in mice.

23.7 SUPERSENSITIVITY OF PRE- AND POSTSYNAPTIC DA RECEPTORS AND THE OCCURRENCE OF (CROSS) TOLERANCE FOR DA SYNTHESIS AFTER CHRONIC HALOPERIDOL AND CLOZAPINE TREATMENT.
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Chronic administration of neuroleptics produces a variety of alterations in the dopamine (DA) system. Among these are increases in the DA receptor density and a decreased effect of these drugs on DA synthesis and turnover. Atypical neuroleptics, such as clozapine have been claimed to differ in the induction of these adaptational changes of the DA system.

We have studied the effect in rats of a 3 week administration of haloperidol (HAL, 2 mg/kg/day in the drinking water) and a behaviourally equipotent dose of clozapine (CLO, 60 mg/kg p.o. twice daily) on several neurochemical parameters. The determinations were made 5 days after the last drug administration.

Both chronic HAL and CLO treatment resulted in an attenuated DA turnover, as revealed by a decreased striatal HVA content (44% and 64% of control resp.). An acute challenge with apomorphine (APO, 2 mg/kg i.p.) further lowered the striatal HVA level to 13% in chronic HAL treated animals, compared to 37% for control animals.

In contrast to the decreased basal DA turnover, the tyrosinehydroxylase (TH) activity was not affected by chronic pretreatment with either HAL or CLO. However, an acute challenge with either HAL or CLO resulted in a smaller increase in TH activity in chronic HAL treated animals (ED_{50} = 1,1 and 160 mg/kg p.o. resp.) than in control animals (ED_{50} = 0.3 and 62 mg/kg p.o. resp.) indicating (cross) tolerance.

Chronic HAL treatment did not affect the K^+-stimulated release of either ^{3}H-DA or ^{3}H-Ach from striatal slices in vitro. However in chronic HAL treated animals we did find a stronger inhibitory effect of 10 nM APO on the ^{3}H-DA release (48% vs 34% inhibition in control) and on the ^{3}H-Ach release (42% vs 28% inhibition in control), indicating supersensitivity of both pre- and postsynaptic D_2 receptors after chronic HAL treatment.

In conclusion, CLO could not be differentiated from the classic neuroleptic HAL as suggested by Guidotti et al (Life Sciences, 23: 501, 1978). Both compounds induced adaptational changes of DA turnover and synthesis. Moreover, in contrast to Arbilla et al (Br.J.Pharmac 79: 16, 1983) chronic HAL treatment induced supersensitivity of both pre- and postsynaptic D_2 receptors.

23.8 FLUNARAZINE LIMITS STRIATAL DOPAMINE RELEASE INDUCED BY HYPOXIA-ISCHEMIA.
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Unilateral carotid artery ligation (UCL), followed by exposure to moderate hypoxia (8% 02), in 7 day old rat pups is a useful model of perinatal hypoxic-ischemic neuronal injury. This preparation yields reproducible acute biochemical changes as well as chronic histologic injury in forebrain (primarily striatum) on the side of UCL. We recently described a time dependent threshold for hypoxic exposure that leads to acute changes in striatal dopamine (D) turnover: on the side of UCL, levels of homovanillic acid (HVA), the major extra-neuronal metabolite of D, rise predictably (by > 60%) after 1 1/2 hrs of 8% 02, coincident with D depletion. The threshold parallels that for production of gross morphological changes in pups raised to maturity and suggests that acute D release is associated with the events that lead to neuronal injury (Ann. Neurol., in press).

We examined the effects of 3 potentially neuroprotective drugs on striatal D metabolism. D & HVA were measured in striatal extracts using HPLC-EC. HVA/D ratio was used as a measure of D turnover; in 20 controls HVA/D = 0.09 ±.01. In each experiment, results from groups of ligates with and without drug treatment (Rx) before hypoxia were compared.

Flunarizine (Flu), a diphenylalkylamine calcium (Ca++) channel blocker which is concentrated in brain after systemic administration, has neuroprotective effects in several other hypoxia models. Flu was tested orally (p.o.) and intra-peritoneally (i.p.). Pre-Rx with 20 and 30 mg/kg p.o. limited D depletion and HVA elevation (by 40%) on the side of ligation. 10 mg/kg i.p. led to a 35% decrease in HVA accumulation (n=6, $p<.05$) and HVA/D was 0.7 vs 0.9 in untreated pups. 20 mg/kg Flu, i.p., divided into 2 doses, consistently attenuated D turnover. With Flu, HVA/D was 0.36 vs 0.77 in untreated ligates (n=16, $p<.025$). In contrast, verapamil, a phenylalkylamine Ca++ channel blocker which enters brain poorly, did not change HVA/D and pups appeared ill (1.5 & 3 mg/kg). Indomethacin, a prostaglandin synthesis inhibitor protective in some models of ischemic tissue injury, also did not alter the striatal D response to hypoxia-ischemia (3,10,& 30 mg/kg i.p.). Flu attenuates hypoxia-ischemia induced D release from striatal nerve terminals in this model, possibly by improving regional cerebral blood flow or by limiting Ca++ entry into ischemic dopaminergic neurons.

23.9 REMOTE FUNCTIONAL DEPRESSION OF GLUCOSE METABOLISM AFTER CORTICAL INJURY IS ALTERED BY AMPHETAMINE AND HALOPERIDOL. D.M.Feeney, M.A.Rodriguez*, D.A.Hovda, and M.G.Boyeson, Departments of Physiology, Psychology and Electrical Engineering, University of New Mexico, Albuquerque, New Mexico 87131.

Metabolism of ^{14}C-2-deoxyglucose (2DG) has been reported to be depressed in areas remote from the the primary site of injury (Hayes, et al. Science 1984; Reinstein, et al., Brain Res., 1979). But whether this remote functional depression (RFD) is simply a correlational phenomena or contributes to symptoms is unknown.

After unilateral sensorimotor cortex injury in rats (Feeney et al., Science, 1982) amphetamine (AMP) accelerates and haloperidol (HAL) retards recovery of locomotor ability. To determine whether these drug treatments which affect behavioral recovery also alter 2DG metabolism in a parallel fashion, computer enhanced autoradiography was conducted. Saline (SAL) or AMP (2 mg/kg, i.p.) was administered to normal uninjured rats or rats with sensorimotor cortex ablation 24 h after surgery. Twenty four hours after drug or SAL treatment the animals were administered 50 uci of 2DG, sacrificed and brain sections analyzed for glucose metabolism using standard autoradiographic procedures. Images were enhanced and quantified using a VAX 11/788 computer and COMTAL image processing system. The results indicated a widespread depression of glucose utilization as compared to normal controls. Areas affected include the ipsilateral and contralateral cortices and the brain stem especially the ipsilateral red nucleus. Quantitative measures of other areas are currently under investigation. Animals treated with AMP showed an alleviation of this RFD and those treated with HAL showed a worsening of RFD compared to saline controls. Amphetamine accelerates behavioral recovery and alleviates 2DG-RFD. Haloperidol retards behavioral recovery and worsens RFD. This suggests a causal relationship between RFD and behavioral symptoms after brain injury. This could be one mechanism for von Monakow's concept of diaschisis. Supported by a grant from Merck Sharp & Dohme Res. Labs.

23.10 IN VITRO MODELS OF MPTP TOXICITY AND PHARMACOLOGY: METABOLIC GENERATION OF A TOXIC METABOLITE AND HIGH AFFINITY BINDING TO BRAIN HOMOGENATES. J.N. Johannessen, S.P. Markey*, L. Kelner*, and M. Shih*. Laboratory of Clinical Science, NIMH, Bethesda, MD 20205

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes irreversible parkinsonism in humans and non-human primates. The syndrome results from the selective toxic action of MPTP on the nigrostriatal dopamine cells, sparing the mesolimbic dopamine cells. Based on results of in vivo experiments we have proposed that the toxic action of MPTP is dependent on its metabolic conversion to 1-methyl-4-phenylpyridine (MPP+). Central to this theory is the ability of primate nervous tissue to convert MPTP to MPP+ in vitro.

Homogenates of monkey brain were incubated with MPTP under various conditions. The presence of MPP+ in the homogenate was confirmed by two methods. 1) HPLC analysis of incubation mixtures containing ^{3}H-labeled MPTP revealed a peak coincident with MPP+ which grew with longer incubation times, but was absent if the homogenate was boiled prior to incubation with MPTP. Reduction of this peak with $NaBH_4$ yielded a hexane extractable material which coeluted with MPTP, the reduction product of MPP+. 2). Concentrated ethanol extracts of tissue homogenate were subjected to FAB-MS. Samples incubated with MPTP displayed a prominent peak at m/z 170, the molecular ion for MPP+. If incubated with equal amounts of MPTP and $^{2}H_2$ MPTP, a doublet at m/z 170 and 172 resulted. No such peaks were seen in control homogenates or boiled homogenates incubated with MPTP. Quantitation of MPP+ formed by brain homogenates after incubation with 1 mM MPTP demonstrated a linear increase in the amount of MPP+ for periods up to 1 hour. The enzyme velocity was calculated to be 6.8 pmoles MPP+ formed/mg tissue/min.

While rodents do not share the primate's high sensitivity to the toxic effects of MPTP, they do share acute pharmacological effects remeniscent of sympathetic discharge. In considering that this effect may be receptor mediated, we examined the binding of MPTP to rat and monkey brain. Homogenates were incubated with varying concentrations of ^{3}H-labeled MPTP in the presence and absence of a high concentration of unlabeled MPTP. A high affinity site was detected which had a K_D of 9.3 nM. Whether this site represents a pharmacologic effector or is indicative of binding to the MPTP converting enzyme is as yet uncertain.

23.11 THE EFFECT OF POSTNATAL HYPOXIA ON BIOGENIC AMINES IN THE BRAINSTEM NUCLEI OF NEONATAL AND OLDER RABBITS. M. Colleen McNamara, J. Gingras-Leatherman and E.E. Lawson*. Dept. of Pediatrics, Univ. of North Carolina, Chapel Hill, NC 27514

Previous studies in our laboratory have demonstrated concentrations of the biogenic amines dopamine (DA), norepinephrine (NE) and serotonin (5HT), in brainstem nuclei of neonatal rabbits are reduced when compared with adults (Dev. Brain Res., 7:181, 1983). In addition younger animals have lower turnover rates (TOR) of NE and 5HT indicating a longer time to replenish these amines (Brain Res. in press). Oxygen is used in the mammal brain for energy production and is a substrate for the rate limiting enzymes of DA and NE (tyrosine hydroxylase) and 5HT (tryptophan hydroxylase) synthesis pathways (Davis, Brain Res., 80:237, 1974). The existence of low amine levels, and lower TOR in the neonate suggests that these animals may be at risk when exposed to factors which lower O_2 availability. We investigated age-related differences in sensitivity to hypoxia within nuclei which give rise to the major neurotransmitter pathways.

Rabbits (3 d/o and 21 d/o) were confined to thermal water-jacketed chambers. They were exposed to 6 h breathing 21% O_2 (grp I), 10m 10% O_2 every .5 h for 6 h (grp II), 2 h 10% O_2 followed by 4 h 21% O_2 (grp III) or 4 h 21% O_2 followed by 2 h 10% O_2 (grp IV). Brainstem nuclei: substantia nigra (SN), dorsal raphe (dr), locus coeruleus (LC) and n. reticularis pontis oralis (rpo) were sampled according to the micropunch technique of Palkovits (Brain Res., 80:237, 1974). Using sensitive radioenzymatic assays, the concentrations of DA, NE, and 5HT were measured in each of the nuclei.

In general, DA was not affected by hypoxia. The changes in NE were inconsistent. The data for 5HT is presented below. (% change from control values). * = $p < .01$

	3 d/o: SN	dr	LC	rpo	21 d/o: SN	dr	LC	rpo
Grp II	-18	-84*	-54	-39	73	64*	50	11
Grp III	-64*	-64*	-76*	-58	351*	36*	28*	209
Grp IV	-88*	-94*	-88*	-79*	275*	95*	113*	141*

n = 6 for both age groups for each condition

Acute hypoxia in the young animals, resulted in decreased 5HT concentration in all nuclei sampled. The findings in group III indicate a failure to recover from these effects. Since 5HT has an influential role in several central control systems: blood pressure, respiration, thermoregulation, nociceptionception, as well as sleep and arousal states, our data suggest a mechanism for the increased susceptibility of newborns to hypoxia.

23.12 MULTIPLE DOSES OF D-AMPHETAMINE ACCELERATES RECOVERY OF LOCOMOTION FOLLOWING BILATERAL FRONTAL CORTEX INJURY IN CAT. R.L.Sutton* D.A.Hovda and D.M.Feeney (SPON: R.A.Fox). Depts. of Psychology and Physiology, Univ. of New Mexico, Albuquerque, NM 87131.

Catecholaminergic agents combined with locomotor experience (EXP) markedly influence recovery of beam-walking locomotor ability following brain injury in rat and cat (Feeney, et al. Sci.,1982,217,855-857; Hovda and Feeney, Brain Res.,1984,298,358-361). Administration of d-amphetamine (AMP) and EXP after unilateral sensorimotor cortex ablation produced an enduring acceleration of beam-walking ability.

One plausible explanation for the drug-accelerated recovery after unilateral injury is that homologous areas contralateral to the injury or tissue adjacent to sensorimotor cortex mediate the drug-induced recovery. To test this hypothesis, cats underwent extensive bilateral frontal cortex ablations after baseline testing of their ability to traverse a 1.1 m X 5.6 cm beam at a height of 1.2 m. Following surgery, beam-walking ability was tested every other day by two raters (one blind to drug treatment) beginning on day 6 post-injury and continuing until day 30 postinjury. The animals were randomly assigned to two groups after surgery and received multiple doses of SAL or AMP (5mg/kg) i.p. on days 12, 16 and 20. Following drug administration, animals were tested on the beam at 1, 2, 3, 6 and 24 h postinjection, thus receiving beamwalking EXP while intoxicated. It was found that multiple doses of AMP accelerated the rate of locomotor recovery in bilaterally injured cats compared to SAL controls. These findings indicate that, in the cat, neither the contralateral homologous cortex nor areas adjacent to sensorimotor cortex are necessary for drug promotion of the recovery of beam-walking ability. We propose that this accelerated recovery of locomotor ability by AMP is due to an alleviation of a transient remote functional depression which occurs in intact, subcortical brain areas following injury to the cerebral cortex (See Feeney et al. Neurosci. Abst., 1984). Supported by Merck Sharp and Dohme Research Laboratories.

23.13 THE ROLE OF NOREPINEPHRINE IN RECOVERY FROM BRAIN INJURY. M.G. Boyeson, and D.M. Feeney. Dept. of Psych., Univ. of New Mexico, Albu., N.M. 87131.

The following experiments (EXP) were conducted to study the biochemical mechanisms involved in recovery of beam walking ability in rats unilaterally ablated in the right sensorimotor (RS) or left cerebellar cortex (LCB). In EXP 1, a single intraventricular infusion of either norepinephrine (NE) or dopamine was given to rats 24h following injury to RS cortex to test which of these neurotransmitters were involved in the amphetamine (AMP)-induced recovery of beam walking ability reported previously (Feeney et al., Sci., 217:855,1982). Only NE was found to mimick the AMP-induced recovery function. In further support of the involvement of the NE system in maintaining recovery was finding that phenoxybenzamine (PBZ; 10mg/kg) reinstated the beam walking deficit in recovered rats. In EXP 2, rats trained on the beam received a single ip. dose of AMP (2mg/kg) or saline (SAL) 24h following a RS cortex ablation, and levels of NE and its metabolite MOPEG were assayed at 2,6, or 18 days postinjury in the locus ceruleus and cerebellum. At 2d postinjury SAL-treated rats had a low turnover rate of NE compared to controls with a unilateral lesion of the caudate nucleus. At the same time postinjury, AMP-treated rats had a significantly higher turnover of NE, suggesting that AMP was acting to remove the NE depression. Behaviorally, AMP-treated rats were significantly improved on the beam at 2d postinjury. No biochemical differences were found at 6d postinjury. At 18d postinjury, the turnover of NE in the LCB was 8X higher than in SAL controls, suggesting that the LCB was compensating for the ablated RS cortex in AMP-treated rats. In EXP 3, rats trained on the beam received a LCB cortex ablation and were tested to 80d postinjury. Starting at 24h postinjury, rats received either SAL, AMP, haloperidol (HAL; .4mg/kg), or AMP& HAL injected every 5d through 30d postinjury. All drugs retarded recovery on the beam compared to SAL controls. Neither PBZ nor propranalol (20mg/kg) were effective in reinstating the beam deficit in the few rats that did recover. In light of the biochemical results of EXP 2, and the poor recovery on the beam after LCB injury, the results suggest that the cerebellum is critical to recovery of beam walking ability. The results also provide a mechanism through which a NE "diaschisis" could operate and influence recovery from brain injury. (Supported by a grant from Pennwalt Corp.)

CATECHOLAMINES: PHYSIOLOGICAL EFFECTS II

24.1 EVIDENCE FOR D-1 RECEPTOR MODULATION OF NEUROTRANSMISSION IN THE SUBSTANTIA NIGRA ZONA RETICULATA. R.T. Matthews and D.C. German, Dept. of Anatomy, Texas A&M Univ. Sch. of Med., College Station, TX 77843, Dept. of Physiol., Univ. of TX Health Sci Center, Dallas, TX 75235.

The substantia nigra zona reticulata (ZR) is an important motor output nucleus of the midbrain. The ZR contains dendrites of the dopamine (DA) containing neurons of the substantia nigra zona compacta (ZC). Evidence suggests that DA released from these dendrites modulates ZR output. For example, microiontophoresis of DA excites ZR neurons (Ruffieux & Schultz, Nature 285:240, 1980) and attenuates GABA inhibition of ZR neuronal impulse flow (Waszcak & Walters, Science 220:218, 1983). D-1 receptors and the associated protein, DARPP32, have been found in the ZR (Ouimet et al., J. Neurosci., 4:111, 1984) whereas a receptor binding site of the D-2 type has been found in the ZC (Seeman, Pharmacol. Rev., 32:229, 1981). Experiments were undertaken to test whether a D-1 type of receptor may mediate these effects of DA on ZR neurons.

It was found that: (1) microiontophoresis of the D-2 receptor agonist LY141865, inhibited spontaneous activity of single substantia nigra DA neurons and increased activity of single ZR neurons (and was slightly more potent on DA neurons); (2) the D-1 receptor agonist SKF38393-A was similar to LY141865 except it was slightly more potent on ZR neurons; (3) cis-flupentixol blocked the effects of microiontophoresed DA on both DA and ZR neurons at low doses (0.5-1.0 mg/kg, i.v.) without affecting inhibition of these neurons by iontophoresed GABA or excitation by glutamate; (4) haloperidol blocked DA effects on DA neurons at a low dose (0.1 mg/kg, i.v.) without affecting GABA inhibition; (5) haloperidol at high doses (1.0 mg/kg, i.v.) did not consistently block DA excitation of ZR neurons; (6) DA attenuated the ability of GABA to inhibit, and glutamate to excite ZR neurons; and (7) cis-flupentixol, at a low dose, blocked DA attenuation of glutamate excitation of ZR neurons.

These data confirm the findings of others that microiontophoresed DA excites ZR neurons and acts as a neuromodulator. The effects of agonists and antagonists with relatively specific affinities for D-1 or D-2 receptors suggests that inhibition of DA neurons by DA is mediated by a D-2 type receptor and excitation of ZR neurons is mediated by D-1 type receptor. Research supported by grant MH-30546.

24.2 BURST FIRING OF NIGRAL DOPAMINERGIC NEURONS IN FREELY MOVING RATS. A.S. Freeman and B.S. Bunney. Depts. Psychiatry & Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510.

In the past decade, many studies have investigated the firing characteristics of central dopamine (DA) neurons in anesthetized or immobilized animals. We have reported that the electrophysiological characteristics of putative DA cells in the substantia nigra (SN) zona compacta (ZC) of freely moving rats are indistinguishable from midbrain neurons conclusively identified as dopaminergic, including their ability to fire in both single spike and bursting modes. In addition to firing rate, the pattern of firing may be a critical factor in determining the manner in which these neurons influence their target cells. For this reason, we have begun to analyze the firing patterns of DA cells in the awake, unrestrained rat.

A stainless steel well was implanted on the skull over the SN of male rats and a microdrive assembly inserted into the well. Glass-coated tungston electrodes recorded putative DA unit activity (signal:noise > 10:1). Neuronal activity was taped for off-line computer analysis. Individual putative DA cells (n=21) fired both single spikes and bursts of up to 20 spikes/burst. On the average, they fired 44% of their spikes in bursts with an average of 3.2 spikes/burst. Within bursts, the mean (+S.D.) interspike interval was 71+15 msec. The shortest intraburst interspike interval was consistently found between the first 2 spikes of a burst (47+9 msec). Following each burst, there ensued a brief period (331+108 msec) in which no neuronal discharge occurred. These characteristics are similar to those we have observed in anesthetized and immobilized rats. Four putative ZC DA cells were observed to be electrically coupled to at least one other neuron with similar characteristics. As has been suggested, coupling may provide a mechanism for bursting and, through synchronization of DA release, a means by which the influence of DA on postsynaptic cells can be enhanced.

Thus, in addition to the previously reported similarities between putative DA neurons of freely moving rats and identified DA cells of anesthetized and immobilized rats, DA neurons of the freely moving rat also burst fire with similar characteristics. Furthermore, investigation of possible differences in the degree of electrical coupling between DA cells in these preparations may reveal differences in the way in which DA cells interact with each other as a function of the physiological state of the animal. Supported by Grants MH-08987, MH-25642, MH-28849 and the State of Connecticut.

24.3 4-AMINO PYRIDINE REDUCES EXCITABILITY OF DOPAMINE TERMINALS. S.F.Sawyer*, J.M.Tepper, S.J.Young* and P.M.Groves. Dept. Psychiatry, Univ. Calif. San Diego, La Jolla, CA 92093.

Increased activation of autoreceptors on dopaminergic terminals in rat neostriatum by local infusion of dopamine agonists, or by spontaneous or stimulus-induced increases in impulse flow, decreases terminal excitability in vivo. These effects can be blocked by local infusion of dopaminergic antagonists. Furthermore, dopamine terminals appear to be under tonic autoinhibitory control, mediated by endogenously released dopamine, since infusions of dopamine receptor blockers alone increase terminal excitability (Tepper et al., Soc. Neurosci. Abstr. 8:791, 1982). We have suggested that the decreases in excitability result from an autoreceptor-mediated hyperpolarization of the terminal regions, consistent with the potassium-dependent hyperpolarizing action of autoreceptor agonists at monoamine cell bodies. Since the ionic basis of autoreceptor-mediated decreases in terminal excitability is unknown, we examined the effect of 4-amino pyridine (4-AP), which blocks voltage-dependent potassium channels, on dopaminergic terminal excitability.

Antidromic responses to neostriatal stimulation were recorded from nigral dopamine neurons in urethane anesthetized rats. Stimulus current just sufficient to evoke 100% antidromic responding (threshold) was determined before and after infusions of 0.31 µl of 4-AP (10 or 100 µM) into the stimulation site. Changes in threshold of less than 10% were considered as no effect. Threshold was increased for five cells (+14.8±1.5%) and unchanged in two (-3.9±3.9%). Since 4-AP decreased terminal excitability, these results argue against a direct effect of autoreceptor stimulation on 4-AP sensitive potassium channels. Instead, 4-AP may decrease excitability by increasing impulse dependent dopamine release. To examine this possibility, 9 animals were treated with 250 mg/kg alpha-methyl-p-tyrosine (AMpT) 6-24 hr. prior to recording, to deplete dopamine. In AMpT animals, striatal infusions of 4-AP increased threshold in 2 cases (+28.6±14.3%), decreased threshold in 1 case (-11.8%), and had no effect in the remaining 6 cases (+1.8±2.0%).

These data suggest that 4-AP sensitive potassium channels are probably not involved in the autoreceptor-mediated hyperpolarization of dopamine nerve terminals, and that in striatal dopaminergic terminals, as in other central and peripheral nerve terminals, 4-AP leads to increased impulse-dependent transmitter release.

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24.4 DOPAMINE-CONTAINING NEURONS IN A SLICE OF THE DORSAL DIENCEPHALIC CONDUCTION SYSTEM. G.R. Christoph* and K.S.Wilcox* (Spon: J.E. Carnahan). Central Research & Devel. Dept., E.I. du Pont de Nemours & Co., Inc., Wilmington, DE 19898.

The dorsal diencephalic conduction system primarily consists of the afferent and efferent connections of the habenula, and this system is one of two major routes linking limbic forebrain with midbrain structures. Habenular efferents project caudally via the fasciculus retroflexus (FR) and a subset of these efferents terminate in the ventral tegmental area (VTA) where perikarya of dopamine-containing neurons (DA-cells) are located. Electrophysiological studies in vivo have shown that electrical stimulation of habenular neurons inhibits most (91%) DA-cells in the VTA (Christoph, G.R. et al., 1983, Neurosci. Abst., 9 #292.10). This orthodromic inhibition of DA-cells could be studied in more detail with the advantages of in vitro methods. The configuration of the habenula, FR, and VTA permits these structures to be included in a 6 mm x 3 mm x 0.4 mm saggital slice of rat brain. Adult rats were decapitated and the brains were removed and mounted on a vibratome stage immersed in 5°C oxygenated medium composed of 124 mM NaCl, 5 mM KCl, 1.25 mM KH_2PO_4, 2.5 mM $MgSO_4$, 2.5 mM $CaCl_2$, 26 mM $NaHCO_3$, 2 mM ascorbic acid, 2mM urea, and 10 mM glucose. One slice per brain contained the habenula, FR, and VTA. The slice was transferred to a chamber (Biela Eng. Co.) where it was perfused with 32-34°C medium at 0.5-1.0 ml/min. Electrical stimulation (50-200 µA, 0.2 msec) of the habenula evoked gross fiber responses along the length of the FR consisting of two components with different conduction velocities (0.51 and 1.05 m/sec). Spontaneously active single neurons in the VTA were recorded extracellularly with glass micropipets (2 M NaCl, 4-6 megohms). Most neurons had action potential durations <1.5 msec with firing rates of 10-20 Hz. A subgroup of neurons (N=15) in the VTA were distinguished by their longer duration action potentials (>2.2 msec) and slow, steady discharge rate (2-5 Hz). The characteristics of this group correspond to those of DA-cells in vivo, except that the firing pattern was much more regular as has been reported for comparisons of nigral DA-cells in vivo and in vitro (Sanghera, M.K. et al. 1983, Neurosci.Abst. 9 #292.3). The firing rate of these neurons was suppressed 50-90% by perfusion with medium containing apomorphine (5-50 µM), and activity returned to baseline after drug wash-out. Two cells (13%) were inhibited by habenular stimulation, and although this is much lower than the incidence of inhibition in vivo, these results demonstrate the feasibility of using the slice to study the synaptic control of DA-cells in the VTA.

24.5 CONTRASTING EFFECTS OF AMPHETAMINE AND 4-OH AMPHETAMINE ON SPONTANEOUS ACTIVITY OF CELLS IN THE LOCUS COERULEUS. R. N. Holdefer, M. Sopko* and R. A. Jensen. Developmental Biopsychology Lab., Southern Illinois University, Carbondale, IL 62901

Post-trial peripheral injections of both d-amphetamine and 4-OH amphetamine enhance the retention of an inhibitory avoidance task despite the limited capacity of 4-OH amphetamine to cross the blood-brain barrier. Much evidence suggests that central noradrenergic pathways may be importantly involved in the capacity of these amphetamines, as well as other drug treatments, to enhance retention performance. This evidence, and the well-known finding that peripherally administered d-amphetamine depresses the spontaneous activity of cells in a major noradrenergic nucleus, the locus coeruleus (LC) (Graham, A.W. and Aghajanian, G.K., Nature, 234:100, 1971), led to the present investigation of the effects of peripherally administered amphetamine and 4-OH amphetamine on spontaneous activity in the LC.

Spontaneous activity of single cells in the LC was monitored in halothane-anesthetized rats before and after the intraperitoneal administration of d-amphetamine (1.0 mg/kg) or 4-OH amphetamine (0.82 mg/kg). These dosages have been shown to enhance retention performance in an inhibitory avoidance task (Martinez, J.L. et al., Brain Res., 195:433, 1980). Cells initially identified by their waveform, firing rate (1.41 Hz, S.E.M. = 0.15) and excitation and subsequent inhibition to paw-pinch were later shown by histology to have been in the LC. Mean firing rates were obtained for a minimum of 5 minutes of stable activity pre-drug and for 10 minute epochs up to 60 min post-drug. Amphetamine significantly depressed activity ($F = 4.71$; $df = 2, 10$; $p < .05$). Five minutes after amphetamine administration activity had decreased by 33% and 10 min later it had decreased by 83%. However, 4-OH amphetamine was without significant effect on the firing rate of these cells ($F = .158$; $df = 3, 17$; p = N.S.). Unlike the effect of d-amphetamine, this drug resulted in a small increase in activity 5 min post-drug, an effect that did not persist 15 min later.

The dosage of 4-OH amphetamine used in this study, which did not change the spontaneous firing rate of neurons in the LC, also has no effect on regional brain NE concentrations (Martinez, J.L. et al., Behav. Neurosci., 97:962, 1983). The absence of these central effects of 4-OH amphetamine underscores the role that peripheral factors must play in the modulation of memory by amphetamines.

24.6 INFLUENCES OF AMPHETAMINE AND HALOPERIDOL ON DOPAMINE TERMINAL AUTORECEPTORS. G. Mereu*, M.J. Meldrum, T.C. Westfall, X.T. Hu*, and R.Y. Wang. Dept. of Pharmacol., St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

Quite recently a number of studies, using both biochemical and electrophysiological methods, are debating the existence and the role of presynaptic and somato-dendritic dopamine (DA) autoreceptors in the meso-cortical DA system.

It has been reported that the intravenous (i.v.) administration of d-amphetamine (d-AMP), a dopamine agonist, or haloperidol (HAL), a DA antagonist, results in a shift of current threshold needed for evoking antidromic action potentials of A9 DA cells from the neostriatum. The results suggest that d-AMP and HAL could exert their effects via DA autoreceptors located on DA terminals, i.e. DA released by d-AMP would hyperpolarize the cell membrane of DA terminals, while the blockade of DA receptors might induce the opposite effect.

Considering that this approach might be useful for identifying DA presynaptic receptors in the meso-cortical and meso-limbic systems, we are currently studying the excitability of the axon terminals of these areas after local infusion of DA-agonists and antagonists.

Preliminary results indicate that i.v. d-AMP significantly increased the threshold current (17.4±4.7%) necessary to evoke 50% of action potentials of meso-limbic A10 neurons. By contrast, local infusion of 0.5 µl of HAL (1 µM in saline, pH 7.2) directly into the nucleus accumbens (NAc) significantly decreased the amount of current (22.2±5.3%) needed for evoking DA antidromic action potentials by NAc stimulation; HAL also reversed the increase of threshold current induced by low doses of d-AMP (0.5-1.0 mg/kg i.v.). No effect was observed after local infusion of 0.5 µl of 2M NaCl. In a few cases (3 out of 8) after HAL infusion, a slight (15%) increase of spontaneous firing rate of A10 DA neurons was observed. In 2 out of 3 tested cells, HAL infusion after i.v. d-AMP (which consistently decreased neuronal activity) led to a reversal of suppression of firing.

The present results, although preliminary, are in agreement with the previous studies on noradrenergic and A9 DA neurons and further support the hypothesis that the activation of presynaptic DA receptors might hyperpolarize the cell membrane of DA terminals thereby reducing the amount of DA released. (Supported by USPHS grants MH-34424, MH-38794, MH-00378 and NS-16215.)

24.7 DOPAMINE RELEASED IN NUCLEUS ACCUMBENS REDUCES AMPLITUDE OF ANTIDROMIC POPULATION SPIKE IN THE AMYGDALA FOLLOWING ACCUMBENS STIMULATION. G. J. Mogenson, C. Y. Yim and M. Wu*, Dept. of Physiology, Univ. of Western Ontario, London, Canada and Dept. of Anaesthesia Research, McGill Univ., Montreal, Canada.

Recent studies showed that dopamine (DA) released in the nucleus accumbens preferentially modulates the response of accumbens neurons to excitatory input from the amygdala without affecting their spontaneous activity (Yim & Mogenson, Brain Res. 1982, 239, 401). The results suggest that DA may act presynaptically to modulate the release of transmitter from the amygdala afferents. To test this hypothesis, the effect of DA on the excitability of amygdala terminals in the accumbens was investigated.

Extracellular field potentials from electrical stimulation of the nucleus accumbens were recorded from the amygdala in 22 urethane-anesthetized rats using glass micropipettes. A population spike of mean amplitude of 4.2 mV and mean latency of 17 ms was observed in the basolateral nucleus and it was shown to be an antidromically evoked population spike (APS) by the high frequency (> 500 Hz) following test. Antidromic spikes from single units with similar latencies were also observed from the same recording sites.

Amphetamine injected either intraperitoneally or into the accumbens reduced the amplitude of the APS by more than 40%. The effect of the injection was prolonged, usually lasting up to 2 hrs. Stimulating the ventral tegmental area (VTA) with a train of pulses (10 pulses at 100 Hz) prior to stimulation of the accumbens produced a similar attenuation of the APS. The effect of VTA stimulation was, however, abolished after injection of 6-OHDA into the VTA 7 days prior to the recording experiment. The threshold for eliciting antidromic spikes from single units was also increased by VTA conditioning stimulation or IP injection of amphetamine.

These results indicate that the APS in the amygdala following accumbens stimulation is modulated by DA released in the accumbens. This attenuation of the APS is probably due to a reduction in the excitability of the terminals in the accumbens by the binding of DA to presynaptic receptors on these terminals. However, whether this phenomenon is related to the ability of DA to selectively modify the response of accumbens neurons to excitatory input from the amygdala remains to be investigated.

(Supported by MRC of Canada)

24.8 VENTRAL TEGMENTAL AREA AND SUBSTANTIA NIGRA DOPAMINE NEURONS: A COMPARISON OF PHYSIOLOGIC CHARACTERISTICS AND EFFECTS OF AMPHETAMINE. R. Shaver* and G. Aston-Jones (SPON: R. L. Isaacson). Center for Neurobehavioral Sciences, SUNY, Binghamton, NY 13901; present address: Dept. Biology, NYU, New York, NY 10003.

Using single unit recordings, putative dopamine (DA) cells were identified in chloral hydrate-anesthetized rats by their characteristic waveform, wide spike duration and histologic localization to the ventral tegmental area (VTA) or substantia nigra zona compacta (SNC). Spontaneous discharge, axonal conduction properties, and responses to sciatic nerve or terminal field stimulation were determined. For one neuron per animal, d-amphetamine (AM) was administered in doses of 1.0 mg/kg, i.p., up to a maximum cumulative dose of 5.0 mg/kg, and the above physiologic testing was repeated.

The results were as follows: (1) Spontaneous rate, discharge pattern, conduction velocity, and refractoriness were similar for DA cells in VTA and SNC. (2) Most VTA (61%) and SNC (78%) DA cells were responsive to sciatic nerve stimulation, exhibiting periods of post-stimulus inhibition or excitation. (3) Nearly every DA cell investigated in VTA (91%) or SNC (95%) exhibited altered activity (inhibition or excitation) following antidromic activation. (4) AM generally decreased spontaneous activity of DA neurons; however, a subgroup of DA cells in both VTA and SNC were unresponsive to this rate-suppressing effect. (5) There was no appreciable change in thresholds or latencies for antidromic activation following AM administration. (6) Responsiveness to sciatic nerve or terminal field stimulation were markedly altered (often enhanced) by AM, independently of AM's effects on spontaneous discharge rates.

These findings indicate that DA cells in VTA and SNC are very similar in certain physiologic characteristics, but that subgroups of DA cells differ in responsiveness to sciatic nerve or terminal field stimulation, as well as in sensitivity to rate-suppressing effects of AM. These studies also reveal that DA neuronal responsiveness to various stimuli is markedly altered by AM administration, independently of its influence on spontaneous discharge. These latter results indicate that synaptic and post-excitation responsiveness may be important indices of AM's effects on midbrain DA neurons.

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24.9 EPINEPHRINE AND NOREPINEPHRINE DISPLAY BOTH EXCITATORY AND INHIBITORY EFFECTS ON FROG MOTONEURONS. C.J. Wohlberg, J.C. Hackman, and R.A. Davidoff. Depts. of Pharmacology and Neurology, Univ. of Miami School of Medicine and Neurophysiology Lab., VA Medical Center, Miami, Fl., 33101.

Numerous reports have described various effects of epinephrine (E) and/or norepinephrine (NE) on motoneurons and evoked reflexes in the lumbar spinal cord. We set forth to study both catecholamines (CAs) in the same preparation in order to determine any pharmacological differences between them.

Effects of CAs were examined in the in vitro, hemisected frog spinal cord. Frogs were placed on ice for 30 min., decapitated, and their spinal cords rapidly removed. The cords were superfused with HCO_3-Ringer's bubbled with 95:5 O_2:CO_2. The motoneuron membrane potential change elicited by exogenous CAs was studied by sucrose gap DC recording from the ventral root (VR). Both NE and E (10 uM, 30 sec. applications) brought about complex changes in the VR membrane potential. There was an initial hyperpolarization followed by a later and longer lasting depolarization. The former response was antagonized in normal Ringer's by yohimbine (1uM) and reduced by ouabain (0.1-10uM), but when synaptic activity was blocked with tetrodotoxin (TTX), the hyperpolarization was dose-dependently antagonized by ouabain, but not yohimbine. The late depolarization was antagonized in both normal and TTX Ringers by propranolol(10-100 uM).

Changes in reflex activity were studied by AC recording from both the flexor and extensor efferent nerves of the frog thigh. At lower concentrations, E (0.1-1uM) and NE (1-10uM) potentiated the early components of the extensor reflex and slightly depressed the flexor reflex. At higher concentrations (100 uM), E and NE reduced the early and late components of both the extensor and flexor reflexes. The potentiation of the extensor reflex was blocked by the addition of 10uM propranolol and was mimicked by the addition of 100uM isoproterenol, indicating that the effect is mediated through activation of beta receptors. The depression seen at higher concentrations was not antagonized by alpha or beta blockers, possibly indicating a non-specific or metabolic effect.

In conclusion, it appears that CAs exert two different effects on ventral horn neurons of the frog spinal cord. The initial hyperpolarization appears to be mediated by alpha$_2$-adrenoceptors located on interneurons or afferent terminals, while the depolarizing and excitatory effect is mediated through beta receptors. In addition, a metabolic effect, occuring directly on motoneurons, is also seen. Supported by NIH Grants HL07188T and NS17577, VAMC Grant MRIS1769 and a grant from the National Parkinson Foundation.

24.10 NOREPINEPHRINE INCREASES SYNAPTIC ACTIVITY IN RAT CEREBRAL CORTEX IN DISSOCIATED CELL CULTURE. P. A. Rosenberg*, J. S. Schweitzer*, and M. A. Dichter. Department of Neuroscience, Children's Hospital, Boston, Mass. 02115.

We have studied the action of norepinephrine and other adrenergic agents on neurons derived from cerebral cortex in dissociated cell culture. Cultures were used from 3-8 weeks after plating cortex derived from E 15 fetuses. Penetrations with microelectrodes were generally restricted to neurons with somal diameters greater than 18 microns, and drugs were applied by pressure perfusion from pipets with tip diameters of 5-10 microns. Norepinephrine, epinephrine, or isoproterenol were applied in concentrations of 1 nM to 20 uM in solutions containing 100 uM EDTA to prevent oxidation, and phenylephrine was applied in concentrations of 1-100 uM. These agents produced no detectable change in membrane potential or input resistance in 120 neurons. There was also no change in the action potential. In 29 of these cells (24%) adrenergic agonists produced an increase in the number of IPSP's and EPSP's observed in the penetrated cell. This effect was observed at concentrations of isoproterenol as low as 10 nM. Addition of explants of locus coeruleus to these cultures did not significantly increase the number of neurons demonstrating this response (13/93).

The basis for the effect of adrenergic agents on synaptic activity in the cortical cultures remains elusive. One explanation is that there exists a directly responding subpopulation of neurons which, because of small size, rarity, or unusual morphology, has never been impaled. A second explanation, which may overlap the first, is that the response is localized to processes on the responding neurons, and not directly observable by penetration of the soma.

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24.11 IONTOPHORESIS IN THE FREELY MOVING RAT: NOREPINEPHRINE IN THE CEREBELLUM. M.O. West and D.J. Woodward, Dept. of Cell Biology, Univ. TX. Hlth. Sci. Ctr., Dallas, TX 75235.

The objective of this study was to determine whether the effects of iontophoretically applied norepinephrine (NE) on cerebellar unit activity in the awake rat are similar to those reported in the anesthetized preparation. We have developed a technique for microiontophoresis in freely moving rats. The present system has matured since our previous reports, and therefore the important improvements that have been made are included here.

Recordings were obtained following 3-4 days' recovery from surgical implantation of a microelectrode drive assembly. Prior to each experiment, the microdrive was attached to a hub cemented to the animal's skull. Inserted into the drive was a 4-barrel micropipet prepared with appropriate drug and salt solutions as follows. Omega dot glass tubing was pulled to a fine taper and the tip was broken to a total diameter of 4-8 micrometers. The 4 barrels were backfilled with a microsyringe, centrifuged (3000 r.p.m.) to remove air bubbles from the pipet, and used immediately. This resulted in ready availability of drugs at the tip, lower impedances, and lower ejection currents (10-40nA) required to produce effects.

NE as a modulatory agent is of interest in characterizing the phasic firing patterns of cerebellar Purkinje neurons. In preliminary studies, iontophoretic application of NE with sufficient ejection current (20-40 nA) produced inhibition of spontaneous unit activity. At lower currents (10-15 nA), NE produced substantial amplification of phasic inhibitory responses to iontophoretically applied GABA, without affecting spontaneous activity. These results clearly demonstrate that some actions of NE can be shown during conscious behavior which previously have been observed in the anesthetized rat.

Beyond this, other forms of phasic cerebellar activity may be investigated. Units in anterior intermediate cerebellum in the rat showed robust increases in activity during treadmill locomotion (compared with motionless behavior), and these units often showed correlations with footfall cycle (Sorensen et al., Neurosci. Abstr., 1984). As a result of our preliminary findings, we anticipate being able to explore additional interactions of NE with such behaviorally specific neuronal activity patterns. Supported by NIAAA 3901, DA02338 and the Biological Humanics Foundation.

24.12 STUDIES OF INHIBITORY MECHANISMS IN LOCUS COERULEUS. M. Ennis* and G. Aston-Jones (SPON: R. J. Valentino). Center for Neurobehavioral Sciences, SUNY, Binghamton, NY 13901; present address: Dept. Biology, NYU, New York, NY 10003.

Previous work has suggested that norepinephrine (NE)-containing locus coeruleus (LC) neurons receive inhibition from adrenergic neurons in the medulla as well as from NE released by LC axon or dendritic collaterals. Therefore, we investigated the effects of lesions to the C2 adrenergic cell group on spontaneous discharge of LC neurons, as well as the effects of near-threshold antidromic or synaptic driving of LC neurons on post-activation inhibition. Single unit extracellular recordings were obtained from adult male albino rats under chloral hydrate anesthesia. All data reported here are from histologically verified NE-LC neurons.

LC neurons recorded 5-8 days after ipsilateral aspiration lesion of the rostral nucleus tractus solitarius (C2 cell group) in 5 rats exhibited a mean discharge rate of 1.5 ± 0.2 Hz, similar to the rate (1.7 ± 0.2 Hz) obtained for LC neurons in 4 intact control rats.

The effects of possible collateral release of NE onto neighboring LC neurons was examined by electrically stimulating the dorsal noradrenergic bundle (DB) or contralateral rear foot (for sciatic nerve activation) at intensites near threshold for driving single LC neurons. Significant inhibition was observed for 6 of 7 neurons for 500 msec following DB stimuli that elicited antidromic driving, as well as for 300 msec following similar stimuli that failed to evoke driven responses. The magnitude of such inhibition was significantly greater following driven responses than on non-driven stimulus trials ($p<.02$). In addition, significant inhibition lasting several hundred msec was observed in 3 of 4 other LC neurons for DB stimuli just subthreshold for eliciting orthodromic activation. Similarly, footshock stimuli elicited inhibition (lasting 200-500 msec) in 12 of 14 neurons following driven responses, and in 7 of these cells following similar stimuli which failed to elicit spikes.

These results indicate that the ipsilateral C2 adrenergic cell group may not have a substantial tonic inhibitory influence on LC neurons. In addition, our studies suggest that LC collaterals innervating neighboring neurons may play a significant role in post-activation inhibition in this nucleus.

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24.13 TETRAHYDROBIOPTERIN AND 6-METHYLTETRAHYDROPTERIN DECREASE RAT SERUM PROLACTIN AND INCREASE HYPOTHALAMIC DOPAMINE SYNTHESIS. J. F. Reinhard, Jr., R. F. Butz*, G. D. Jahnke* and C. A. Nichol*
The Wellcome Research Laboratories, Research Triangle Park, NC 27709.

As part of a study on the ability of tetrahydropterin cofactors for tyrosine hydroxylase to accelerate catecholamine synthesis *in vivo*, relatively small responses to tetrahydrobiopterin (BH_4) were observed in brain striatum (Reinhard *et al., J. Neurochem.* **41**:581, 1983). Since brain catechol synthesis is tightly controlled by pre- and post-synaptic receptors, we examined the effects of exogenous pterin cofactors on serum prolactin (PRL) levels as an indicator of changes in hypothalamic dopamine synthesis. Release of PRL from the pituitary is controlled by dopamine neurons in the arcuate nucleus of the hypothalamus which lack presynaptic receptors and respond slowly to circulating levels of PRL. Serum PRL levels were measured by radioimmunoassay (NIADDK rPRL-RP3) after injecting rats intraperitoneally with either BH_4 or 6-methyltetrahydropterin (6-MPH_4). Administration of 6-MPH_4 (250 µmol/kg) decreased serum PRL for at least 4 hours (to 47.8 ± 16% of control values at 1 hr), and the decrease in PRL was dose-dependent both for 6-MPH_4 (ED_{50} = 256 µmol/kg) and for BH_4 (ED_{50} = 144 µmol/kg). Direct effects of 6-MPH_4 on the pituitary were tested by incubating anterior hemipituitaries with either dopamine or the pterin. While dopamine decreased PRL release by 85%, 6-MPH_4 was without effect. Injection of rats with the serotonin precursor 5-hydroxytryptophan (5-HTP) substantially increased serum PRL levels; this effect was antagonized by 6-MPH_4. The decrease in PRL produced by 6-MPH_4 in saline-treated and in 5-HTP-treated rats was antagonized by haloperidol. The effects of 6-MPH_4 on brain and hypothalamic catechol synthesis were evaluated by measuring the rate of accumulation of DOPA after m-hydroxybenzylhydrazine. Ninety minutes after 6-MPH_4 hypothalamic DOPA was increased by 36% (from 1.33 ± 0.11 to 1.81 ± 0.24 nmol/g x 30 min). In contrast, DOPA accumulation following 6-MPH_4 in the remainder of the brain increased by only 15% (from 0.875 ± 0.05 to 1.01 ± 0.04 nmol/g x 30 min). These results suggest that hypothalamic tyrosine hydroxylase is not saturated with BH_4. Furthermore, changes in serum PRL can provide an *in vivo* model for evaluating the effects of pteridine cofactors on dopamine synthesis.

24.14 Hypothalamic monoamines and catabolites in the female rat analysis by HPLC: H.•H. Osterburg*, C.E. Finch. Andrus Gerontology Ctr., U. Southern Cal., Los Angeles, CA 90007, E. Cohen-Becker*, and P. M. Wise*, Dept. Physiology, U. Maryland, Baltimore, MD 21201.

Monoamines were measured in the range of 20-1000 pg per sample, using a Bioanalytical Systems LC-4A electrochemical detector with glassy carbon electrode and a Brownlee 5 micron, C-18 reversed phase column. Tissue was homogenized in 0.1 N HCl, 1 mM EDTA, 0.1 mM ascorbate centrifuged 15,000 g x min. Monoaminergic compounds were analyzed in 3 groupings: 5-HT and 5-HIAA, by direct injection of supernate in a mobile phase of 0.05 M K-phosphate, 2 mM EDTA, pH 3.0 with 12% methanol; DOPAC, MOPEG, HVA, DOPET were extracted into ethyl acetate, evaporated, and injected in a mobile phase of 0.05 M Na-acetate, 2mM EDTA, pH 4.65, with 6% methanol. From the residual aqueous phase of the preceeding extraction, DA and NE were isolated by binding to alumina, followed by elution with 0.1 N HCl, and injection with the indole mobile phase. Compounds were identified by retention time and voltametry. Internal standards corrected for recovery, which was particularly important for MOPEG: N-methyl 5-HT (indoles), vanillyl alcohol (MOPEG), caffeic acid (DOPAC, HVA), dihydroxybenzylamine (DA,NE). This procedure permitted measurement of MOPEG in trace quantities in the presence of predominating amounts of DA and NE. MOPEG in MBA (medial-basal hypothalamus) was 150 pg/mg protein and 250 pg/mg protein in POA (preoptic area). MOPEG/NE in MBA was 0.8%; in POA, 0.4%. DOPEG was not reliably measured because of interference from the solvent front, but is present at 500 pg/mg tissue; DOPEG/NE, 2%. DOPET was at the limit of detectibility, 20 pg/mg tissue; DOPET/DA, 0.4%. Levels of other compounds were as previously reported. Thus, of the non-sulfated neutral catabolites of NE and DA, only MOPEG as accurately measured in this protocol. Estradiol treatment to induce an afternoon LH surge decreased, between morning and afternoon, the ratios of DOPAC/DA in MBH (-35%, $p<0.01$ and POA (-10%. $p<0.01$); however MOPEG/NE increased 40% ($p<0.1$) relative to non-surging controls in the POA. These data are consistent with previous findings that DA turnover decreases, whereas NE turnover increases just before the LH surge.

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24.15 NOREPINEPHRINE ENHANCEMENT OF GABAERGIC THALAMIC INPUTS TO THE LATERAL HYPOTHALAMUS OF RAT. J-T Cheng and B.D. Waterhouse. Dept. Cell Bio., UTHSCD, Dallas, TX 75235.

The present studies were conducted as part of an ongoing investigation of the physiological actions of norepinephrine (NE) in local neuronal circuits of the mammalian brain. In this report, we describe noradrenergic actions in the lateral hypothalamus (LH), an area which has been implicated in controlling ingestive behaviour. Microiontophoretically applied NE was interacted with LH neuronal responses to iontophoretically applied GABA and activation of previously identified (Cheng, 1982) GABAergic pathways from the reticular (RT) and ventral (VT) thalamic nuclei. Extracellular activity of LH cells was recorded from halothane anesthetized Long-Evans hooded rats. Inhibitory responses of LH neurons (n=16) to electrical stimulation of RT (or VT) (25-350 μA) or iontophoretic pulses (10 sec duration at 40 sec intervals) of GABA (2-30 nA) were examined before, during and after NE microiontophoresis (8-50 nA). Peri-event histograms were used to quantitate effects of NE on spontaneous activity and synaptically mediated or GABA-induced responses. As demonstrated previously, electrical stimulation of RT or VT produced a short latency suppression of LH neuron spontaneous discharge. In 13 of 16 LH cells tested, microiontophoretically applied NE markedly prolonged the duration of this stimulus bound inhibition by 46%, from a mean of 28 to 41 ms. Recovery to the control pattern of response was observed following cessation of NE administration. Local application of NE also enhanced inhibitory responses induced by GABA iontophoresis in 80% of LH neurons tested (n=20). In all cases GABA-mediated responses were augmented ($\bar{X}$=75%) above control levels at doses of NE which had little (<15%) or no effect on SA. In 5 cells, firing rate during the GABA response epoch was decreased during NE more than spontaneous activity, such that a net enhancement of GABA-induced inhibition was observed. Recovery to the control level of response was seen in all cases after termination of the NE ejection current. In summary, these results indicate that as in other noradrenergic target regions of the CNS, NE can augment GABAergic synaptic actions in the LH. These observations suggest that NE plays an important role in the synaptic transfer of information within LH circuits, and consequently may affect important homeostatic functions mediated by this structure. (Supported by NINCDS NS 18081 and the Klingenstein foundation to BDW)

24.16 FURTHER STUDIES ON THE ACTIONS OF PROGLUMIDE IN THE CNS. L.A. Chiodo, A.S. Freeman and B.S. Bunney. Depts. Psychiat. & Pharmacol., Yale Univ. Sch. Med., New Haven, CT 06510.

We have recently reported that the glutaramic acid derivative, proglumide (PROG), appears to be a selective and specific antagonist of cholecystokinin's (CCK) effects in the CNS (SCIENCE 219:1449, 1983). Given the potential importance of such an antagonist as a tool to aid in elucidating the biochemical, electrophysiological and behavioral actions of CCK, we conducted a series of electrophysiological studies designed to further characterize the actions of PROG in brain. The actions of proglumide, alone and in combination with other drugs, are examined on CCK-sensitive DA neurons in the midrain (medial zona compacta region of the substantia nigra), CCK- and DA-sensitive prefrontal cortex neurons, and neurons in the somatosensory cortex of chloral hydrate anesthetized rats.

PROG alone (0.2-1.0 mg/kg) had no effect on the firing rate or firing pattern (degree of bursting) in any region. However, PROG was again observed to block CCK, but not glutamate, induced increases in spontaneous neuronal activity in all three regions. This effect was maximal at a dose of 0.2 mk/kg. We also examined the ability of PROG to block the actions of several putative neurotransmitters which were iontophoretically applied. In every case, PROG had no effect. Thus, PROG did not alter the excitatory effects of neurotensin on midbrain DA neurons, the inhibitory actions of MET-enkephalin or excitatory actions of substance P on DA-sensitive prefrontal cortex neurons, nor the inhibitory effects of histamine in the somatosensory cortex. The acute oral administration of PROG (1 and 10 mg/kg) did not alter the number of active DA neurons encountered in either A9 or A10. However, after 21 day administration of two oral doses of PROG, the following was observed: 1 mg/kg caused an increase in cells/track in A9 (not A10) while 10 mg/kg increased the number of DA neurons encountered in both regions. Acute oral PROG (1 mg/kg) was also able to significantly attenuate the inhibitory effects of intravenous apomorphine on DA neurons, while in chronically treated animals the response to apomorphine was no different from control.

In summary, PROG appears to be a specific antagonist of CCK in the CNS. This agrees with studies in the periphery which demonstrate that PROG is a competitive antagonist for CCK receptors. The actions of acute and chronic PROG administration on the physiology of midbrain DA neurons suggests that these cells may be under the tonic influence of CCK. Supported by MH-28849, MH-08848 and State of CT.

NEURAL PLASTICITY IN ADULT ANIMALS: SPINAL CORD AND MOTONEURONS

25.1 GROWTH OF MULTITERMINAL INNERVATION IN LOBSTER MUSCLE: POSSIBLE FUNCTIONAL AND PROLIFERATIVE MECHANISMS. C.K.Govind, J.Pearce*and D.E.Meiss. Zoology Dept.,Univ. of Toronto, West Hill M1C 1A4, Ontario, Canada.

Transmitter output and fine structure of identified neuromuscular synapses of the single excitor axon to the limb accessory flexor muscle was examined during growth of the lobster, Homarus americanus. The mean quantal output of synapses on the proximal fibers increased over three-fold (from 0.15 to 3.3) for an approximate ten-fold increase in body weight of lobsters ranging from 0.5 to 5 kg. This growth in synaptic transmission is due to a proliferation of multiterminal innervation as seen by comapring an equivalent length of muscle fiber between the 0.5 and 5 kg animals. A single branch of the axon innervates the muscle fiber in the small lobster compared to several branches in the large lobster. This gives rise to an approximate four-fold increase in the number of nerve terminals, synapses, and dense bars between small and large lobsters. Concurrently the mean size of synapses increases three-fold, though the length of dense bars and their number per synapse remains constant. Additionally, synapses show distinct areas of non-specialized membrane which are larger and more abundant in the enlarged synapses of the 5 kg lobster. The occurrence of dense bars (active sites) adjacent to these synaptic interruptions creates functional sub-units which may facilitate the addition and retrieval of vesicular membrane during endo- and exo-cytosis. This is similar to the frog and moth neuromuscular junction which consist of a series of regularly repeating units, each made up of an active site bordered by glial membrane. Finally, in both small and large lobsters, the ultimate endings of the multibranched innervation are synapses, contained within terminals densely packed with vesicles. Since the innervation continues to proliferate with growth i.e. with each molt, these endings represent a potential growing tip. Like the plant apical meristem which grows by the addition of cells proximal to the tip, so the addition of membrane via exocytosis at these terminal synapses could result in elongation of the axon branch. Such a process would resemble sprouting from existing neuromuscular terminals in vertebrate muscle.

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25.2 SOLEUS MOTOR UNIT ANATOMY IN NORMAL AND ADULT SPINAL CATS S.C. Bodine, T.C. Cope, T.P. Martin, and V.R. Edgerton Brain Research Institute and Dept. Kinesiology, UCLA, Los Angeles, CA. 90024

Soleus muscle unit (MU) anatomy was studied in normal (N) and 4-month spinal-cord transected (T) adult cats. Since the Sol is a homogeneous muscle of type S units, it has been assumed that Sol MU SpT should be identical to whole muscle SpT which has a reported range of 1.6-2.3 kg/cm^2 (Close, Physiol. Rev, 52, 1972). Based on this assumption innervation ratios have been calculated using a SpT value of 2.0kg/cm^2. (Burke et al., J. Physiol., 238, 1974) In this study, all fibers belonging to the MU were counted in serial sections taken along the entire length of the muscle. Reconstruction of the muscle allowed for a direct determination of innervation ratio from which SpT was calculated. In addition, the effect of spinal cord transection on fiber cross-sectional area and SpT was assessed.

Motoneurons were stimulated repetitively in order to deplete the muscle unit of its glycogen. Fibers were assessed for glycogen content using an image processing computer which allowed for the measurement of single fiber optical densities and a more precise identification of the fibers belonging to the stimulated muscle unit.

EXP	MYOSIN ATPase	CT (ms)	P_o (g)	FIBERS	AREA (um^2)	SpT$_2$ (kg/cm^2)
N	L	107	11.2	157	3201	2.23
N	L	63	13.7	177	1969	3.94
N	L	65	15.6	316	2148	2.30
N	L	72	15.6	227	2240	3.07
T	L	67	6.3	199	1757	1.81
T	D	45	2.2	186	1083	1.09
T	D	54	6.5	409	1443	1.10

Within a MU, fiber sizes were distributed over approximately a 2-fold range in both N and T cats. Mean fiber size was lower in T ($p<.001$). In addition, fibers that stained dark (D) for alkaline myosin ATPase were significantly smaller than fibers that stained light (L) in both N and T cats.

Both muscle atrophy and reduced SpT seem to contribute to a reduction in motor unit force after transection. These values on normal MUs in the SOL are similar to or slightly higher than has been reported for whole SOL muscles in the cat. Supported by NIH Grant NS16333.

25.3 FIBER SIZE AND MEATABOLIC VARIABILITY WITHIN CAT TIBIALIS ANTERIOR MOTOR UNITS R.R. Roy, T.P. Martin, S.C. Bodine, E. Eldred, and V.R. Edgerton Brain Research Institute and Dept. Kinesiology, UCLA, Los Angeles, CA. 90024.

Current evidence suggests that there are remarkable similarities in enzyme properties of muscle fibers belonging to the same motor unit (MU) (Nemeth et al. J.Physiol., 1981) thus implying a strong neural influence of motoneurons on the muscle unit. To further examine the extent of this metabolic homogeneity in a mixed, predominately fast muscle , MUs in the cat tibialis anterior (TA) were studied.

MUs were isolated from the ventral root and the contractile properties measured. The MUs then were depleted of glycogen by repetitive stimulation of the isolated motor axon. The TA was removed immediately, weighed and prepared for quantitative histochemical analyses. The periodic acid Schiff (PAS) method for glycogen was used to identify the fibers belonging to a MU. Serial sections were stained for myosin ATPase (pH=8.8), succinate dehydrogenase (SDH) and alpha-glycerophosphate dehydrogenase (GPD). SDH and GPD optical densities as well as fiber areas were determined using an image processing system. Mean optical densities and fiber areas and their coefficients of variation (CV) were compared between the MU fibers and a mixed population of non-motor unit (non-depleted,NMU) fibers located in the region of the MU.

Generally, the fiber area and metabolic data showed a marked variability both within and among MU types. MUs representing each of the major MU types were glycogen depleted. CV's for areas ranged from 15-47% for MU fibers and 25-68% for NMU fibers. For SDH, the CV ranged from 13-38% within a MU and from 37-78% across MUs. Similar values for GPD were 20-95% and 55-90%. In addition, there was considerable variation in the relationship between fatigue index (initial tension:tension at 2 min) and SDH activities across MUs.

These data suggest that the size and the metabolic potentials of the fibers within a MU vary to a similar degree as that found among fibers of different units of the same general MU type (ie, FF,FR, and S). These findings are similar to those observed in the soleus, a more homogeneous muscle (Martin et al. Neurosci. Abstr.,1984). Supported by NIH Grant NS16333.

25.4

WITHDRAWN

25.5 ALTERED SPINAL DISTRIBUTION OF LEUCINE ENKEPHALIN (LE), SOMATOSTATIN (SS), VASOACTIVE INTESTINAL POLYPEPTIDE (VIP), AND SUBSTANCE P (SP) IMMUNOREACTIVITY (IR) IN CHRONIC SPINAL CATS. K.B. Thor, M. Kawatani, J.R. Roppolo, S.L. Erdman* and W.C. deGroat. Dept. Pharm., Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Spinal injury produces marked changes in the reflex control of the urinary bladder (UB). These changes might be mediated by alterations in spinal peptidergic pathways associated with sacral (Sc) parasympathetic (PSYM) outflow to the UB. This was examined using immunohistochemical techniques to compare LE, SS, VIP, and SP IR in normal (N) and chronic spinal cats (CS, 11-18 months following T_{13} transection).

LE and SS IR in the spinal cord of N cats exhibited staining patterns similar to previously published reports. No LE IR cells and only a few weakly stained SS IR cells (2 cells/section, 28 µm thick) were seen in N cats. In CS cats LE and SS IR showed marked increases in the density of terminal-like staining and in the number of LE and SS IR cells (10-20 cells of either type/section) in the dorsal horn (DH) and intermediate gray (IG). These cells were not localized to the PSYM nucleus. SS IR cells were also located in lamina (lam) VIII of the ventral horn (VH) and in the ependymal layer. Unique to the Sc cord of CS cats were clusters of 2-3 SS IR cells organized in a longitudinal column at the lateral borders of lam I and V.

VIP and SP IR also exhibited differences between N and CS cats. In N cats VIP IR in medial lam V was weak in comparison to dense VIP IR in medial lam VII. SP IR had the opposite distribution. In contrast, VIP and SP IR were nearly equal in both medial lam V and VII of CS cats. In addition, VIP and SP IR projections along the lateral margin of the DH were 30% wider in CS cats than N cats. Wide-spread SP IR was present throughout the VH of N cats while CS cats had very little SP IR except in Onuf's nucleus (ON). Little VIP IR was seen in the VH of N or CS cats except in ON.

These results show that increases in LE, SS, VIP and SP IR occur in certain regions of the spinal cord in CS cats. Increases in peptides might account for the changes in spinal reflexes in paraplegic animals. The ability of naloxone, an enkephalin receptor antagonist, to induce micturition in CS cats supports this idea. Sprouting of afferent terminals which contain SP and VIP might also account for the appearance of excitatory reflexes to the UB in CS cats which are uncommon in N cats. The overlap of VIP and SP IR with preganglionic neurons supports this idea.

25.6 SEASONAL VARIATION IN MOTONEURON MORPHOLOGY AND STRIATED MUSCLE MASS IN A MAMMAL. Nancy G. Forger & S.M Breedlove. Dept. Psychology, U. of California, Berkeley, CA 94720.

White-footed mice (Peromyscus leucopus) are seasonal breeders that reproduce during the long day lengths of spring and summer and undergo gonadal regression in the fall. Photoperiod is a proximate cue governing cycles of the reproductive apparatus; testis activity and quiescence can be induced with appropriate lighting conditions in the laboratory. In rats, the perineal muscles bulbocavernosus (BC) and levator ani (LA) are innervated by the spinal nucleus of the bulbocavernosus (SNB). Both the SNB cells and their target muscles possess androgen receptors and are reduced in size following castration in adult rats. We examined the possibility that BC/LA muscles and their motoneurons undergo morphological changes in concert with variation in testicular function and androgen production in the seasonal breeder, P. leucopus.

Motoneurons innervating the BC in P. leucopus were identified by HRP retrograde labelling, and found to occupy a more lateral position than the SNB in rats. Despite the disparity in anatomical position, we retain the designation "SNB".

Male white-footed mice were exposed to a sequence of photoperiods simulating two breeding seasons separated by a winter. Four to 6 animals were sacrificed at the conclusion of each of the three "seasons". The first season began at birth and consisted of 9 weeks of long days. Males sacrificed at the end of this period were designated LD1 (N=6). Eleven weeks of exposure to short day lengths followed, after which group SD (N=6) was killed. Remaining animals were re-introduced to long day lenghths and killed on week 31 (LD2, N=4).

	LD1 (wk 1-9)	SD (wk 9-20)	LD2 (wk 20-31)
Testes (mg)	266 ±11.0	54 ±13	432 ±38
SV (mg)	56 ± 7	12 ± 1	159 ±29
BC/LA (mg)	92 ± 6	20 ± 2	202 ±16
SNB nuclei (μm^2)	203 ± 9.6	150 ± 9.7	177 ±13.2
SNB somas (μm^2)	875 ±46.6	740 ± 9.7	807 ±127.4

Testes, seminal vesicles (SV) and perineal muscles of each long day group were heavier than those of SD mice (t-tests, $p<.001$). In addition, both the nuclei and somas of SNB motoneurons in SD animals were reduced compared to LD1 mice ($p<.03$). SNB nuclei and somas were increased in LD2, however neuronal increases did not reach statistical significance by week 31. In conclusion, photoperiod-induced changes in testicular function are accompanied by alterations in perineal musculature as well as SNB cell size; seasonal variation in these parameters in the field are, therefore, likely. To our knowledge, seasonal variation in neural morphology has not previously been demonstrated in a mammal.

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25.7 M. TIBIALIS ANTERIOR: ANALYSIS OF FLEXION REFLEX AND MOTOR NERVE INDUCED CONTRACTIONS IN ACUTE AND CHRONIC SPINAL CATS. R.G. Durkovic and K.E. Misulis*. Dept. of Physiology, Upstate Med. Ctr., Syracuse, N.Y. 13210.

Contraction properties of tibialis anterior (TA) muscles were examined in unanesthetized cats with T-10 spinal cord transections made three months, two weeks or just before anemic decerebration. A hind limb was anchored and the distal tendon of the TA muscle was cut and attached to a force-displacement transducer for monitoring tension. Experiments began more than two hours following decerebration.

Flexion reflexes were produced by repetitive electrical stimulation of the cutaneous superficial peroneal nerve (30/s) or saphenous nerve (10/s) of the same limb. Stimulus intensities were supramaximal for Aα and Aδ fibers of each cutaneous nerve. Reflex tensions were similar in all three groups. Motor nerve (MN) induced contractions were then obtained using supramaximal electrical stimulation of the cut common peroneal nerve at the same frequencies used to induce the reflex contractions. The mean reflex/MN tension ratios were 0.54 (acute), 1.04 (2 wk) and 0.75 (3 mo) indicating, as expected, that cutaneous nerve stimulation evokes proportionally larger flexion reflex responses in chronics compared to acutes. However, the chronic data are contradictory to what was expected based upon behavioral observations: the three month chronics typically exhibited a hyperreflexia not observed in 2 week chronics and, therefore, a larger reflex/MN ratio was expected in 3 month chronics.

This contradictory finding may be in part explained by the changes in TA muscle size as a function of time after spinal transection, i.e., TA muscle weight in acutes (4.98 gms) and 3 month chronics (4.72 gms) were significantly different from 2 week chronics (3.54 gms) reflecting muscle atrophy in 2 week chronics. Also influential may be the reversal of normal motor recruitment order that is observed only in 2 week chronics (e.g., Durkovic and Misulis, Neuroscience 7 (suppl.): 61[1982]) which might increase the reflex/MN ratio in 2 week chronics.

TA muscles of 3 month chronics also tended to produce more force per gram of muscle weight. This may be a result of the alterations in muscle fiber type in these muscles (see accompanying abstract).

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25.8 M. TIBIALIS ANTERIOR: ANALYSES OF MORPHOLOGICAL AND HISTOCHEMICAL CHARACTERISTICS IN ACUTE AND CHRONIC SPINAL CATS. K.E. Misulis* and R.G. Durkovic (SPON: M.M. Mozell), Upstate Medical Center, Syracuse, New York 13210.

The flexion reflex involves activation of many hindlimb muscles including the tibialis anterior (TA). This is a mixed muscle composed of both type I (slow) and type II (fast) fibers. The output characteristics of the TA in response to reflex activation are altered by chronic spinal cord section (see accompanying abstract). The present study examined the structural and histochemical changes in this muscle with time after spinalization.

At time 0, 2 weeks, and 3 months after T-10 spinal cord section TA muscles of adult cats were removed for histological analysis. Trichrome and Actomyosin ATPase reaction were used to determine the morphological and fiber type characteristics, respectively.

There were no signs of muscle fiber necrosis or denervation and the total number of fibers appeared to be the same in all animals $p>.05$. However, muscle fiber diameters were smaller in two week chronics. Furthermore, the fiber type profile changed as shown below:

	I	IIa	IIb	
acute (control)	15.3%	31.0%	53.7%	
2 week spinal	9.3%**	38.3%**	52.4%	*p<.05
3 month spinal	14.3%	25.8%*	60.0%*	**p<.005

These data indicate that compared to controls there is a decrease in type I and an increase in the IIa (fast twitch, high oxidative) populations in 2 week spinal animals. In three month chronics there is a decrease in type IIa and an increase in the type IIb (fast twitch, low oxidative) fiber populations compared to controls. This is felt to represent an actual conversion in fiber type characteristics since there is no dropout or proliferation of muscle fibers. Understanding the mechanisms of these changes will be important not only for the study of the flexion reflex, but also for the study of nerve and muscle plasticity.

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25.9 AGEWISE CHANGES IN C57BL/6J MOUSE NEUROMUSCULAR JUNCTIONS: A LIGHT MICROSCOPIC STUDY OF THE SOLEUS AND EXTENSOR DIGITORUM LONGUS. M.H. Andonian* and M.A. Fahim. (SPON: C. Ko), Dept. of Biol., Andrus Gerontology Center, Univ. of So. Calif., L.A., CA 90089-0191

The morphology of the phasic extensor digitorum longus (EDL) and tonic soleus (SOL) motor endplates was examined in young mature (7-8 month) and old (24 month) male C57BL/6J mice. This strain was selected because it is a widely used strain in aging studies, and is also one of the parents of the previously characterized CBF-1 hybrid mouse (Fahim and Robbins, J. Neurocytol., 10:13-25, 1983).

EDL and SOL muscles were dissected from young and old mice under Metofane anesthesia, and stained with zinc-iodide osmium. Camera lucida drawings were made of endplates from 25-30 fibers of each muscle from 3 animals of each age. Morphometric measurements were made by digitizing the drawings on a computer controlled bit pad. Measurements included nerve terminal perimeter, area, and length along with fiber diameters.

In the EDL, there were slight, but insignificant decreases in the perimeter (2.7% ↓), area (10% ↓), and length (3.4% ↓) of the endplates, and no change in the fiber diameters in the older animals. In the SOL, there was an insigificant decrease in the perimeter (13% ↓), but sigificant ($p < .05$) decreases in area (19% ↓) and length (35% ↓), as well as a significant decrease in the fiber diameter (31% ↓) in the old animals.

Qualitatively, the junctions of both muscles appeared more complex and more highly branched in the old animals. Extraterminal sprouting was observed in all groups, however group or age differences were not appearant.

Analysis of these preliminary data suggest that the neuromuscular morphometry of the phasic EDL muscle in the C57BL/6J remains relatively unchaged with age, whereas that of the tonic SOL muscle undergoes some compensatory decreases. These changes may suggest a general decrease in the overall activity of the SOL with advancing age.

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25.10 **MOTOR AXON SPROUTING IN FROG SARTORIUS MUSCLES IS NOT ENHANCED BY CONTRALATERAL DENERVATION.** **A.A. Herrera and D.R. Scott*. Neurobiology Section, Dept. Biological Sciences, University of Southern California, Los Angeles, CA, 90089.**

In an earlier study we reported (A.A. Herrera & A.D. Grinnell, Nature 291, 495, 1981) that unilateral denervation of the frog sartorius muscle causes a large (3-8X) and persistent increase in transmitter release from nerve terminals in the contralateral unoperated sartorius. We also reported preliminary findings that, unlike the frog cutaneous pectoris muscle (S. Rotshenker, J. Physiol. 292, 535, 1979), unilateral denervation in the sartorius does not cause contralateral motor axon sprouting. In view of a conflicting report (G. Ring et al., Brain Res., 260, 313, 1983), we now report results of a more extensive and better controlled histological study.

From a large batch of northern variety Rana pipiens 50 matched pairs of individuals were selected. In each pair the left sartorius nerve was surgically exposed and either cut (experimental) or not (control). 57 to 133 days later, right unoperated sartorius muscles were removed from a pair. Muscle pairs were stained with either silver plus cholinesterase or with nitroblue tetrazolium (NBT) plus cholinesterase. Unoperated control muscles were also examined. The incidence of normal endplates and of 7 different categories of motor axon sprouts was independently scored by 2 observers who did not know the identity of the muscles examined. Endplates that met strict criteria for visibility were scored in 41 silver stained and 45 NBT stained muscles. Results determined by the two observers were in good agreement.

All endplates, even in unoperated muscles, showed a high incidence (20-30%) of remodelling or morphological plasticity. There were no significant differences between experimental and control muscles either overall or within matched pairs, and likewise no differences between results obtained with the two different staining procedures. We conclude that sprouting is a frequent occurrence in normal frog sartorius endplates and that this ongoing remodelling is not enhanced by contralateral denervation.

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25.11 GABA MEDIATED CHANGES IN POSTCRUSH SPINAL CORD OF MICE. L.J. Fisher, J.N. Adler* and M.W. Luttges. Aerospace Engineering Sciences, University of Colorado, Boulder, Colorado 80309.

Several laboratories have documented time-dependent changes in spinal cord activity following unilateral sciatic nerve damage. These alterations include somatotopic reorganization within the ipsilateral dorsal horn, changed cross-cord responses and differing pharmacological responses from ipsilateral and contralateral spinal cord. The physiological processes underlying such changes remain elusive. In the present study, response profiles from various depths of the lumbar region of mouse spinal cord were examined following sciatic nerve crush. A lumbar laminectomy was performed on mice anesthetized with chloral hydrate (700 mg/kg, ip). Tungsten electrodes (25 μm tip) were used to record extracellular responses elicited using single pulse and high frequency stimulation of sciatic nerves ipsilateral to the nerve crush. Responses were recorded at 100 μm depth increments from the surface of the cord at various postcrush periods. In addition, amino-oxyacetic acid (AOAA) and picrotoxin were used to evaluate the involvement of GABA in postcrush alterations. Cholinergic modifications were evaluated also. Spinal cord responses typically contained the afferent volley, a short latency negative waveform and a later, slow positivity. The after-positivity was sensitive to high rates of stimulation and was obtained predominantly in the middle and lower regions of dorsal horn (layers IV-VI). This positivity was demonstrably enhanced 9 days after nerve crush. In addition, increased after-positivity was observed in both normal and nerve-damaged mice following AOAA administration, while reduced positive components were apparent following picrotoxin. Cholinergic drugs did not induce significant alterations in the spinal response profiles. These results are consistent with previous work showing altered GABA compartmentalization in spinal tissues as a consequence of time postcrush. Possible alterations in other transmitters cannot be discounted. But, the role of GABA in postcrush spinal cord alterations provides a model for both spinal plasticity and dysfunctions.

LONG TERM POTENTIATION

26.1

WITHDRAWN

26.2 QUESTIONABLE EFFECTIVENESS OF NITRENDIPINE AND VERAPAMIL IN SELECTIVELY BLOCKING POSTSYNAPTIC CALCIUM CHANNELS IN HIPPOCAMPUS. J.S. Taube and P.A. Schwartzkroin. Dept. of Neurological Surgery, Univ. of Washington, Seattle, WA 98195.

Several investigations have suggested that Ca^{++} is necessary for the postsynaptic component (EPSP-spike potentiation) of LTP. We wanted to further test the hypothesis that functional postsynaptic, voltage-dependent Ca^{++} channels are critical for development of LTP. A selective block of these Ca^{++} channels (without interfering with Ca^{++} channels involved in synaptic transmission) has been suggested in other studies employing verapamil (Sastry et al., Life Sci. 34: 385). However, investigators disagree on the selectivity and effectiveness of such blockers (Dingledine, J. Physiol. 343:385). We examined the effectiveness of nitrendipine and verapamil in: 1) blocking synaptic transmission, 2) blocking postsynaptic Ca^{++} channels, and 3) preventing development of LTP.

Intracellular recordings were obtained from CA1 pyramidal cells in guinea pig hippocampal slices. Cells were tested with nitrendipine (1mM dissolved in 95% ethanol and then diluted in the balanced salt solution to 10 µM) and verapamil (1 mM dissolved in distilled water and drop applied onto the somatic/apical dendritic region).

Following application of nitrendipine in ethanol there was no consistent change in the AHP (following current-induced repetitive spiking) or the orthodromic response to Schaffer collateral stimulation. Ca^{++} spikes (evoked by depolarizing current in the presence of 100 µM TTX) showed either no change or a small reduction in amplitude. Tetanization of the Schaffer collaterals produced no change or a small decrease in the population spike in 7/9 slices; in the other two slices there was a two-fold increase in the amplitude of the population spike. Similar results were obtained when only ethanol was added to the bathing medium, suggesting that the absence of potentiation in the nitrendipine-treated cells was due to effects of the ethanol. Verapamil application had no effect on the AHP and Ca^{++} spike, but did produce a consistent decrease in the Schaffer-evoked population spike. Tetanization produced potentiation of the population spike in 5/7 slices.

These results suggest that the organic Ca^{++} channel blockers, nitrendipine and verapamil, are not selective against postsynaptic Ca^{++} channels in hippocampus. They thus cannot be used to demonstrate a specific postsynaptic calcium role in LTP.

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26.3 GABA SENSITIVITY DOES NOT CHANGE DURING LONG-TERM POTENTIATION IN RAT HIPPOCAMPAL SLICES. H. E. Scharfman and J. M. Sarvey, Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814

Long-term potentiation (LTP) in the hippocampus is a long-lasting enhancement of synaptic efficacy, following high frequency, repetitive stimulation. Changes in excitatory or inhibitory pathways may mediate LTP. Evidence that the IPSP decreases in amplitude during LTP suggests that an alteration of inhibitory processes could be important to the production of LTP. We asked whether a decrease in postsynaptic sensitivity to the major inhibitory transmitter in the hippocampus, gamma-aminobutyric acid (GABA), could explain the depression of the IPSP during LTP.

Extracellular recordings were taken from the CA1 cell body layer of 375μm hippocampal slices. Stimulation of the stratum radiatum was used to produce a population spike. GABA (10mM) was pressure-ejected through a micropipette placed within 50μm of the recording electrode. Postsynaptic sensitivity to GABA was tested at the soma, since the IPSP is thought to be produced by somatic GABA receptors. GABA produced a dose-dependent, reversible inhibition of the population spike amplitude, without affecting the extracellularly recorded EPSP. A fixed amount of GABA inhibited the same percentage of the population spike, with a similar time course of recovery, regardless of the amplitude of the spike. In some cases, the short period of inhibition was immediately followed by an equally short period when the population spike showed a rebound increase in amplitude, and then returned to control amplitude.

In most experiments, repetitive stimulation (100 Hz for 2 sec) produced short-term potentiation of the population spike (STP; 1-15 min duration; mean % of control amplitude ± SEM : 202% ± 35.0) followed by LTP (duration $\geq$ 1 hr; 209% ± 17.0). Neither the inhibition, duration of effect, dose-dependence, nor rebound (when it occurred) changed after repetitive stimulation, whether STP and LTP occurred or not (n=12). Preliminary evidence suggests that the same is true in field CA3 (n=2) and the fascia dentata (n=2). This suggests that a decrease in postsynaptic sensitivity to GABA is not the mechanism for the depression of the IPSP which occurs during LTP. A decrease in transmitter release may be an alternative explanation.

26.4 INHIBITION OF PROTEIN SYNTHESIS SPECIFICALLY BLOCKS NOREPINEPHRINE-INDUCED LONG-LASTING POTENTIATION IN THE FASCIA DENTATA OF RAT HIPPOCAMPAL SLICES. P.K. Stanton and J.M. Sarvey. Dept. of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Hippocampal long-term potentiation (LTP) is a long-lasting enhancement in synaptic efficacy after brief, high frequency repetitive stimulation. We have provided evidence that ongoing protein synthesis is required for LTP to occur. Other groups have shown that depletion of forebrain norepinephrine (NE) by 6-hydroxydopamine reduces the amplitude of LTP in the fascia dentata, and NE potentiates the evoked population response in the same area. Our study was undertaken to better characterize the NE-induced potentiation in the fascia dentata, and to test the ability of the protein synthesis inhibitor emetine to block this potentiation as it does LTP.

Extracellular potentials evoked in the dentate by stimulating the perforant path were recorded in hippocampal slices (400μm thick). NE was bath perfused for 30 min at 1,5, 10,50 or 100 μM. The increase in spike amplitude after 30 min of NE perfusion was defined as NE-induced potentiation (NEP), and potentiation after an additional 30 min drug-free wash was defined as NE-induced long-lasting potentiation (NELLP). NE produced a dose-dependent potentiation of the population spike during the 30 min perfusion, which increased after the 30 min wash (% of control ± SEM; * $p<.05$, paired t-test).

[NE] (μM)	N	30 min NE (NEP)	30 min WASH (NELLP)
1	4	108.9 ± 4.6	105.0 ± 15.4
5	4	99.6 ± 12.9	122.0 ± 22.2
10	8	143.7 ± 13.4*	149.8 ± 19.9*
50	7	146.2 ± 13.1*	164.6 ± 27.8*
100	6	140.8 ± 11.0*	178.2 ± 25.2*

Both NEP and NELLP were completely antagonized by the β antagonist propranolol (50μM,N=4), and were specific to the fascia dentata, since NE (50μM,N=3) produced only a slight, transient depression in spike amplitude in field CA1. The protein synthesis inhibitor emetine (15μM, which inhibits protein synthesis in slices by >95%) perfused for 30 min before, and throughout the NE perfusion (50μM) and wash, did not alter the NEP seen during perfusion of NE (146.0 ± 21.8%, N=6), but blocked NELLP after the wash (113.1 ± 3.8%,N=6).

These results parallel blockade of LTP by protein synthesis inhibitors, and suggest a necessity for newly synthesized or rapidly turned over proteins in the production of NELLP.

26.5 Voltage-clamp analysis of excitatory and inhibitory conductances during long-term synaptic potentiation in hippocampus. W.H. Griffith, T.H. Brown and D. Johnston. Dept. of Med. Pharm. and Toxicol. Texas A&M Univ., College Station, TX 77843; Div. of Neurosci., Beckman Res. Inst. of the City of Hope, Duarte, CA 91010; and Dept. of Neurology, Baylor Coll. of Med., Houston, TX 77030.

The application of voltage-clamp techniques to the study of synaptic conductances in the mammalian central nervous system has provided a powerful tool for analyzing the mechanisms of synaptic plasticity (Brown and Johnston, J. Neurophysiol. 50, 1983; Johnston and Brown, in: Brain Slices, 1984). Long-term synaptic potentiation (LTP) has generated a great deal of interest as a possible cellular mechanism for certain aspects of memory. Recent work has shown that LTP has associative properties (Barrionuevo and Brown, Proc. Nat. Acad. Sci. 80, 1983) as well as being regulated by noradrenergic receptors (Hopkins and Johnston, Neurosci. Abstr., this volume).

We have been particularly interested in the mossy fiber synapses onto CA3 pyramidal neurons in the hippocampus. These synapses have been demonstrated to be within about 6% of a length constant from the cell bodies of CA3 neurons and thus amenable to voltage-clamp analysis (Johnston and Brown, J. Neurophysiol. 50, 1983).

In this study we investigated whether changes in the biophysical properties of the mossy fiber evoked excitatory and/or inhibitory synaptic inputs occur with LTP. We utilized the in vitro rat hippocampal slice preparation in normal saline and single-electrode voltage-clamp methods. Current-voltage relationships of the mossy fiber evoked synaptic input were analyzed before and 15 min after a 100 Hz, 1 sec stimulation of the mossy fibers. The conductance of the inhibitory input showed little or no change during LTP - if anything, there was a slight increase in conductance that may have been due to contamination by the excitatory input. The measured conductance of the excitatory input, however, increased dramatically - usually in the range of 50-100%. These results, as well as other (Barrionuevo and Brown, Neurosci. Abstr., 1983), suggest that changes in inhibition are neither necessary nor sufficient to explain LTP. The temporal overlap of the excitatory and inhibitory inputs, and the problem this causes in the analysis of synaptic changes during LTP, will be discussed. (Supported by NIH Grant NS15772, AFOSR R49620 and McKnight Scholars and Development Awards).

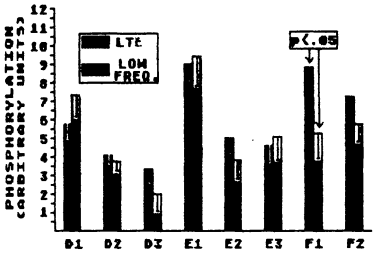
26.6 LONG-TERM CHANGES IN THE PYRIFORM CORTEX EVOKED POTENTIAL PRODUCED BY STIMULATION OF THE OLFACTORY BULB. J. S. Stripling, D. K. Patneau, and C. A. Gramlich*. Dept. of Psychology, University of Arkansas, Fayetteville, AR 72701.

Electrical stimulation of the olfactory bulb produces an evoked potential (EP) in the pyriform cortex characterized by an initial surface-negative wave representing activation of the principal neurons of the pyriform cortex via the lateral olfactory tract, followed by a surface-positive wave which is associated with recurrent inhibition. The experiment reported here examined the changes which occur in the pyriform cortex EP following a pattern of stimulation which has been found to produce short- and long-term potentiation in other areas of the forebrain.

Male Long-Evans rats with electrodes in the olfactory bulb and pyriform cortex were divided into two groups. Experimental animals received high-frequency stimulation of the olfactory bulb in the form of 30 trains delivered at 10-sec intervals; each train consisted of ten 0.2 msec pulses at a frequency of 100 pulses/sec. Control animals received the same number of pulses delivered at a lower frequency (1 pulse/sec). This procedure was repeated six times at 2-day intervals using current intensities of 67, 200, or 600 microamperes. Neither the Experimental nor the Control treatment altered the initial component of the pyriform cortex EP, indicating that the synaptic input to this area arriving via the lateral olfactory tract was unaffected. However, the Experimental animals exhibited a marked increase in the duration of the second component of the EP which appeared to reflect two separate processes: a short-term change which peaked within 30 min of the trains, and a long-term change which accumulated across the six treatments. The Control animals exhibited no short-term change in the EP, but did show a long-term increase in the duration of the second component which, while smaller than that seen in Experimental animals, reached statistical significance. These results suggest that repeated stimulation of the olfactory bulb, especially at high frequencies, causes a persistent alteration in the way information is processed by the pyriform cortex. The nature of this change is difficult to determine from the present experiment, but it may represent an increased inhibitory response, since paired-pulse inhibition of the EP was enhanced in the Experimental group (and to a lesser extent in the Control group) in association with the long-term increase in the duration of the second component of the EP.

26.7 **PROTEIN F1 (47kD,4.5pI) IN VITRO PHOSPHORYLATION INCREASED BY AND DIRECTLY RELATED TO THREE DAY GROWTH OF LONG TERM SYNAPTIC ENHANCEMENT.** D.M. Lovinger, R.F. Akers, R.B. Nelson, Cresap Neurosci. Lab. (CNL), Northwestern Univ., Evanston, IL 60201, C.A. Barnes, B.L. McNaughton, Dept. Psych., Univ. of Colorado, Boulder CO 80309, and A. Routtenberg (CNL).

Increased in vitro phosphorylation was only observed (Fig.1) in Protein F1 in dorsal hippocampal tissue from chronically implanted animals exhibiting long term enhancement (LTE) three days after high frequency stimulation, compared to tissue from low frequency stimulated or unoperated animals. F1 phosphorylation appears to be related to LTE rather than mere activation of perforant path-dentate gyrus synapses. Protein F1 phosphorylation was directly related to the 3-day growth of LTE of population spike amplitude (r=+0.66, p<.05), or population EPSP amplitude (r=+0.64, p<.05). Protein F1 may regulate growth or retard decay of long lasting synaptic plasticity. This relationship between LTE and phosphorylation was selective for Protein F1. Protein F1 is a substrate for the calcium/ phospholipid-dependent protein kinase C (see Nelson et al., this meeting). The LTE-related increase in F1 phosphorylation lasting days may involve a long term alteration in the activity of this novel kinase, which has been related to growth (Boynton, A.L. et al., Biochem. Biophys. Res. Comm., 1983). (Supported by MH2521, and AFSOR 83-0335 to A.R.)



26.8 DETERMINANTS OF ASYMPTOTIC LIMITS OF LONG-TERM POTENTIATION. B.Burger, R.Dickstein and W.B Levy (SPON: J.Jane). Dept. of Neurosurgery, UVa Sch. of Med., Charlottesville, Va 22908.

The asymptotic limits of long-term potentiation are well known. Combined with other findings, i.e. long-term depression and associative requirements of synapse modification, such limits are central to algebraic descriptions of the synaptic modification process. In one formulation[2] the asymptotic limits could be dictated by specific or non-specific inputs. Specific means a process controling each synapse individually; non-specific implies an analog to arousal. The data here argue that these limits are not set by non-specific influences. The experiments resemble L&S[1] except for the injection of tetrodotoxin into the entorhinal cortex to reduce recurrent activity. Experiments used stimulating electrodes bilaterally placed in the angular bundles(AB). Extracellular field potentials(pEPSP) were monitored in the dentate gyrus(DG) on both sides, recording a contralateral and ipsilateral pEPSP for each AB stimulation. 10 rats received 2 distinct conditioning periods separated by an intermediate baseline. The 2 periods shared the same conditioning parameters (30 trains @ 400Hz, 1/145s), but while conditioning in the first period was unilateral, it was simultaneously bilateral in the second. Test stimulation monitored synaptic efficacy between each 400 Hz train and during periods of no conditioning (15m initial baseline, a 10m intermediate baseline, and a 15m postconditioning period). Results are for pEPSP's in the DG which experienced conditioning to its ipsilateral inputs during both conditioning periods. Comparison between initial and intermediate baselines showed significant potentiation of the conditioned ipsilateral pEPSP (261%;t=5.8;df=9) and significant depression of the converging contralateral pEPSP (80% of baseline;t=2.6;df=9). The second period with its paired conditioning gave little increase in the ipsilateral pEPSP (109%) thus establishing the asymptotic limit of ipsilateral potentiation. Yet simultaneously, the contralateral pEPSP potentiated significantly (157%;F=11;df=9). Thus synaptic potentiation occurs within microns of synapses that have reached their asymptotic limit of potentiation. The results are consistent with the synaptic modification form $\Delta m_i = \varepsilon f(y)(x_i - cm_i)$ where ε is a non-specific factor; $f(y)$ is a postsynaptic term involving integration of converging inputs. The parenthetical term depends on afferent activity and gives specificity to each synapse. It is the final determinant of the asymptotic limits of synapse modification. Supported by NIH NS15488 & AFOSR 830236. [1]Levy and Steward, Br.Res. 175(1979)233. [2]Levy et al., Neurosci. 8(1983)799.

26.9 LONG-TERM REVERSIBLE CHANGES IN THE TRANSLATION OF SYNAPTIC CURRENT INTO CELL FIRING. W. B Levy. Dept. of Neurosurgery, U. Va. Sch. Med., Charlottesville, VA 22908.

Production of long-term potentiation (LTP) of the synaptic response (pEPSP) in the ipsilateral entorhinal cortex (EC) - dentate gyrus (DG) system is well reported. Concomitant with this pEPSP-LTP is potentiation of the translation of synaptic current into cell firing (SC→CF).[1,2] This altered translation is removed with paired ipsilateral angular bundle (AB) + DG commissural (COMM) conditioning.[3] The study here independently replicates both of these findings and additionally shows that 1) this depressed translation can be induced by COMM conditioning alone as well as by paired AB+COMM conditioning; 2) the translation can be depressed simultaneously with pEPSP-LTP; and 3) this depression does not require prior LTP.

This study includes 17 rats prepared as usual[1,2]. A recording electrode was placed in the DG cell layer. Ipsilateral to this electrode was a stimulating electrode in the AB positioned to produce a medial EC-type response. A COMM stimulating electrode was placed contralateral and anterior to the recording electrode and was positioned to maximize inhibition of the AB-induced population spike (giving reductions of at least 90%). For conditioning stimulation of either AB or COMM alone, 8 trains (1/10s) of 8 pulses were delivered @ 400 Hz. For paired AB+COMM conditioning, the COMM train length was extended by 2 pulses and preceded AB stimulation by this amount. The 7 animals receiving paired conditioning received electrolytic EC lesions prior to the electrophysiological measures and manipulations. All testing was to AB stimulation alone. Test pulses were delivered 1/30s at one of four sequenced intensities for 20 to 30m postconditioning. SC→CF translation is quantified as in Wilson[1].

AB conditioning increased the translation of SC→CF in 5 of 6 animals. Following this conditioning procedure, COMM conditioning alone depressed translation in 6 of 6 animals. In fact, in 4 of the 5 animals where comparison is possible, the depression not only removed the effects of potentiation but depressed translation beyond the original baseline values. Paired conditioning also depressed translation in 6 of 7 rats. In 6 of these animals pEPSP-LTP was also observed, thus indicating the independence of the two forms of modification. Over all animals without prior AB conditioning, translation was depressed beyond baseline levels 7 out of 9 times using either COMM alone or COMM+AB conditioning.

[1]Wilson. J. Neurophysiol. 46(1981)324. [2]Wilson et al. Ibid 339. [3]Brassel et al. Neurosci. Abstr. 8(1982)740.

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26.10 LTP-ASSOCIATED CHANGES IN POSTSYNAPTIC DENSITY LENGTH AND THE LENGTH OF THE SYNAPTIC INTERFACE IN THE DENTATE GYRUS. N. L Desmond and W. B Levy. Dept. Neurosurgery, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908.

Following brief, high-frequency conditioning trains, the size of dendritic spines in the central zone of synaptic activation in the dentate gyrus increases.[1] Here we report that the postsynaptic densities and the interface between the pre- and postsynaptic elements, the apposed length, increase. This increase is specifically associated with concave spine heads. These results are compatible with the interpretation that LTP results from bigger and more efficacious synapses.

Eleven animals were studied. Group 1 further analyzes the 6 animals of D&L[1]. In Group 2 (N=5), the conditioning stimulation delivered to one angular bundle consisted of 24 trains (1 train/5s) of 8 pulses @ 400 Hz. These animals were sacrificed 10 min after the 24th train. Response amplitude was required to increase at least 50% over the preconditioning baseline response amplitude for an animal to be included in the study. Animals were perfused with mixed aldehydes and blocks of dentate gyrus were prepared conventionally for transmission EM. Asymmetric shaft and spinous synapses were quantified double-blind on montages of the dorsal leaf molecular layer. Spinous synapses were categorized by shape[1] and their PSD and apposed lengths were quantified. The middle third of the molecular layer is the region of primary synaptic activation during conditioning stimulation.[1] In the middle third of the molecular layer, the total apposed length of all identified synapses increases 32% (t=3.84, p<.05) for Group 1 and 12% (t=1.78,NS) for Group 2. Total PSD length also increases following conditioning stimulation, averaging 15% (t=1.65,NS) for Group 1 and 10% (t=2.04, p<.05) for Group 2. The increased apposed and PSD lengths overall following conditioning stimulation are associated with the population of U-shaped spinous synapses in the middle third of the molecular layer. In this region, the total apposed length of U-shaped spines increases 112% (t=3.76, p<.05) for Group 1 and 41% (t=2.24, p<.05) for Group 2. Total PSD length of U-shaped spines increases similarly in the middle third of the molecular layer, averaging 89% (t=3.46, p<.05) for Group 1 and 44% (t=4.24, p<.05) for Group 2. Although stereological corrections are incomplete, the data clearly show that more surface area per dendritic spine is associated with LTP and not more spines in the region of primary afferent activation.

[1]Desmond & Levy. Brain Res. 265 (1983) 21-30.

Supported by grants (NIH NS15488 & AFOSR 83-0236) to WBL.

26.11 TEMPORAL CONTIGUITY REQUIREMENTS FOR ASSOCIATIVE LONG-TERM POTENTIATION IN HIPPOCAMPAL SLICES. S. R. Kelso and T. H. Brown, Div. of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Associative long-term potentiation (LTP) has been suggested as a possible mechanism for aspects of learning and memory. In the in vitro hippocampal slice preparation, a conditioning stimulus train delivered to a weak synaptic input to the CA1 region produced LTP in that pathway only if a separate stronger input to this region was concurrently stimulated with a conditioning train (Barrionuevo & Brown, Proc. Natl. Acad. Sci. 80:7347, 1983). This phenomenon was termed associative rather than heterosynaptic LTP because presenting the same conditioning train to the strong input alone did not produce LTP in the weak input.

We were interested in the timing rules governing the induction of associative LTP. To what extent do the temporal contiguity requirements resemble those observed behaviorally in classical conditioning studies? Can associative LTP be induced if the conditioning trains that are presented to the two pathways are separated in time? More generally, how does the interstimulus interval affect the occurrence, magnitude and duration of associative LTP? Such information is relevant to the possible role of associative LTP in learning and memory and also to the design of experiments aimed at understanding the biophysical and biochemical mechanisms responsible for this intriguing phenomenon.

We have begun to investigate formal analogs to forward, backward, and trace classical conditioning. Before and 20 min after presenting a 100 Hz conditioning train to the two synaptic inputs to the CA1 region, the amplitude of the response to the weak input was tested every 6 seconds. The conditioning trains presented to the weak and strong inputs lasted 650 and 450 msec, respectively. In the forward conditioning paradigm, the onset of the weak-input conditioning train preceded the onset of the strong-input conditioning train by 200 msec. In the backward conditioning paradigm, the onset of the conditioning train delivered to the strong input preceded that presented to the weak input by 750 msec. In the trace conditioning paradigm, the onset of train delivered to the weak input preceded that delivered to the strong input by 750 msec.

Our preliminary results indicate that the forward but not the backward conditioning paradigm is effective in inducing associative LTP. We are uncertain about the trace conditioning paradigm. Supported by NIH grant NS07408, AFOSR Contract F49620 and a McKnight Foundation Scholar's Award.

26.12 ROLE OF DENDRITIC SPINES IN MODULATING SYNAPTIC EFFICACY. T. H. Brown, S. Kelso, D. Johnston and R. A. Fricke. Div. of Neurosciences, Beckman Res. Inst. of the City of Hope, Duarte, CA 91010; Dept. of Neurology, Baylor Col. of Med., Houston, TX 77030; and Dept. Anatomy, Emory Univ. Schl. of Med., Atlanta, GA 30322.

The role of dendritic spines in synaptic signalling has been a mystery that has invited considerable speculation and theoretical analysis. Our initial computer simulations of hippocampal neurons quickly demonstrated that certain interesting effects of the spines depend critically on the synaptic conductance waveform--information that was previously unavailable for any vertebrate central neurons. Now that this key information is available for hippocampal neurons (Brown & Johnston, J. Neurophysiol., 1983; Johnston & Brown, In: Brain Slices, 1984; Kelso & Brown, unpublished), we can evaluate certain hypothesized roles of dendritic spines.

We have been particularly interested in Rall's proposal that changes in the shapes of dendritic spines could modulate synaptic efficacy and that such shape changes could underlie long-term forms of synaptic memory (Rall, In: Studies in Neurophysiology, 1978). The feasibility of this hypothesis depends on whether spines actually cause significant attenuation of the excitatory postsynaptic potential (EPSP) in the parent dendrite. Our computer simulations have shown that the amount of attenuation depends on two parameters--the peak synaptic conductance G_s and the reciprocal of the spine axial resistance G_a. If G_s is small relative to G_a, the amount of attenuation is negligible. In this case, reasonable variations in the spine shape would have little effect on synaptic efficacy, and the Rall hypothesis would have to be abandoned or modified. However, we find that apparently realistic values of G_s can result in significant EPSP attenuation. A spine shape change that increased G_a would be manifest as an increase in the measured synaptic conductance G_m and therefore an increase in the mean quantal size. If a voltage clamp were applied to the parent dendrite, at the site of the spine attachment, the value of $G_m = G_a G_s/(G_a+G_s)$.

The significance of this and other effects of the dendritic spines will be discussed in relation to our findings and current hypotheses regarding long-term synaptic potentiation. Supported by NIH grants NS07190 and NS18861, McKnight Scholars and Development Awards, and AFOSR Contract F49620.

26.13 HIPPOCAMPAL NEURON INPUT IMPEDANCE AND SPIKE THRESHOLD BEFORE AND DURING LONG-TERM SYNAPTIC POTENTIATION. G. Barrionuevo, and T. H. Brown. Div. of Neurosciences, Beckman Res. Inst. of the City of Hope, Duarte, CA 91010.

Long-term potentiation (LTP) is a use-dependent form of enhanced synaptic efficacy that can persist for hours or longer and can be induced by high-frequency activation of the synapses for only a few seconds or less. This great asymmetry between the duration of the synaptic activity and the duration of the subsequent synaptic change is the defining characteristic of LTP--a property that makes this phenomenon a possible candidate for certain forms of information storage in the nervous system.

We were interested in knowing whether LTP might be due in part to changes in the postsynaptic active or passive membrane properties. Our previous studies of LTP in hippocampal slices found no increase in the postsynaptic input resistance or membrane time constant, measured by applying inward current steps (Barrionuevo & Brown, Proc. Natl. Acad. Sci.,80:7347-7351, 1983; Barrionuevo, et al., Soc. Neurosci. Abst., 9:103a, 1983; Barrionuevo, et al., in preparation). In the present study we investigated the postsynaptic input impedance and spike threshold, before and during LTP, by injecting an outward current waveform (an alpha function) that mimicked the actual synaptic current waveform that we have measured under voltage-clamp conditions (Brown & Johnston, J. Neurophysiol., 50:487-507, 1983; Barrionuevo, et al., Soc. Neurosci. Abst., 9:103a, 1983; Barrionuevo, et al., in preparation). For comparison, we also measured the input resistance using inward current steps. Values of the resting membrane potential and the excitatory postsynaptic potential (EPSP) amplitude were also measured.

Results of intracellular measurement on 9 hippocampal neurons from regions CA1 and CA3 are summarized below. Only the EPSP increased significantly. In some cells LTP was accompanied by a slight excitability increase, but additional experiments are needed to verify this result.

Measurement	Control	During LTP	% Change
EPSP amplitude, mV	7.0 ± 1.1	11.8 ± 1.3	+68.6
Resting Potential, mV	81.9 ± 2.3	82.0 ± 2.4	+0.1
Input Resistance, MΩ	52.8 ± 6.5	53.0 ± 6.0	+0.4
Input Impedance, MΩ	9.7 ± 1.8	9.9 ± 1.9	+2.1

Supported by NIH grant NS18861, a McKnight Foundation Scholar's Award, and AFOSR Contract F49620.

26.14 EFFECT OF PRIOR INDUCTION OF LTP IN THE TEMPORODENTATE PATHWAY ON SUBSEQUENT KINDLING. T. Sutula, O. Steward, Depts. of Neurosurgery, Neurology, and Physiology, University of Virginia, Charlottesville, VA 22908

Kindling refers to progression of electrographic and behavioral seizures as a consequence of repeated stimulation of certain brain structures. The phenomenon has been studied as a model of focal epilepsy, and its cellular mechanism is of interest. One of the proposed mechanisms of the kindling effect is long-term potentiation(LTP) which is an increase in synaptic efficacy induced by brief trains of stimulation. In a previous study (T. Sutula and O. Steward, Neurol 33 (Suppl 2):188, 1983),we demonstrated that kindling induced by stimulation of the entorhinal cortex(EC) was accompanied by an increase in synaptic efficacy of the monosynaptic projection from EC to the dentate gyrus(DG), which was similar to LTP. To more directly test the hypothesis that LTP might represent a mechanism of kindling, we attempted to determine a) whether chronic stimulation designed to produce LTP but no afterdischarges is sufficient to induce kindling, and b) if LTP did not by itself produce kindling, whether subsequent kindling in this pathway occurred more rapidly after induction of LTP.

Using chronic neurophysiological techniques, the EC was stimulated, and evoked responses and afterdischarges(AD) were recorded in the DG. One group (LTP-Kindle, n=9) received potentiating stimulation (8 trains of 8 pulses at (400HZ) for 20 days, followed by daily kindling stimulation (1 train of sixty 1 msec pulses at 60HZ). A control group (Control-Kindle, n=8) received only test pulses for 20 days (64 pulses at 0.33HZ), followed by daily kindling stimulation. A third group received only kindling stimulation beginning one week after surgery (Kindle, n=11).

Delivery of potentiating stimulation(LTP-Kindle group) failed to produce AD or seizures during the first 20 days. After initiation of kindling stimulation, this group required an average of 9.66 AD's before exhibiting class V seizures. The Control-Kindle group developed class V seizures after 26.63 AD's, while the Kindle group developed class V seizures after 18.36 AD's. The LTP-Kindle group reliably developed AD with the first kindling stimulation, but the other groups required an average of 2.77 stimuli to induce AD. (Differences significant at ($p < 0.05$).

The results suggest that while LTP is not sufficient for kindling, it may facilitate subsequent kindling.

26.15 INTERACTIONS BETWEEN SEPTODENTATE AND PERFORANT-PATH INPUTS TO THE DENTATE GYRUS. G.B. Robinson and R.J. Racine. Dept. of Psychology, McMaster University, Hamilton, Ontario, L8S 4K1.

One of the characteristics of long-term potentiation (LTP) is its dependence on cooperative interactions between neural pathways. Concurrent high-frequency activation of perforant path and septodentate (SD) afferents, for example, results in significantly greater LTP of the perforant path-granule cell (PP-GC) population spike than results from PP trains alone (Robinson, G. & Racine, R. Brain Res., 249:162, 1982). This associative interaction may result from a suppression or blockade of the GC recurrent inhibitory circuits by the SD inputs.

If the SD input blocks or reduces recurrent inhibition, then it should also block or reduce paired pulse depression in the PP-GC circuit. We utilized a 3 pulse paradigm to investigate this possibility. SD pulses were applied 1) 5 ms prior to the conditioning pulse, 2) 5 ms following the conditioning pulse, or 3) 5 ms prior to the test pulse of a perforant path pulse pair. The interval between the perforant path pulses ranged from 20 ms to 10 sec. If the SD input interferes with recurrent inhibition, then the first pattern (SD pulse preceding PP conditioning pulse) should block the interneurons involved before the granule cell collaterals activate them. The 2nd and 3rd patterns should have little effect because the interneurons will already have been activated by the collateral discharge from granule cells (evoked by the conditioning pulse).

SD pulses prior to the conditioning pulse either had no effect on paired pulse depression or increased it slightly. SD pulses following the conditioning pulse significantly increased paired pulse depression effects, particularly the late component (interpulse intervals of 100 ms to 2 sec). SD pulses prior to the conditioning pulse produced a large decrease in the late phase of depression.

These results appear to be more consistent with a direct action of SD afferents on granule cells rather than an indirect action via inhibitory interneurons.

26.16 PAIRED-PULSE POTENTIATION AND SUMMATION OF PERFORANT PATH POTENTIALS. R.M.Douglas, Dept. of Physiology, McGill University, Montreal, Canada H3G 1Y6.

When the perforant path is stimulated twice in close succession, the field EPSP evoked by the second stimulus is often larger than that evoked by the first. The amount of potentiation observed is less however when using higher stimulation intensities (Steward et al, EBR, 1976). Whether this decrease is due to nonlinear summation in the postsynaptic granule cells was examined in the present study by independently varying the intensities of the first (conditioning) and second (test) pulses.

That postsynaptic summation is, in fact, quite linear was most evident in experiments in which a low intensity pulse was used to condition a small population of synapses which were then interrogated in the presence of additional synaptic inputs brought in by higher intensity test pulses. The absolute value of the increment in EPSP size so produced was relatively independent of test intensity.

In contrast, alterations in conditioning intensity had a marked effect on the amount of potentiation measured. Low intensity conditioning stimuli produced 30-40% increases, while the potentiation after high intensity stimuli typically was only 5-10%. When the intensity of the conditioning stimuli was varied over a wide range, the effect on the measurable potentiation was most marked for intensities evoking a population spike threshold. Inhibition evoked by commissural stimulation also affected the size of the perforant path response, suggesting that the presence of little apparent potentiation after high intensity conditioning stimuli is due, at least in part, to shunting of the postsynaptic potentials by inhibitory synapses. Since the reduction for a given conditioning intensity was about the same for both high and low intensity test pulses, this possible action of inhibitory synapses is divisive.

In conclusion, while summation in the dendrites of the granule cells is essentially linear, at least for synaptic inputs produced by single shocks, measurement of the magnitude of paired-pulse potentiation (and presumably also of longer-lasting potentiations) is highly dependent on the state of the postsynaptic cells.

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26.17 ADRENAL HORMONE MODULATION OF LONG-TERM SYNAPTIC ENHANCEMENT IN THE DENTATE GYRUS. Dana*, R. C. and Martinez, J. L., Jr., (SPON: M. R. Rosenzweig), Department of Psychology, University of California, Berkeley, CA 94720.

The effect of adrenalectomy (ADX) on long-term synaptic enhancement (LTE) of hippocampal dentate gyrus synapses was studied in pentobarbital anesthetized adult male Sprague-Dawley rats in vivo. Extracellular field potentials were recorded in the hilus of the dentate gyrus by stimulating the medial perforant path with monophasic test pulses once every 10 s. The stimulation intensity was set (5.9 +/- 0.3 V, mean +/- S.D.) to produce a 1 mV population spike. Following a 10 min baseline recording period, LTE was induced by presentation of 4 high-frequency trains (200 Hz, 250 ms) with 5 s intertrain intervals. The magnitude of LTE was assessed as the mV increase in population spike amplitude measured 20 min following tetanization at different times of the day. Animals were maintained on a 12 h lights-on, 12 h lights-off schedule.

The results from experiments conducted on 23 control and 15 ADX rats revealed that there was a circadian rhythm in the magnitude of LTE in both groups. Control animals studied during the dark phase demonstrated a 7.9 +/- 2.4 mV increase in population spike amplitude, which was significantly greater than that obtained for control animals studied during the light phase (1.8 +/- 0.8 mV). The opposite results were obtained for ADX rats, in that, significantly less LTE was obtained during the dark (2.1 +/- 1.0 mV) than during the light phase (6.6 +/- 2.2 mV). These results do not appear to be due to changes in the level of baseline excitability since there were no significant group differences in the stimulation intensity required to produce a 1 mV population spike.

Our previous report of an impairment of LTE in ADX rats (Dana et al., Neurosci. Abst., 8:316, 1982) may now be explained on the basis of a circadian effect, since in the absence of the secretions from the adrenal gland, there is a reversal in the phase of the circadian rhythm. Our earlier studies were conducted during the dark phase of the light-dark cycle. During this period ADX animals demonstrate the smallest amount of LTE, while normal rats demonstrate the greatest magnitude of LTE in the dentate gyrus.

Since ADX animals demonstrate substantial LTE during the light phase we suggest that other hormonal systems than adrenal hormones are involved in regulating the circadian rhythm of LTE. (Supported by ONR Contract N00014-83-K-0408 to JLM and the Rennie Foundation).

26.18 LONG-TERM POTENTIATION IN THE INTERPOSITUS AND VESTIBULAR NUCLEI. R.J. Racine, D.A. Wilson*, R. Gingell* & D. Sunderland*. Dept. of Psychology, McMaster Univ., Hamilton, Ontario L8S 4K1.

High frequency stimulation of forebrain pathways can produce a long-term potentiation (LTP) effect, which can last for several days or weeks. Many forebrain pathways support LTP (R. Racine, W. Milgram & S. Hafner, Brain Res., 260:217, 1983). Until recently, however, we have not been able to produce LTP effects in cerebellar circuitry, even though this structure is believed to be involved in learning. We have tested about 40 animals in a search for LTP in the cerebellar cortex. The only effect of trains applied to surface pathways or to deep white matter has been to produce a depression lasting for several sec to several min.

Recently, we have been looking at the deep nuclei (interpositus and vestibular) and we have found reliable LTP effects when trains are applied to the cerebellar peduncles. Animals were prepared with indwelling recording electrodes in either interpositus (3 animals) or the vestibular nucleus (5 animals). Stimulating electrodes were aimed at the inferior peduncle, but ended up deep in the white matter where the peduncle fibers enter the cerebellum. Tests pulses were delivered to the input pathway at a frequency of one every 10 sec. After recording 120 pre-train responses from the nucleus, the first set of 5 trains were applied. The test pulses were monitored for the following 10 min. This procedure was repeated until a total of 10 sets of trains had been applied. Train intensity was set at 100 uA for the first set of trains and was increased in 100 uA steps until the final set of trains.

The responses evoked in the nuclei consisted of one to three population spikes. That these were population spikes was confirmed (at least for the vestibular nucleus) by single cell recording. The application of the stimulation trains resulted in an increase in both the number and the size of the population spikes. This increase lasted for several weeks.

26.19 ON THE MECHANISM BY WHICH LONG-TERM POTENTIATION IS INDUCED IN THE RAT SUPERIOR CERVICAL GANGLION. C.A. Briggs, D.G. McKenna* and D.A. McAfee. Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Long-term potentiation (LTP) in the superior cervical ganglion is an increase in nicotinic synaptic transmission that lasts hours or longer, yet it is induced by only a few seconds of repetitive preganglionic stimulation. The present studies examine the processes underlying the induction of LTP. Ganglia were maintained in vitro at ambient temperature (21-23°C) by superfusion (1 ml/min) of oxygenated Locke's medium. The postganglionic compound action potential was recorded in response to preganglionic test stimuli at 1/min (Brown & McAfee, Science 215:1411, 1982). Atropine (2 uM) was present to block any muscarinic effects. CALCIUM: LTP could not be induced when Ca^{++} was absent from the bathing medium. ACTIVATION OF NICOTINIC RECEPTORS: Blockade of nicotinic receptors during the stimulus train by 3 mM hexamethonium did not reduce LTP. Likewise, stimulation of cholinergic receptors with bath-applied carbachol (100-1000 uM), instead of a conditioning train, did not induce a potentiation resembling LTP. However, transient depolarization with high K^+ (50 mM) did substitute for a stimulus train in inducing LTP. Because nonsynaptic (antidromic or intracellular current injection) stimulation of the postganglionic neuron does not potentiate nicotinic transmission, we suspect that the K^+ effect results from presynaptic depolarization. ACTIVATION OF ADRENERGIC RECEPTORS: Isoproterenol (3 uM pulsed for 3 min) caused a subsequent potentiation of ganglionic transmission that lasted for at least an hour; this was blocked by 1 uM propranolol. However, the LTP induced by a preganglionic stimulus train was not reduced by a variety of adrenergic antagonists (1 uM propranolol, 10 uM sotalol, or 1 uM phentolamine). When one branch of a divided preganglionic nerve was conditioned, test responses in the other branch were not potentiated. This suggests that LTP is not induced by a releasable factor that can diffuse throughout the ganglion. We conclude that LTP depends upon depolarization and Ca^{++} influx into presynaptic terminals. However, we cannot yet determine whether Ca^{++} itself induces LTP or supports the release of a non-adrenergic, non-cholinergic substance which in turn induces LTP. Supported by American Heart Association Fellowship #766F1, NSF Grant BNS 81-12414, NIH Grant NS-18966.

LIPIDS AND MYELIN

27.1 MORPHOLOGICAL AND IMMUNOCYTOCHEMICAL INVESTIGATIONS OF SPINAL NERVE ROOTS FROM NORMAL AND MYELIN DEFICIENT (MLD) MUTANT MICE: B. Droz* and F.X. Omlin* (SPON: C. M.-F. Marchand). Institut d'Histologie et d'Embryologie, Université de Lausanne, CH-1011 LAUSANNE-CHUV (Switzerland)

To define an unequivocal comparison between the central (CNS) and the peripheral (PNS) nervous system, we investigated the transitional zone of cervical spinal nerve roots. Myelin deficient (mld) is an autosomal recessive mutation; homozygout mice have both a severe myelin deficit in the CNS and an altered myelin composition (Matthieu, J.-M. et al., Develop. Brain Res., 13: 149, 1984). At 15 days of age, mld and age matched control animals were killed by intracardiac perfusion with a fixative containing glutaraldehyde/paraformaldehyde for electron microscopic (EM) investigations or $HgCl_2$/formaldehyde for immunocytochemistry of myelin-associated glycoprotein (MAG) at the light microscopic (LM) level.

The sections of mld tissue revealed no evident morphological alterations of myelin and glial cells in the PNS region of both dorsal and ventral roots. In contrast, the CNS region of the mld-roots showed axons ensheathed by thin and/or loose myelin. Within the ventral root these axons, which could be followed from the CNS to the PNS, showed a drastic swelling in the CNS. The axonal diameter of the same fiber was in these cases 4-5 times larger in the CNS than in the PNS. It is of interest, that these axons were ensheathed by only 1-2 turns of oligodendroglial membranes. Furthermore, the axoplasm of these fibers showed the following alterations compared to the control animal: bundles of neurofilaments, disorganized appearance of axoplasmic reticulum and accumulation of large vesicles. 20-µm thick vibratom sections were immunostained for MAG. Within the CNS part of mld dorsal and ventral roots, most of the oligodendrocytes were enlarged and more intensely immunostained than in sections obtained from control animals. The peripheral part of the mld roots showed a normal pattern of MAG immunostaining. MAG was restricted to the paranodal loops and Schmidt-Lantermann incisures.

In conclusion, a defect of CNS myelin coincides with dramatic alterations of motor axons in their CNS environment when they reach the transition zone prior to entry into the PNS.

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27.2 EFFECTS OF COMPLETE ISCHEMIA ON RAT BRAIN MITOCHONDRIAL LIPIDS. C. Viereck* and R.F. Del Maestro* (SPON: M.P. Rathbone), Brain Research Laboratory, Victoria Hospital, University of Western Ontario, London, Ontario N6A 4G5.

The mitochondria, in providing brain cells with their primary source of metabolic energy, are an absolute requirement for normal cerebral function and structure. It is known that phospholipids form a vital structural and functional part of mitochondrial membranes by helping the organelle provide a selectively permeable barrier essential for the maintenance of ionic gradients. Phospholipids also provide many mitochondrial enzymes with the proper viscosity and surface ionic environment for maintaining optimal function. In order to investigate the effect of ischemia at the membrane level, mitochondrial lipid analyses of normal and ischemic rat brains were undertaken.

The rat model of complete cerebral ischemia involved sacrificing animals by decapitation and placing the brains in a nitrogen bubbled 37°C saline bath. Ischemic periods of 15 and 30 minutes were studied.

It was found that total phospholipid phosphorus levels did not change during cerebral ischemia. Resolution of mitochondrial phospholipid species and determination of phospholipid phosphorus shows a significant 15-20% decrease in cardiolipin levels following 15 minutes of ischemia. Phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylinositol and sphingomyelin levels showed no significant changes. The total cholesterol levels increased 25% following 15 minutes of ischemia. Free fatty acid levels were characterized and quantitated by GLC. Of the fatty acids measured, arachadonic acid and docosahexaneoic acid levels were found to increase 300% following 30 minutes of ischemia. Bound fatty acids of the phospholipids resolved above were characterized and no change in the ratio of saturated fatty acids to unsaturated fatty acids occurred during cerebral ischemia.

The lipid changes found may help provide a greater understanding of events occurring in cerebral mitochondria during ischemia.

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27.3 DIVIDING OLIGODENDROCYTE PRECURSORS DO NOT STAIN FOR MYELIN BASIC PROTEIN. V.L. Friedrich, Jr. and N.H. Sternberger. Dept. of Biobehav. Sci., Univ. of Connecticut, Storrs, CT and Center for Brain Res., Univ. of Rochester Sch. of Med. and Dentistry, Rochester, NY 14642.

Mouse and rat pups were injected with tritiated thymidine 3 days after birth and were sacrificed 2h to eight days later by perfusion. Vibratome sections of spinal cord and brainstem were stained by the PAP procedure using antisera against myelin basic protein (MBP, kindly supplied by Drs. S.R. Cohen and R.M. Herndon) and embedded in epoxy resin. Two um thick sections were cut from the embedded material and processed for autoradiography.

At 3 days after birth, all areas contained a moderate number of immunostained cell bodies, cell processes and myelin sheaths. No myelin sheaths were present in much of the midbrain. Nevertheless, immunostained cell bodies were demonstrated there, indicating that our method is sensitive. The number of stained cell bodies increased substantially during the subsequent week, as did the number of myelin sheaths. All areas contained many labelled nuclei.

At 12h and 24h after thymidine injection, no radioactive cells were immunostained and no immunostained cells were radioactive. By contrast, profiles both radioactive and immunostained were common at 4 days after injection. At 2 days after injection, such profiles were present but relatively rare.

The present results indicate that the dividing precursors of oligodendrocytes do not stain for MBP during normal development. Stainable levels of MBP are present only in cells which are postmitotic, and appear 2-4 days after final division of the precursor cell.

As previously shown, oligodendrocytes in developing animals express stainable MBP before they make myelin sheaths. It follows that, at least in normal development, the dividing oligodendrocyte precursor does not bear myelin sheaths.

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27.4 BIOCHEMICAL AND IMMUNOCHEMICAL CHARACTERIZATION OF THE MYELIN-ASSOCIATED GLYCOPROTEIN FROM HUMAN TISSUE. A.B. Noronha*, T.J. Tolliver*, P.L. Grojec*, M.A. Curtis* and R.H. Quarles* (SPON: J. Hofteig) NINCDS & NIDR, National Institutes of Health, Bethesda, MD 20205.

The myelin-associated glycoprotein (MAG) has been implicated in pathological changes occurring in human demyelinating diseases including multiple sclerosis and progressive multifocal leukoencephalopathy. Recently it was shown to be an antigen reacting with IgM paraproteins associated with neuropathy (Braun et al J. Neurochem. 39: 1261, 1982; Steck et al Neurology 33:19, 1983.; Ilyas et al Proc. Natl. Acad. Sci., USA 81: 1225, 1984) and also to react with the mouse monoclonal antibody, HNK-1, that identifies a surface antigen on human natural killer cells (McGarry et al Nature 306: 376, 1983). For these reasons, it is important to characterize human MAG both chemically and immunologically. MAG was extracted from human brain myelin with lithium diiodosalicylate and further purified by gel filtration on Sepharose CL-6B. Amino acid and carbohydrate analyses revealed a composition similar to that previously described for rat MAG (Quarles et al. Biochim. Biophys. Acta 757: 140,1983). Human MAG contains about 23% glutamic plus aspartic acids, 11% basic amino acids and 23% nonpolar amino acids. GLC revealed the presence of Fuc, Man, Gal, GluNAc, and NANA. Human CNS MAG is rapidly converted to a slightly lower M_r derivative (dMAG) by a neutral protease in myelin, and the activity of this protease is elevated significantly in myelin from multiple sclerosis brains (Sato et al Ann. Neurol. 15: 264, 1984). The amino acid and carbohydrate composition of isolated dMAG was similar to that of intact MAG, except that preliminary results indicated a slightly higher content of nonpolar amino acids. Two dimensional polyacrylamide gel analysis showed that both MAG and dMAG separated into two major components with pI values between 3 and 4.5. Limited proteolysis of purified human MAG with trypsin produced fragments with M_r's of 68K, 25K, 16K, 14K and several below 10K. Concanavalin A bound primarily to the 16K fragment. The carbohydrate epitope that is recognized by human paraproteins and HNK-1 was primarily in the 68K and 25K fragments. The 25K fragment containing the antigen reacting with human paraproteins and HNK-1 exhibited a low pI similar to that of intact MAG and work to purify and characterize it is in progress. (This work was supported in part by a postdoctoral fellowship awarded to A.B.N. by the National Multiple Sclerosis Society.)

27.5 BINDING OF MONOCLONAL ANTIBODIES AGAINST MYELIN-ASSOCIATED GLYCOPROTEIN (MAG) TO A LOW MOLECULAR WEIGHT PEPTIDE IN PERIPHERAL NERVES (PN). L.S. Marton* and K. Stefansson* (SPON: B.G.W. Arnason). Dept. of Neurology and The Brain Research Institute, University of Chicago, Chicago IL 60637.

A rat monoclonal antibody (mAb) and a human mAb that recognize different epitopes on MAG were obtained. Both of the antibodies stain immunohistochemically the peri-axonal portion of CNS myelin but not intensely. On Western blots of adult CNS tissue they react specifically with MAG (mw 100,000) and dMAG (mw 90,000), a breakdown product of MAG produced by an endogenous protease. Immunohisto-chemically, both antibodies stain intensely myelin in PNs. It has been suggested that the greater staining of PN myelin than CNS myelin with some anti-MAG antibodies may be due to binding to a carbohydrate moiety shared by MAG and a PN ganglioside (Ilyas et al. PNAS 81:1225, 1984). We have shown that in addition to MAG/dMAG both of the mAbs react on Western blots of PN with a lower mw peptide. This peptide migrates on SDS polyacrylamide gels close to but just ahead of the Po protein.

This low mw peptide does not appear to be a breakdown product of MAG since incubation at 35°C for 5 hours does not result in a shift of the antigen from the position of MAG/dMAG to the low mw peptide. The low mw PN antigen does not co-purify with MAG in the Lis-phenol extraction procedure. We propose that the difference in intensity of immunohistochemical staining for MAG in the CNS and PN could in part be due to a low mw peptide in PN that shares antigenic determinants with MAG.

27.6 GLYCOLIPIDS OF THE BOVINE PINEAL. M.M. Whalen*, G.C. Wild and W.D. Spall* (SPON: J. Wallace). Dept. of Biochemistry, University of New Mexico School of Medicine, Albuquerque, NM 87131 and Toxicology Group, Los Alamos National Laboratories, Los Alamos, NM 87544.

As an initial phase of an investigation of synaptic function, we are focusing on glycolipids which are enriched in synaptic membranes. Bovine pineals contain a large volume of synaptic endings in association with pinealocytes. The synapses are relatively homogeneous, being noradrenergic with large amounts of serotonin. Knowledge of the glycolipids of pineal may increase understanding of pineal function.

Pineal acetone powder (either purchased or prepared from fresh frozen tissue) was chloroform: methanol extracted. The extract was applied to a silicic acid column and eluted batchwise with chloroform:acetone and chloroform: methanol mixtures. Column fractions were further purified by either thin layer chromatography (TLC) or a high-performance liquid chromatographic procedure (HPLC) which we have developed. The TLC or HPLC preparations were analyzed by gas chromatography.

The major glycolipid of pineal shows chromatographic behavior and molecular composition consistent with standard galactocerebroside at a concentration lower than in normal gray matter. 1.9% of the isolated, purified molecule contains a tetradecylglyceryl ether instead of sphingosine.

The major gangliosides have chromatographic behavior and component ratios consistent with GD3 and GM3. GD3 is present at 2-3 times the concentration of GM3. There is also evidence for gangliosides with high glucose content, one of which has the chromatographic characteristics of a disialoganglioside. This molecule yields a glucose/galactose ratio of 2.96.

We have also found an acetone-soluble molecule which, after methanolysis and trimethylsilylation, yields a ratio of total sugar to cholesterol of 1.14. The sugars are glucose, galactose, mannose and an unidentified peak. This molecule has an Rf of between 0.2 and 0.4 on thin-layer chromatography on silica in chloroform-methanol-2.5M NH3 (60:35:8). This polar form of cholesterol may be novel to mammalian tissue.

The ganglioside content of pineal is significantly different in pattern from that of whole brain. High glucose gangliosides which may be novel to mammalian tissue are also present. Other glycolipids include cerebrosides, glycosyl glyceryl ethers and a molecule which has both cholesterol and saccharide.

27.7 STRUCTURAL BASIS FOR SPECIFICITY OF AFFINITY PURIFIED ANTIBODIES TO GM1 GANGLIOSIDE AND RELATED GLYCOLIPIDS.
Y. Huang* and M.M. Rapport. Div. of Neuroscience, N.Y. State Psychiatric Inst. and Dept. of Biochem., College of Physicians and Surgeons, New York, N.Y. 10032

Since cell surface glycolipids may play a role as regulators of cell growth and differentiation, antibodies directed against these substances may be useful for investigating the mechanisms involved. We here report the reactivities of anti-glycolipid antibodies purified by elution from glycolipid-containing liposomes. Antisera were raised in rabbits by immunization with 4 glycolipid antigens: gangliosides GM1 and GM2, mixed brain gangliosides, and asialo GM1. The antibodies were purified by absorption on and elution from the respective glycolipid-containing liposomes (PNAS 79, 6080, 1983), labelled with radioiodine, and tested by RIA. Alternatively they were tested by ELISA using a commercial second antibody. Tests by ELISA and by RIA gave similar results. Antibodies to GM1 cross-reacted with asialo GM1 and GD1b (25% and 20% respectively of the GM1 reactivity) and gave little reaction (below 5%) with GD1a, GM2, GD3, cytolipin K (galNAc-gal-gal-glc-ceramide) or cytolipin H (gal-glc-ceramide). Anti-asialo GM1 antibodies and anti-GM2 antibodies did not cross-react (< 5%) with the other glycolipids. These results indicate that a major immunodeterminant of anti-GM1 antibodies is the hydroxyl group at C-3 of the terminal galactose residue. When NANA is attached to this group (as in GD1a), the antibodies no longer recognize the gal-β1→3galNAc structure which is present in GM1, GD1b and asialo GM1. Since affinity-purified polyclonal antibodies can be obtained to both GM2 and asialo GM1 which have much greater specificity than antibodies to GM1, we believe that securing specific antibodies with a high degree of specificity for GM1 (and some of the other gangliosides) may present a special problem. This problem probably results from steric configuration of the ganglioside antigens that affect both immunogenicity and cross-reactivity.

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27.8 MONOCLONAL ANTIBODIES TO GM1 GANGLIOSIDE. H. Laev*, S.P. Mahadik, and M.M. Rapport. Div. of Neuroscience, NYS Psychiatric Inst. & Depts. Biochemistry & Psychiatry, Coll. of Phys. & Surg., Columbia U., New York, N.Y. 10032.

Gangliosides are thought to play a critical role in various processes such as cell growth, differentiation, and synaptic function. These acidic glycosphingolipids are members of a large family of substances with a high degree of structural similarity and low immunogenicity, posing a special problem in obtaining conventional (polyclonal) antibodies specific for individual species. We have applied hybridoma methodology to obtain monoclonal antibodies to GM1 ganglioside, since polyclonal antibodies against this molecule have been useful in numerous biological studies over the past 10 years. Monoclonal antibodies were prepared by hybridizing myeloma cell lines (either P3X63Ag8 or X63Ag8.653) with spleen cells from BalbC mice immunized with GM1. Recloned cells producing antibodies to GM1 were detected by ELISA. Cell supernatants or ascites fluids were tested for specificity under optimal conditions to minimize the non- specific binding of different types of hybridoma immunoglobulins. Ascites fluids and supernatants representing 11 different clones were screened for reactivity with GM1, GM2, GD1a, GD1b, GT1b and asialo GM1. Two clones reacted almost exclusively with GM1, seven reacted predominantly with GM1, and two showed poor discrimination. We conclude that at least three different types of monoclonal antibodies to GM1 were obtained with different degrees of specificity depending on the portion of the molecule serving as the determinant. Antibodies with such selective differences in specificity may be useful for demonstrating the participation of individual molecules in biological processes.

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27.9 JIMPY-SHIVERER DOUBLE MUTANT MOUSE: CNS MORPHOLOGY.
A.-L. Kerner[1]*, D.A. Kirschner[1]*, J.H. Carson[2]*, J. Rosenfeld[3], S. Billings-Gagliardi[4], and M.K. Wolf[4]. [1]CHC and HMS, Boston, MA 02115; [2]U. Conn. Health Ctr., Farmington, CT 06032; [3]U. Conn., Storrs, CT 06268; [4]U. Mass. Med. Sch., Worcester, MA 01605.

Jimpy (jp) and shiverer (shi) both produce CNS hypomyelination, but have two distinct biochemical and morphological phenotypes. We sought additional information about expression of the two mutations by determining whether and how they interact in the same mouse. Three consecutive cycles of crossing proven, genetically marked Ta jp/+ females and their proven female offspring to shi/shi males produced marked, doubly affected Ta jp/Y - shi/shi males and crossover controls. Doubly affected animals were prepared both in Farmington and in Worcester on two different genetic backgrounds. Myelin basic protein (MBP), proteolipid protein (PLP) and 2',3'-cyclic nucleotide-3'-phosphohydrolase (CNP) had previously been quantitated in Farmington animals (Kerner and Carson, Trans. Am. Soc. Neurochem. 15: 235, 1984) and had showed, in percentages of wild-type:

	Ta jp/Y	shi/shi	Ta jp/Y - shi/shi
MBP	8-10%	<1%	5%
PLP	<1%	30%	25%
CNP	8%	100%	36%

Farmington and Worcester double mutant animals show similar ultrastructure. Myelin is present in the corpus callosum at postnatal day 24 (P24) and in the optic nerve at P20, but in the reduced amounts typical of all CNS hypomyelinated mutants. The myelin profiles are grouped in clusters as in Ta jp/Y. Most of the sheaths include cytoplasm between lamellae, as in all shi/shi sheaths from these regions at P20 to P24. However, a small number of sheaths show short segments of major dense line (MDL), which were never seen in shi/shi animals at this age. Rarely, the MDL is more extensive and has an abnormal radial component. The white matter contains bundles of oligodendrocyte microprocesses characteristic of shi/shi but never seen in Ta jp/Y. Finally, the lipid-filled cells of Ta jp/Y white matter have not been seen in the double mutants. Thus morphological and biochemical data agree in suggesting that each mutation reduces the severity of the other. The resulting double mutant phenotype shows features of each single mutation by itself, but in milder form. Supported by NIH Grants NS20824 to D.K., NS15190 to J.C., and NS11425 and a Javits Award to S.B.-G. and M.K.W.

27.10 SHIVERER AND ITS ALLELE, MYELIN-DEFICIENT: ALTERATIONS OF MORPHOLOGY WITH ADVANCING AGE. S. Billings-Gagliardi and M.K. Wolf. Department of Anatomy, University of Massachusetts Medical School, Worcester, MA 01605.

The allelic mouse mutations shi and shi^{mld}, compared on B6C3H stocks, produce morphologically similar diseases throughout life in all aspects except for the time of appearance of the major dense line of myelin (MDL) and the amount of MDL at any age. At postnatal day 21 (P21) the percents of myelinated optic nerve axons are similar (20% shi^{mld}, 24% shi). Different axonal diameters are equally represented in both mutants and controls; however, small axons are seldom myelinated in either mutant. The cytology of oligodendrocytes in both mutants differs from controls primarily in the prominence of the Golgi apparatus and associated structures. In P20 optic nerve, 47% of shi and 49% of shi^{mld} axons are associated with 0.5 um diameter oligodendrocyte microprocesses not found in controls. Inappropriate myelination of oligodendrocyte somas and microprocesses and myelin incompletely surrounding axons are seen in both shi and shi^{mld}. However, in P21 animals, no true MDL is seen in shi optic nerve or cerebellar cortex, while in shi^{mld}, about 0.5% of myelin sheaths in those regions show MDL, most often in one or two lamellae, but rarely in an entire myelin profile of 6 - 7 layers. The numbers of myelin sheaths do not increase grossly with age in either mutant, but changes in their compaction occur. At P50 to 53 there are short lengths of MDL in a few shi sheaths. In shi^{mld}, the number of sheaths showing MDL increases, and a tendency emerges for such sheaths to cluster together. Animals at P103 to 106, and P139 to 140 show continuing increase in numbers of MDL-containing sheaths; however, shi always lags significantly behind shi^{mld}. All other abnormalities persist in both mutants at all ages studied. Thus, with increasing age, there is increase in the amount of MDL in both shi and shi^{mld}. However, MDL appears later in shi than in shi^{mld}, and is present in smaller amounts at all ages studied. Other abnormalities are comparable in amount and constant during aging in both mutants. We believe that levels of CNS myelin basic proteins, now being measured, will parallel the increase in MDL. However, the constancy of the other mutant defects during aging suggests either (1) that the amounts or kinds of proteins produced are insufficient to correct the other defects or (2) that the other defects are separate effects of the primary mutations. Supported by NIH Grant #NS-11425 and a Javits Award from the NINCDS.

27.11 MYELIN PROTEOLIPID BIOSYNTHESIS IN PRIMARY CULTURES OF FETAL RAT BRAIN. Wendy B. Macklin and Steven T. Gremillion*, Dept. Biochemistry, LSU Medical Center, New Orleans, LA 70112.

Mixed primary cultures of fetal rat brain have been shown to produce many myelin-specific markers, e.g. myelin basic protein, galactocerebroside, CNPase, myelin-specific cholesterol ester hydrolase and myelin proteolipid (PLP). The present studies were initiated to investigate the biosynthesis and post-translational processing of PLP in these cells, in particular, studying the processing of PLP through cellular membranes and assessing whether post-translational acylation of PLP occurs in these cultures. Cells expressing PLP were incubated with ^{35}S-methionine for 15 min, 1 hr, 4 hr, or 24 hr. Immunoprecipitation of ^{35}S-methionine-labelled PLP indicated that ^{35}S-methionine incorporation into PLP peaked at approximately four hr. Labelled cells were homogenized in 0.32M sucrose and centrifuged at 12,000 xg for 20 min. The supernatant was then centrifuged at 100,000 xg for one hr to produce microsomes while the pellet was applied to a discontinuous sucrose gradient containing 0.9M and 1.2M sucrose and centrifuged at 100,000 xg for one hr. Three fractions were isolated from the gradient: the 0.32/0.9M and the 0.9/1.2M sucrose interfaces and the pellet. The fractions were analyzed by SDS polyacrylamide gel electrophoresis to assess the membrane localization of PLP. As in brain slice systems, no newly synthesized PLP was found in the myelin-like fraction (0.32/0.9M sucrose interface) after a 15 min pulse of label. After a four hr pulse, ^{35}S-methionine-labelled PLP appeared in this fraction. When cells were labelled in the presence of monensin, which blocks processing of proteins through the Golgi system, the appearance of newly synthesized PLP in this fraction was reduced. This suggests as *in vivo*, that PLP is processed through the Golgi system. Using ^{3}H palmitic acid, the post-translational fatty acid esterification of PLP was demonstrated in these cells. Analysis of subcellular fractions after a 15 min pulse of ^{3}H palmitic acid indicated no newly acylated PLP in either the myelin-like fraction or the microsomes, although acylated PLP appeared in these membranes after a four hr pulse. Another membrane fraction was identified which contained newly acylated PLP within 15 mins of labelling. This membrane, the 0.9/1.2M sucrose interface, may contain the acylating enzyme for PLP. This is an important observation which will be studied in future investigations. Supported by PHS grants # NS 18732 and T5HL-07495.

REGULATION OF PITUITARY FUNCTION I

28.1 ADRENALECTOMY-INDUCED ENHANCEMENT OF CRF- AND VASOPRESSIN-IMMUNOREACTIVITY IN PARVOCELLULAR NEUROSECRETORY NEURONS: ANATOMIC, PEPTIDE AND STEROID SPECIFICITY. P.E. Sawchenko and L.W. Swanson. The Salk Institute, La Jolla, CA 92037.

Following adrenalectomy (ADX), CRF- and vasopressin-immunoreactivity (VAS-IR) are jointly expressed in parvocellular neurosecretory neurons in the paraventricular nucleus (PVH). These cells stain with antisera against CRF, but not VAS, in colchicine-treated rats, suggesting a steroid-dependent plasticity in the expression of peptides by neuroendocrine neurons. This study sought to determine (1) which adrenal steroids mediate the effect, (2) whether staining for other neuropeptides that have been co-localized with CRF in the PVH of colchicine treated rats is also enhanced by adrenalectomy, and (3) whether the ADX-induced co-expression of CRF- and VAS-IR is limited to the cells in the PVH. Adult male rats (n=45) were adrenalectomized and osmotic minipumps were implanted subcutaneously. Minipumps were filled with a vehicle or with dexamethasone, corticosterone, deoxycorticosterone or aldosterone so as to deliver 10 or 50 µg steroid/100 g BW/day for 7 days. Normal (n=6) and colchicine-treated rats (n=8) provided comparisons. The animals were perfused and multiple series of sections through the PVH were stained immunohistochemically using antisera against CRF, VAS, met-enkephalin or neurotensin. Sequential double staining techniques were used to confirm indications that two or more peptides might be co-localized in individual neurons. Staining for CRF- and VAS-IR was compared in other brain regions in vehicle-treated ADX, normal and colchicine-treated rats. The results confirmed that CRF- and VAS-IR are enhanced and jointly expressed in cells in the parvocellular division of the PVH of ADX rats; neither met-enkephalin- nor neurotensin-IR was affected. CRF-IR was also augmented in cell bodies in other brain regions of ADX rats, but VAS-IR was never observed in CRF-stained neurons in these regions. Lower doses of dexamethasone attenuated, and higher doses abolished, the ADX-induced increase of both CRF-and VAS-IR in the PVH. The relative efficacy of the steroids in antagonizing the effects of ADX was dexamethasone> corticosterone > deoxycorticosterone>aldosterone. CRF- and VAS-IR in the PVH were not influenced differentially by any of the steroid replacement regimens. These results suggest that the ADX-dependent enhancement of CRF-and VAS-IR in the PVH is at least somewhat specific to these peptides and to this cell group. Moreover, the expression of CRF- and VAS-IR at this locus appear to be regulated similarly by adrenal steroids, with glucocorticoids playing a primary role.

28.2 CRF RELEASES BETA-ENDORPHIN/BETA-LPH IN NORMAL HUMANS. J. F. Lopez*, S. J. Watson, E. Young*, M. Knobloch*, G. Weinberg*, W. Vale, J. Rivier*, J. Greden*, and H. Akil. Mental Health Research Institute, Department of Psychiatry, University of Michigan, Ann Arbor, MI 48109.

It is known that Beta-Endorphin (B-END) and Beta-LPH (B-LPH) are coreleased with ACTH from the corticotrophic cells in the anterior lobe of the pituitary. The release of these peptides is in turn controlled by, among other things, Corticotropin Releasing Factor (CRF) from the hyothalamus (Vale, W., Speis, J., Rivier, J., and Rivier, C., Science, 213:1394-1397, 1981). To our knowledge, there has been no previous studies in human subjects measuring the response of B-END/B-LPH to intravenous CRF infusions. We studied several healthy subjects with no history of psychiatric illness, as part of a study investigating the HPA axis in depression. The subjects received an IV infusion of ovine CRF at 14:30 hrs, blood samples were taken at frequent time inervals before and after infusion and measured for plasma B-END/B-LPH immunoreactivity, cortisol and CRF.

The amount of CRF infused was 0.03 ug/kg; this was corrected for preparational loss as estimated by RIA. The mean B-END/B-LPH baseline plasma concentration was 3 fmoles/ml. Ten minutes after CRF infusion B-END/B-LPH peaked to 7 fmoles/ml, dropped rapidly at 30 minutes to 5 fmoles/ml and then decreased slowly back to baseline during the next 150 minutes. The B-END/B-LPH peak preceded the rise in plasma cortisol by 20 minutes. Plasma cortisol concentration (6 ug % basal) reached a peak of 18 ug% 1 hour after CRF infusion and then decreased to 7 ug% 3 hrs after infusion. Plasma CRF measurements showed an average initial peak of about 65 fmoles/ml 5 minutes after infusion. The CRF levels dropped to < 10 fmoles in 30 minutes, but increased again in a second minor peak 60 minutes after infusion.

To summarize, we have shown that in healthy humans, minute amounts of CRF produce a significant (rapid) increase in plasma B-END/B-LPH within 10 mins of administration, and that plasma cortisol rises shortly afterwards. We also showed that CRF rise precedes the rise in B-END by 5 minutes and that a second peak, perhaps related to binding of plasma protein, is observed 1 hr after infusion. The relative release of B-END itself versus its precursor B-LPH is currently under investigation and will be reported.

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28.3 A POSSIBLE ROLE FOR THE CALCIUM/PHOSPHOLIPID-DEPENDENT PROTEIN KINASE IN ACTH SECRETION FROM AtT20-D16V CELLS. L.M. Bilezikjian and W.W. Vale. (SPON: C. Rivier). Peptide Biology Laboratory, The Salk Institute, La Jolla, CA 92037.

The effect of phorbol esters on the cytosolic calcium/phospholipid-dependent protein kinase (C-kinase) was determined in the AtT20-D16V cell line, a mouse pituitary cell line. These cells secrete ACTH in response to corticotropin-releasing factor and phorbol esters. Treatment with increasing doses of phorbol myristate acetate (PMA) stimulates ACTH secretion with an EC_{50} value of 5-10 nM as is observed in cultured normal pituitary cells. The cytosolic fraction of these cells contains both cAMP-dependent protein kinase and C-kinase. Since C-kinase has been implicated to mediate the action of phorbol esters in a number of cell types, its possible role in mediating PMA-stimulated ACTH secretion was investigated. The activity of the C-kinase was monitored using lys-rich histone (0.5 mg/ml) as substrate in the presence or absence of 100 µg/ml phosphatidylserine (PS) and 2 µg/ml diolein (DG), a diacylglycerol. In the presence of PS and DG, ^{32}P incorporation into histone was stimulated by 2.2-fold from a basal value of 487 ± 28 to 1085 ± 130 pmol ^{32}P/mg cytosolic protein. Incubation of cells with 100 nM PMA resulted in a significant decrease of cytosolic C-kinase activity. This effect was time- and concentration-dependent. The decrease in C-kinase activity occurred with an EC_{50} of 5-10 nM and was evident within 5 min of treatment with 100 nM PMA. Following partial purification of the cytosolic enzyme from control or PMA-treated cells on DEAE-cellulose, similar results were obtained as with the crude cytosolic fraction. A possible translocation of the C-kinase from a cytosolic to a membrane-bound form has been demonstrated previously in EL4 mouse thymoma cells (J Biol Chem 257:13193, 1982). A similar phenomenon is suggested in this study. These results suggest that the secretion of ACTH by PMA may be mediated by C-kinase.

28.4 SUBSENSITIVE PITUITARY CYCLIC AMP RESPONSE TO STRESS OR ADRENERGIC, CHOLINERGIC AND DOPAMINERGIC STIMULATION IN VIVO FOLLOWING ADRENALECTOMY. G.J.Kant, C.J. Nielsen* and J.L. Meyerhoff. Neuroendocrinology and Neurochemistry Br, Dept. Med. Neurosciences, Div Neuropsychiatry, Walter Reed Army Institute of Research, Washington DC, 20307.

We have previously reported that various stressors or administration of adrenergic, cholinergic, or dopaminergic agonists markedly increased levels of pituitary cyclic AMP in vivo. We also reported that bilateral adrenalectomy abolished footshock-induced elevations in pituitary cyclic AMP when rats were tested at 7 and 30 days post-adrenalectomy, although plasma prolactin response to the stress was intact. Since stress releases adrenal epinephrine and epinephrine has been shown to increase pituitary cyclic AMP in vitro as well as in vivo, we tested the hypothesis that loss of adrenal epinephrine was the cause of the lack of pituitary cyclic AMP response to footshock.

We administered one of six doses of epinephrine (0.01 to 1.0 mg/kg IP) to adrenalectomized rats. Rats were sacrificed by high-power microwave irradiation 15 min post injection. In sham-operated rats, the epinephrine-induced increase in pituitary cyclic AMP levels was proportional to the dose injected. Pituitary cyclic AMP was non-responsive to epinephrine administration in adrenalectomized rats. In a second experiment splanchnic nerve-sectioned rats were subjected to intermittant footshock for 15 min. Control and splanchnic nerve-sectioned rats demonstrated a similar elevation in pituitary cyclic AMP following footshock. Thus, elimination of stress-induced epinephrine release via splanchnic-nerve section does not prevent stress-induced pituitary cyclic AMP response. The mechanism by which adrenalectomy abolishes the pituitary cyclic AMP response to stress is not via adrenal epinephrine.

In later experiments, pituitary cyclic AMP in adrenalectomized rats was found to be non-responsive to isoproterenol, nicotine, oxotremorine and apomorphine, at doses that markedly increased levels of pituitary cyclic AMP in sham-operated rats.

Finally, we tested adrenalectomized and sham-adrenalectomized rats at various times following adrenalectomy. We found that footshock elevated pituitary cyclic AMP at 24 and 48 hrs after adrenalectomy but the responsiveness was lost by the 3rd day following surgery. Compared to sham animals, the response at 24 hrs was similar, but the 48 hr response was attenuated.

Adrenalectomy causes changes in levels of hypothalamic CRF and plasma ACTH due to lack of feedback from circulating corticosterone. Studies examining the possible involvement of these compounds in regulating the sensitivity of the pituitary cyclic AMP system are currently underway.

28.5 EFFECT OF ADRENALECTOMY ON CRF STIMULATION OF RAT ANTERIOR PITUITARY ADENYLATE CYCLASE. M.A. Oleshansky,* G.J. Kant and J.L. Meyerhoff (SPON: L.N. Neckers). Dept. of Med. Neurosci., Div. of NP, Walter Reed Army Institute of Research, Washington D.C. 20307.

Our laboratory is currently investigating the mechanism of the stress-induced increase in rat anterior pituitary cyclic AMP in vivo. We have demonstrated that adrenalectomy eliminates the increase in pituitary cyclic AMP following footshock. This effect of adrenalectomy is not mimicked by splanchnic nerve section or adrenal demedullation, suggesting that adrenal catecholamines are not responsible for the stress-induced rise in pituitary cyclic AMP. Additionally, we have shown that the effect of adrenalectomy is not initially apparent one to two days after adrenalectomy, at a time when circulating levels of epinephrine and corticosterone are essentially undetectable. This further suggests that adrenal factors are not directly modulating the footshock-induced increase in pituitary cyclic AMP. Recent work by Swanson et al. has demonstrated that corticotropin releasing factor (CRF) levels are increased in hypothalamus following adrenalectomy. These findings have led us to examine the effect of adrenalectomy on CRF stimulation of anterior pituitary adenylate cyclase in vitro.

Adrenalectomized and sham operated male Sprague-Dawley rats were obtained from Zivic Miller and supplied with food/0.9% saline ad-lib for at least one week in our animal quarters. The rats were then decapitated, pituitaries removed and anterior pituitaries dissected. The anterior pituitaries were individually homogenized and cyclic AMP accumulation assayed in the presence and absence of CRF. The assay mixture contained 50 mM Hepes (pH 7.4), 0.25mM EGTA, 1mM DTT, 2mM $MgCl_2$, 0.5 mM IBMX, 0.17 TIU aprotinin, 50 µg BSA, 10 µM GTP, 0.5mM ATP, 5mM creatine phosphate and 100 units CPK. Cyclic AMP was assayed by RIA. CRF increased cyclic AMP accumulation in anterior pituitary homogenates in a dose related manner from 10 to 500 nM in both sham-operated and adrenalectomized rats. Preliminary findings indicate that adrenalectomy tends to shift the dose response curve for CRF to the right, suggesting that pituitary adenylate cyclase is less responsive to CRF after adrenalectomy. We are currently following up this preliminary finding and plan to examine the effect of adrenalectomy on the responsiveness of pituitary adenylate cyclase to other peptides and neurotransmitters.

28.6 LONG TERM EFFECTS OF THE ORGANOPHOSPHATE DFP ON HORMONAL RHYTHMS IN THE RAT. E.H. Mougey*, L.L. Pennington*, G.J. Kant, J.R. Leu, T.G. Raslear*. (Spon: G.R. Sessions) Dept. of Medical Neurosciences, Div. of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, D.C. 20307.

Organophosphate exposure has been reported to cause long term behavioral effects in humans (EEG changes, insomnia) and in animals. Recent experiments in our laboratory have demonstrated that a single injection of the irreversible cholinesterase inhibitor diisopropylflurophosphate (DFP) disrupts normal circadian patterns of eating and other activity in rats for several weeks. We have previously reported that the injection of the cholinergic agonists physostigmine, neostigmine, oxotremorine and nicotine produces an immediate and pronounced increase in plasma levels of prolactin (Prl), corticosterone (CS), beta-endorphin (β-EP) and beta-lipotrophin (β-LPH) in rats. As these hormones are also known to exhibit circadian rhythms, we undertook a study of the effects of DFP exposure on circadian hormonal patterns and whether antidote pretreatment alters these effects.

Male Sprague-Dawley rats (300±25g) were individually caged and kept in a room which was on a controlled light/dark cyle (0600-1800 light/1800-0600 dark). The animals were divided into three groups: saline injection; DFP injection (2.6 mg/Kg); and atropine sulfate (25 mg/Kg) plus 2-Pam chloride (12.5 mg/Kg) followed 15 min later by DFP (2.6 mg/Kg). All injections were given IP. The animals receiving DFP alone had a 35% mortality rate while the animals preteated with the antidote had only a 2.5% mortality rate. All of the saline injected animals survived. Two weeks following the injections, 18 animals (6 from each group) were sacrificed by decapitation every four hours starting at 0600 hrs. Trunk blood was collected in an heparinized tube and centrifuged for 15 minutes at 4°C. Two ml of plasma were transferred to a tube containing 50 µl (.85 TIU) of aprotinin for β-EP and β-LPH assay. An extraction procedure employing PrepPak-500 C18 was used to separate β-EP and β-LPH for subsequent measurement by RIA. The remainder of the plasma was transferred to a separate tube for CS and Prl measurement by RIA. Rectal temperature measurements were taken immediately following sacrifice.

Body temperatures followed the reported diurnal rhythm with a nadir at 1400 hrs and the highest values recorded at 0200-0600 hrs. Plasma β-EP levels appeared to follow this same pattern with no significant differences among the three treatment groups. Peak values for CS, Prl and β-LPH were seen at 1800 hrs. The lowest values for CS and Prl were seen at 0600 hrs whereas the lowest values for β-LPH were found at 1000 hrs. There were no significant differences in the three groups for CS and β-LPH levels. Prl levels were higher for DFP treated rats at all time periods.

28.7 EVIDENCE FOR PULSATILE ACTH RELEASE IN INTACT DOGS AND MODULATION OF SECRETORY PARAMETERS BY GLUCOCORTICOIDS. A. Negro-Vilar, E. Spinedi*, M.T.B. Bedran de Castro* and H.F. Downey*. Rep. Neuroendo. Sect., Lab. Reprod. Develop. Tox., NIEHS, NIH, Res. Tri. Park, NC 27709 and UTHSCD, TX 75235.

The secretion of adrenocorticotropin (ACTH) has been shown to have both circadian and ultradian rhythms. Although a great deal is known about the mechanisms that regulate circadian ACTH rhythmicity, much less is known about ultradian ACTH rhythms. Scant reports in the literature, particularly in human subjects, provide some evidence for episodic release of ACTH, but no studies have performed a thorough characterization of the pulsatile pattern of ACTH release and of the factors that modulate that pattern. In order to study these important aspects of ACTH secretion, we performed a series of experiments in intact, unanesthetized dogs, chronically implanted with indwelling venous catheters and conditioned to the sampling process. Blood samples were collected every 2 min for periods of up to 90 min. Plasma was assayed for ACTH using a direct RIA. A total of 6 intact dogs analyzed showed a characteristic pulsatile pattern of ACTH release, with a mean peak frequency of 4.8/hr and marked pulse amplitude as evaluated by peak-trough differences (see Table). Dexamethasone (DEX) treatment (2 hr before sampling period) reduced significantly mean ACTH levels as well as mean peak and trough values. A small but significant reduction in pulse frequency was also observed. This latter observation may be considered as indicative of a central inhibitory effect of the corticoid on CRF and consequently ACTH release. The marked changes in mean and peak ACTH levels may also indicate a decreased pituitary sensitivity induced by DEX. In conclusion, an episodic pattern of ACTH release has been characterized in the dog, and the pulsatile release of ACTH has been shown to be markedly inhibited by a glucocorticoid.

Parameters of pulsatile ACTH release

Group	Mean ACTH (pg/ml)	Mean Peak Values	Mean Trough Values	Pulse Duration (Min)	Pulse Interval (Min)	Pulse Freq. (#/hr)
INTACT	336.8 ± 33.5	497.0 ± 56.0	280.0 ± 34.0	6.7 ± 0.4	12.0 ± 0.6	4.8 ± 0.3
DEX	102.3* ± 27.6	133.3* ± 32.3	82.5* ± 21.2	8.8 ± 1.8	16.7* ± 0.6	3.0* ± 0.0

*Values are Mean ± SEM. *P < 0.05 vs intact group.

28.8 DIFFERENTIAL PLASMA CORTICOSTERONE RESPONSES TO ELECTRICAL STIMULATION OF LIMBIC FOREBRAIN AREAS. Jon D. Dunn, Dept. Anat., Sch. Med., Oral Roberts Univ. Tulsa, OK 74171.

Previous studies reported from this laboratory have shown that electrical stimulation of cytoarchitecturally distinctive sites within the amygdala and hippocampus results in differential plasma corticosterone responses. To further pursue the question of differential limbic influences on pituitary-adrenal function, plasma levels of corticosterone (cpd B) obtained prior to and following sham or electrical stimulation were determined fluorometrically for adult female rats which had been anesthetized with urethane (1.3 g/kg). All rats were tracheotomized and connected to a respirator, placed on a heating pad and subsequently positioned in a stereotaxic apparatus. Hippocampal EEG, ECG, heart rate, blood pressure and respiration were routinely monitored; timed blood samples (0.2ml) were obtained from a catherized femoral artery or ventral tail artery. Samples were taken at 0.5 min. prior to and at 5, 10, 15 and 30 min. after initiation of stimulation (monophasic square waves, 100μA, 50HZ, 0.5 or 1.0 msec, 1 sec on/ 1 sec off for 30 min). A change in plasma cpd B was considered different from no change when the average of the 5,10,15 and 30 min samples deviated by more than 10% from the pre-stimulus level.

Whereas no change in plasma cpd B levels were observed following sham stimulation, increased plasma cpd B levels followed stimulation of the diagonal band of Broca, bed nucleus of the stria terminalis, medial mamillary nucleus and zona incerta. Stimulation of the medial forebrain bundle resulted in decreased plasma cpd B. In contrast no change in cpd B levels were observed following stimulation of the corpus callosum, fornix, caudate nucleus or ventral posterior thalamic nucleus.

Collectively these data indicate that differential plasma cpd B responses can be evoked from limbic forebrain areas other than those of the amygdala and hippocampus. Additionally, these data along with our previous observations, lend considerable support to the hypothesis that differential control mechanisms reside within as well as between limbic forebrain areas.

28.9 LOW CONCENTRATIONS OF ASCORBATE INHIBIT THE SECRETION OF PRO-OPIOMELANOCORTIN (POMC) DERIVED PEPTIDES FROM ANTERIOR AND INTERMEDIATE LOBES OF MOUSE PITUITARY. L.P. Dwoskin*, J. Stack*, R.G. Allen* and J.W. Kendall* (SPON: L. Gronke). Department of Medicine, Oregon Health Sciences University, Portland, OR 97201.

POMC is a precursor polyprotein that contains amino acid sequences of several pituitary (PIT) hormones including corticotropin (ACTH), ß-endorphin and melanotropin. Differential processing of POMC produces different end-product hormones in anterior (ANT) and intermediate (INT) lobes of the PIT. It has been suggested that POMC peptide secretion from the ANT lobe is positively regulated by peptidergic factors (corticotropin releasing factor), synthesized in the hypothalamus and released into the ANT PIT vasculature. INT lobe peptide secretion appears to be tonically inhibited by dopamine, released from nerve endings which originate in the hypothalamus and terminate in the INT lobe. Recent reports of high concentrations of ascorbate in brain and PIT prompted us to examine the ability of ascorbate to regulate the secretion of POMC peptides from ANT and INT PIT.

Mouse PITs were separated into lobes, enzymatically dispersed, loaded into cell chambers (25 lobes/chamber) and perifused continuously for 7 hrs with Dulbecco's Modified Eagles Medium. During perifusion, the cells were exposed to concentrations of ascorbate (10^{-16}M - 10^{-7}M) in an ascending order of presentation. Concentrations of ascorbate were presented for 20 min, and 20 min elapsed between ascorbate presentations. Secreted POMC peptides were measured in the perifusate by RIA.

When the amount of peptide hormone secreted during stimulus presentation was expressed as a percent of the baseline secretion rate immediately preceding the stimulus, concentration-response relationships were obtained. ß-endorphin was secreted at a base-line rate of 4-5 ng/0.5 ml/min from the INT lobe cells. ACTH was secreted at a base-line rate of 300-400 pg/0.5 ml/min. INT lobes cells were more sensitive to the inhibitory effect of ascorbate than were ANT lobe cells. The lowest concentration of ascorbate (10^{-16}M) inhibited secretion of ß-endorphin from the INT lobe 34% of control and inhibited secretion of ACTH from the ANT lobe cells 16% of control. The IC50 for ascorbate was approximately 10^{-10}M in the INT lobe cells and was approximately 10^{-7}M in the ANT lobe cells. We suggest that ascorbate may be an endogenous regulator and tonic inhibitor of POMC peptide secretion from both the ANT and INT lobes of the mouse PIT.

28.10 ALTERATIONS IN PITUITARY SENSITIVITY TO CORTICOSTEROIDS IN CHRONICALLY STRESSED RATS. E. Young* and H. Akil (SPON: J. Woods). Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan 48109.

The activation of the hypothalamic pituitary adrenal axis by stress is well-known. Using inescapable intermittent footshock as a stressor in rats, we have demonstrated a rise in plasma Beta-endorphin/ Beta-LPH which parallels the rise in plasma ACTH, the primary POMC derived peptides released by anterior lobe (AL). The rise in ACTH is accompanied by approximately a tenfold rise in plasma corticosteroids. Short term AL cultures from rats who have received intermittent footshock for 30 minutes show a blunted dose response curve to the ACTH secretagogues arginine vasopressin (AVP) and ovine corticotropin releasing factor (oCRF). Similarly blunted dose response curves to secretagogues can be seen by either the addition of dexamethasone (0.5 nM) to the culture medium or pretreatment of the rats with 1 mg dexamethasone 90 minutes prior to decapitation. Thus, glucocorticoids may play a role in the blunted response to secretagogues seen in AL cultures from acutely stressed rats.

In contrast, short term AL cultures from rats who have received one half hour of intermittent footshock daily for fourteen days, show normal or increased responsiveness to AVP or oCRF. This increased responsiveness is seen in cultures from chronically stressed rats who received their last stress either immediately prior to decapitation (chronic stress/acute stress) or 24 hours prior to decapitation (chronic stress/rest). This lack of blunting in AL cultures from chronically stressed rats could be due to two possible mechanisms: a) increased content of peptides in the gland, b) decreased sensitivity to corticosteroid negative feedback. To test the latter hyothesis, we examined the release of ACTH and Beta-endorphin to oCRF in the presence and absence of dexamethasone (0.5 nM) in AL cultures from chronically stressed rats and naive unhandled control rats. As hypothesized, the supersensitivity to ACTH releasers in AL cultures from chronically stressed rats was accompanied by a decreased sensitivity to dexamethasone. Thus, the pituitary appears to adapt to the increased demands of chronic stress by 1) an increase in biosynthesis and content of the POMC derived peptides and 2) by a decreased sensitivity to corticosteroid negative feedback on release.

28.11 MATERNAL CONTACT INHIBITS PITUITARY-ADRENAL ACTIVITY IN PREWEANLING RATS. Mark E. Stanton and Seymour Levine. Dept. of Psychiatry and Beh. Sciences, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Developmental studies have demonstrated postnatal changes in the activation of the pituitary-adrenal (p-a) system by psychological factors. Relatively little is known, however, about the inhibition of p-a activity during development. In adult rats, consummatory behaviors (e.g., eating, drinking) are potent inhibitors of the p-a axis. Recently, we reported that maternal suckling and/or contact is sufficient to inhibit the elevation of plasma corticosterone that occurs in infant rats that are placed in a novel test chamber (Stanton, Wallstrom & Levine, Annual Meeting of the International Soc. for Developmental Psychobiology, 1983). The present experiments sought to further investigate this effect.

The first experiment asked whether contact with a lactating female is necessary for p-a inhibition. As in the earlier experiments, pups were taken from the nest, placed in a heated incubator for 24 hr, and then placed in a heated test chamber for 30 min at 12, 16 or 20 days of age. Four independent groups of pups encountered an anesthetized adult female in the test chamber. These 4 groups were formed by a 2 (Lactating vs. Virgin female) x 2 (Contact vs. No Contact) factorial design. Contact was prevented by a wire mesh partition. Pups in a fifth group (Pup Alone) were placed in the chamber alone. At the end of the 30-min test period, pups were decapitated for blood collection. The corticosterone levels of these 5 groups were compared with those of a basal group which was blood sampled immediately before the test session. At all ages, corticosterone elevations occurred in the Pup Alone and the two No-Contact conditions. In the Contact conditions, virgin and lactating females were equally effective in inhibiting corticosterone secretion in the 12- and 16-day-old pups, but virgin females were somewhat less effective in 20-day-old pups.

A second experiment showed that if corticosterone levels are first elevated by 15 min exposure to novelty, contact with a lactating female reduces corticosterone levels within 30 min (but not within 5 or 10 min). This "active" form of p-a inhibition was particularly evident in 20-day-old pups.

The implications of these findings for the ontogeny of p-a function and for the notion of maternal regulation of the infant's physiology are discussed.

28.12 ^{3}H-NITRENDIPINE BINDING TO CALCIUM CHANNELS IN BOVINE AND RAT PITUITARY. M. Titeler, E.B. De Souza, and M.J. Kuhar. Dept. Neuroscience, Johns Hopkins University Sch/Med., Balto., MD 21205.

We have used ^{3}H-nitrendipine to label sites in homogenates of bovine anterior and neurointermediate lobes of the pituitary gland. The amount of specific binding in the anterior lobe was 2.14 $\pm$ 0.30 pmol/gram wet weight and the K_D was 1.70 $\pm$ 0.10 x 10^{-10} M. Preliminary experiments indicated a similar amount of binding in bovine neurointermediate lobe. In competition studies nimodipine and nisoldipine (two potent voltage-sensitive calcium channel blockers) displayed IC_{50}'s of 8 x 10^{-11} M and 4 x 10^{-10} M, respectively. Diltiazem was found to marginally increase binding, while the diphenylalkylamine calcium channel blockers D-600, tiapamil, and verapamil competed in a complex manner in both tissues. The properties of ^{3}H-nitrendipine binding in the pituitary appear to be very similar to the properties of ^{3}H-nitrendipine binding in brain tissue, which is believed to be to voltage-sensitive calcium channels. Preliminary results of autoradiography experiments indicate the similarity of ^{3}H-nitrendipine binding in rat pituitary and brain. These results provide important support for the hypothesis that calcium channels are involved in pituitary hormone secretion and that drugs that interact with calcium channels may modulate the secretory process directly at the level of the pituitary. (Supported by USPHS grants MH25951, MH00053 and a grant from the McKnight Foundation).

28.13 **β_2-ADRENERGIC RECEPTORS IN PITUITARY: IDENTIFICATION, CHARACTERIZATION AND AUTORADIOGRAPHIC LOCALIZATION.** **Errol B. De Souza and Michael J. Kuhar. Department of Neuroscience, Johns Hopkins Univ Sch Med, Baltimore, MD 21205.**

Catecholamines have been shown to regulate pituitary hormone secretion both through an effect in brain and by a direct action on β-adrenergic receptors in the pituitary. Radioligand binding studies in homogenates have identified and characterized β-adrenergic receptors in anterior and intermediate lobes of the pituitary; however, the presence of β-adrenergic receptors in the posterior lobe and the precise anatomical distribution of these receptors in the pituitary remain to be demonstrated. In the present study, we have used ^{125}I-iodocyanopindolol (ICYP) to identify, characterize and localize β-adrenergic receptors in bovine, rat and human pituitary glands by an in vitro labeling light microscopic autoradiographic method.

In biochemical experiments carried out in slide-mounted bovine pituitary sections, the binding of ICYP was saturable and of high affinity with an apparent K_d of 0.2nM. Cyanopindolol, DL-propranolol and zinterol inhibited ICYP binding with IC_{50} values of 0.5, 30 and 88 nM, respectively. Isoproterenol inhibited ICYP binding stereoselectively; (-)isoproterenol had an IC_{50} value of 211nM and the inactive isomer (d)isoproterenol inhibited 50% of the binding at a concentration of 10 μM. The selective β_1-adrenergic receptor antagonist practolol did not alter ICYP binding. These data demonstrate that the β-adrenergic receptors in the pituitary gland are predominantly of the β_2 subtype.

Rat pituitary autoradiograms show specific binding sites for ICYP in anterior, intermediate and posterior lobes with highest concentrations (grains/500 μm ; mean $\pm$ SEM) found in the intermediate lobe (104.5 $\pm$ 3.5), and lower concentrations in the posterior (34.6 $\pm$ 2.2) and anterior (23.0 $\pm$ 1.3) lobes. Autoradiograms of ICYP binding in human pituitary show significantly higher concentrations of ICYP binding (OD;mean $\pm$ SEM) in posterior (0.127 $\pm$ 0.006) than in anterior (0.035 $\pm$ 0.001) lobe of the pituitary. There is an even distribution of β_2-adrenergic receptors within each lobe of both rat and human pituitary glands.

In summary, our results provide the first visualization of β_2-adrenergic receptors in rat and human pituitary and demonstrate the presence of β_2-adrenergic receptors in the posterior pituitary. The data support a role for epinephrine and norepinephine in modulating pituitary function.

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28.14 SEROTONERGIC ELEMENTS OF THE MAMMALIAN PITUITARY. R. Payette*, M.D. Gershon and E.A. Nunez*. (SPON. K. Pfenninger). Dept. of Anatomy and *Cell Biology, P&S of Columbia University New York, N.Y. 10032 .

Although serotonin (5-HT) is found in the pituitary gland the cells that contain it have not definitely been identified. 5-HT has been immunocytochemically detected in pituitary nerve fibers, but investigators have disagreed as to its localization. Exogenous 5-HT is specifically taken up by gonadotrophs of rats and bats; however, these cells have not previously been shown to store endogenous 5-HT. We have therefore studied the location of 5-HT immunoreactivity in the pituitary glands of bats, mice, guinea pigs and rats using several different antisera to 5-HT. In addition, we radioautographically examined the uptake of ^{3}H-5-HT and ^{3}H-dopamine (^{3}H-DA). The location of neural elements was established using antisera to each of the 3 components of the neurofilament triplet. As expected, neurites were found in large numbers in the posterior lobe, in smaller quantities in the intermediate lobe, especially near the marginal cells bordering the pituitary cleft, but were virtually absent from the anterior lobe. Immunoreactivity of 5-HT was found in neurites in the posterior lobe in all species. Fewer 5-HT immunoreactive neurites were seen in the intermediate lobe and none were found in the anterior pituitary. Large numbers of intermediate and posterior lobe neurites were labeled by ^{3}H-DA. The neurotoxin, 6-hydroxydopamine (6-OHDA) did not reduce the number of 5-HT immunoreactive neurites. The different patterns of 5-HT-immunoreactive and ^{3}H-DA-labeled nerve fibers, and the resistance of the former to 6-OHDA, indicate that the two neural elements are distinct. In the anterior lobe 5-HT immunoreactivity was found in scattered epithelial cells in the mouse and bat pituitary but not in guinea pig or rat. These cells were less numerous than cells displaying B-LH immunoreactivity and, in correlation with 5-HT content, were more numerous in aroused than in hibernating bats. In the mouse, 5-HT immunoreactivity was found by electron microscopy exclusively in the granules of cells morphologically identified as gonadotrophs. Uptake of ^{3}H-5-HT was also demonstrated in epithelial cells of the murine anterior pituitary. It is concluded that endogenous 5-HT is stored in neurites of the posterior and, to a lesser extent, the intermediate lobe of the pituitary. In at least some species 5-HT is also present in epithelial cells of the anterior lobe which may be a subset of gonadotrophs.

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28.15 EFFECTS OF ANTISEROTONIN AND ANTICATECHOLAMINE AGENTS ON SEROTONIN-IMMUNOREACTIVE NERVE FIBERS IN RAT PITUITARY. L.C. Saland, J.A. Wallace and F. Comunas*. Department of Anatomy, University of New Mexico School of Medicine, Albuquerque, New Mexico, 87131.

Several investigators have demonstrated the presence of serotonin (5-HT) immunoreactive fibers in the mammalian pituitary gland. We have confirmed these findings in paraffin-embedded rat pituitary, using an antibody to serotonin, utilizing an avidin-biotin-peroxidase technique. The majority of immunoreactive fibers are present within the pars intermedia, with some in pars nervosa, but little or no staining is observed in the anterior lobe. To determine if staining is susceptible to pharmacologic manipulation, two drugs which deplete 5-HT stores in terminals, p-chlorophenylalanine (PCPA) or p-chloroamphetamine (PCA), were administered intraperitoneally to adult male Sprague-Dawley rats. Groups of 3 animals were injected with sequential doses of 300, 100 and 100 mg/kg PCPA. At one week survival from the first injection, PCPA induced a loss of immunostaining in the entire gland as compared to controls. In contrast, the glands of 3 animals receiving 3 sequential 10 mg/kg doses of PCA did not exhibit altered staining when compared to saline-injected rats. In light of the latter results, and in view of studies demonstrating catecholamine (CA) innervation to pars intermedia, we examined effects of the CA neurotoxin 6-hydroxydopamine (6OHDA) on 5-HT immunostaining. Three rats received 2 sequential 150 mg/kg intravenous injections of 6OHDA, which reduced or eliminated 5-HT-staining in pars intermedia, but did not affect pars nervosa staining. The latter doses of 6OHDA have been found to destroy pars intermedia CA innervation (Saland et al., 1983, Soc. Neurosci. Abst. 9:704). We have also shown PCA to have no toxic effect on pars intermedia innervation, although it destroys fibers in the hypothalamus (Saland et al., 1980, J. Neurobiol. 11:57). Our present results suggest that different projections may exist to the intermediate versus the neural lobe. In addition: 1) 5-HT may be taken up by catecholamine fibers in the intermediate lobe; and/or 2) 5-HT may co-exist in CA cell groups projecting to the intermediate lobe, including neurons in the midbrain or dorsomedial hypothalamus (Mezey et al., 1984, Brain Res. 294:231). Supported by NIH RR 08139, NIH NS 20039 and NSF BNS 82-08433.

28.16 DOPAMINE AGONIST BLOCKS E_2-INDUCED TUMORIGENESIS AND ARTERIOGENESIS IN THE RAT ANTERIOR PITUITARY (AP). K.A. Elias* and R.I. Weiner. Dept. OB/GYN and Repro. Sci., UCSF, San Francisco, CA 94108

We have previously demonstrated that the formation of estradiol (E_2) induced prolactin (PRL) secreting tumors is accompanied by the formation of a direct arterial blood supply to the AP (arteriogenesis). These new arteries carry systemic blood containing low dopamine (DA) concentrations. We hypothesize that in this manner regions of the AP escape DA regulation. In this study we simultaneously administered CB-154 (a DA agonist) and E_2 to Fischer 344 rats. Rats were ovariectomized and implanted with a 1 cm blank or E_2-filled silastic capsule. Some rats also received a 5 mg CB-154 pellet. After 21 days, rats were anesthetized, the thoracic cavity opened and microspheres (15 um in diameter) injected into the left ventricle of the heart. The pituitary was weighed, fixed, cleared in methyl salicylate and the number of microspheres in the AP counted. Because of their size, microspheres are trapped at the primary portal capillaries and normally don't reach the AP. Microspheres reach the AP following formation of a direct arterial blood supply.

	Control	CB-154	E_2	E_2 + CB-154
Pit. Wt.(mg)	12 ± 1	10 ± 1	55 ± 5	19 ± 1
Serum PRL (ng/ml)	27 ± 13	12 ± 4	3350 ± 601	212 ± 17
AP Microspheres	9 ± 1	9 ± 2	1215 ± 308	59 ± 13

E_2 increased the size of the pituitary gland and stimulated serum PRL levels 124 fold. The E_2-induced tumorigenesis was accompanied by a 135 fold increase in the number of microspheres found in the AP. Administration of CB-154 significantly decreased the effects of E_2 on pituitary wt., serum PRL and number of microspheres found in the AP. CB-154 treatment permitted only a small increase in the pituitary wt. in response to E_2 which was correlated with a small increase in number of microspheres observed in the AP. These results clearly indicate that the formation of E_2-induced PRL-secreting tumors can be partially inhibited by CB-154, a DA agonist, and that the inhibition is correlated with the blockade of arteriogenesis. These results are consistent with the hypothesis that arteriogenesis plays an important role in E_2 induced AP tumor formation.

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28.17 AGING ALTERS THE RESPONSE OF ANTERIOR PITUITARY DOPAMINE LEVELS TO OVARIECTOMY. N.Telford*, C.V.Mobbs, and C.E.Finch. (SPON: W.L.Byerly) Dept. of Biol. Sci. and Andrus Gerontology Ctr., USC, Los Angeles, CA 90089-0191.

As female mice age, the levels of dopamine (DA) in the anterior pituitary (AP) increase dramatically. This increase, like many other age-related changes in the hypothalamic-pituitary axis, is attenuable by long-term ovariectomy (LTO). We assessed the effect of age and LTO on changes of DA levels in the AP of female C57BL/6J mice following ovariectomy (OVX) or removal of estradiol (E2)-containing implants. Two groups of mice (young, Y, age 7 mo at sacrifice; old, O, age 17 mo at sacrifice) were OVX 1, 4, or 8 wk before sacrifice. A third group (long-term OVX, LTO, age 17 mo at sacrifice, OVXed at 4 mo) was given (E2)-containing implants (ca. 20 pg E2/ml plasma) for 3 wk. The implants were removed 1, 4, or 8 wk before sacrifice. DA in the AP was measured by HPLC. Values are given as ng DA/mg protein (mean ± SEM).

Intact Y mice had lower levels of DA (1.90±0.23) than did O intact mice (9.58±0.92) or LTO mice with 3 wk of E2 implants (10.5±1.24). Following OVX or removal of implants, DA declined in all groups; Y mice had lower levels than the O and LTO mice at all times. In the Y group, this decline was complete by 4 wk. The post-OVX values were: 1 wk, 2.34±0.12; 4 wk, 1.11±0.15; 8 wk, 1.09±0.16. One wk following removal of the implants, the LTO animals showed a marked decrease in DA (4.19±0.69) which declined further by 4 wk (2.00±0.18) and remained unchanged at 8 wk (1.79±0.22). Levels at 4 and 8 wk following implant removal were comparable to those seen in unimplanted LTO animals (1.56±0.26). Old animals had higher levels of DA than the LTO group 1 wk (6.84±0.62) and 4 wk (2.71±0.32) following OVX, and did not reach levels comparable to unimplanted LTO mice until 8 wk after OVX (1.70±0.12).

Aging produces an increase in DA content and in the time required to completely decrease AP DA following OVX. Changes in the rate of decline of DA levels in the AP with age appear to be ovarian dependent. These changes in AP DA may be due to intrinsic changes in the pituitary or to age-related alterations in the supply of DA from the arcuate-median eminence.

This work was supported by NIA grant AG-00446 (CEF), NIA training grant AG-00093 (NT), and NIA training grant AG-00037 (CVM).

28.18 PANCREATIC BOVINE TRYPSIN INHIBITOR-LIKE IMMUNOREACTIVITY IS LOCALIZED IN MAST CELLS IN BOVINE PITUITARY. M. Schäfer* and R. Martin* (SPON: G. Quarton). Sektion Elektronenmikroskopie, Universität Ulm, Federal Republic of Germany.

Recently, a trypsin inhibitor apparently identical to the Kunitz and Northrop factor was isolated from bovine pituitary gland. Its involvement in control and regulation of neuropeptide precursor processing has been suggested (Li, C. H., and Chung, D., PNAS., 80:1204-1206, 1983). Thus, its localization in neurosecretory cells would be of interest. In the present study, we raised antibodies against pancreatic bovine trypsin inhibitor (pBTI), affinity purified them, and used them as the primary antibody in immunocytochemical studies of semi-thin (0.5 micrometers) sections.

Our results indicate that pBTI-like immunoreactivity is found exclusively in mast cells in the connective tissue; not in any neurosecretory endings or endocrine cells of bovine pituitary. Furthermore, in rat and guinea pig pituitary pBTI-like immunoreactivity was not detectable. Pancreatic bovine trypsin inhibitor seems specific, therefore, to systemic bovine mast cells. For pBTI to play a general role in the inhibition of processing enzymes appears unlikely.

29.1 IMPAIRED OSMOREGULATORY MECHANISM IN THE RAT TREATED NEONATALLY WITH MONOSODIUM GLUTAMATE. R. Gerstberger*, T. Di Paolo and N. Barden, Lab. of Molec. Endocrinology, Centre Hospitalier de l'Université Laval, Quebec G1V 4G2, Canada.

Adult rats treated neonatally with monosodium glutamate (MSG) have lesions in certain brain areas which lack a blood-brain barrier and include the arcuate nucleus (ARN) and circumventricular organs. Since some of these areas are involved in osmoregulatory mechanisms, we investigated the response of several parameters to osmotic stress (dehydration following 60 h water deprivation) in control and MSG-lesioned animals. Newborn rats were injected s.c. with MSG (4 mg/g body weight on days 2, 4, 6, 8 and 10) or saline vehicle and experiments carried out at the age of 10 weeks. Mean arterial blood pressure (MABP) was measured via an indwelling carotid artery canulla, plasma or tissue arginine vasopressin (AVP) concentrations by specific radioimmunoassay and tissue catecholamines and their metabolites by HPLC with electrochemical detection. In control animals the MABP significantly increased from 99 ± 4 mmHg to 116 ± 2 mmHg ($p < 0.05$) following dehydration while in MSG-lesioned animals dehydration failed to modify the already elevated MABP of 127 ± 4 mmHg. Similarly, in MSG-lesioned animals ($n = 23$), the plasma AVP concentration (13.5 ± 1.8 pg/ml) was significantly ($p < 0.01$) greater than that of controls (3.2 ± 0.3 pg/ml, $n = 40$) but was not further increased by dehydration while in control animals this produced a five fold elevation of plasma AVP. AVP concentrations in the neuro-intermediate lobe of the pituitary gland were decreased by 33% in MSG-lesioned animals but, in these animals, dehydration caused no further decrease while in controls dehydration decreased the AVP content by 75%. MSG treatment decreased the AVP concentration of the supraoptic nucleus (SON), (70 ± 6 pg/mg protein vs 128 ± 11 pg/mg protein in controls, $p < 0.01$) but not that of the paraventricular nucleus (PVN) and dehydration was without effect in both control and MSG-treated animals. As expected following MSG treatment, the norepinephrine (NE) and especially the dopamine (DA) contents of the ARN were greatly reduced but no differences were apparent in the SON and PVN. The increases in NE content and DA content and turnover, as indicated by the ratio DOPAC/DA, seen in the PVN and ARN and elicited by dehydration were not present in MSG-lesioned animals. We conclude that neonatal MSG treatment abolishes normal osmoregulatory responses and that this model may be useful to elucidate the regulatory mechanisms involved.

29.2 ANGIOTENSIN II: AN IMMUNOHISTOCHEMICAL STUDY OF ITS DISTRIBUTION IN THE PARAVENTRICULO-HYPOPHYSIAL SYSTEM AND ITS CO-LOCALIZATION WITH VASOPRESSIN AND CRF IN PARVOCELLULAR NEURONS. R.W. Lind, L.W. Swanson, D.A. Chin*, T.O. Bruhn*, and D. Ganten*, The Salk Institute, La Jolla, CA 92037 and The University of Heidelberg, Germany.

The distribution of angiotensin II (AII)-immunoreactive cells and fibers in the paraventriculo-hypophysial system was examined in the normal Sprague-Dawley rat and in the Brattleboro rat under various conditions. In the normal rat without colchicine pretreatment light staining was present in magnocellular neurons and in both laminae of the median eminence. Increased cell staining in both magnocellular and parvocellular parts of the PVH was seen following colchicine pretreatment, and fiber staining in the internal and external laminae of the median eminence was selectively increased by water deprivation and adrenalectomy, respectively. The Brattleboro rat lacked AII staining in the magnocellular part of the PVH and in the internal lamina, but evidenced normal staining in the parvocellular-external lamina projection which was increased by adrenalectomy. Staining was blocked by pre-incubation of the AII antiserum with synthetic AII, but not vasopressin.

The enhancement of AII staining in the PVH and median eminence following adrenalectomy suggests that AII may modulate ACTH secretion via projections from parvocellular neurons to the neurohemal zone of the median eminence. Several labs have demonstrated the ability of AII to stimulate ACTH release, and the present study employed electrolytic lesions to suggest that the AII immunoreactivity in the external lamina of the median eminence arises largely, and perhaps solely, from the PVH. Since recent work from this lab suggests that the joint action of CRF and vasopressin in stimulating ACTH release is coordinated in large part by release of these two peptides from individual parvocellular neurons, the possibility that these same cells may also contain AII was examined. Using a monoclonal antibody to vasopressin (provided by E.A. Zimmerman) in combination with polyclonal antibodies to CRF (provided by Wylie Vale) and AII, and by employing an elution procedure allowing sequential staining for these peptides, a substantial number of parvocellular neurons in the PVH was found to contain immunoreactivity for all three peptides in the adrenalectomized, colchicine-treated rat. When taken together with other recent findings on the distribution of AII immunoreactivity in the rat brain, the present results suggest that AII may be a neuropeptide with a number of special functions in homeostatic regulation.

29.3 ACUTE EFFECTS OF LESIONS OF THE PERIVENTRICULAR TISSUE SURROUNDING THE PREOPTIC RECESS (AV3V REGION) ON PARAVENTRICULAR NUCLEI IN RATS. J. Carithers and A.K. Johnson. Dept. of Veterinary Anatomy, Iowa State Univ., Ames, IA 50011 and Dept. of Psychology and the Cardiovascular Center, State Univ. of Iowa, Iowa City, IA 52242.

Lesions of the AV3V region have severe effects on body fluid homeostasis. These include acute adipsia and failure to develop an antidiuretic response to the dehydration which results. Because neurosecretory cells in supraoptic nuclei comprise the major source of antidiuretic hormone (ADH) in this species, we have previously observed the fine structure of supraoptic nuclei in rats with AV3V lesions (Carithers et al, Brain Res. 220:13). Degenerating axons and axon terminals were present, and although the rats had not drunk for 3 days, there was no morphological evidence of hypertrophy in neurosecretory cells. Paraventricular nuclei are the other major source of ADH in rats. Therefore, in this investigation we compared the fine structure of paraventricular nuclei in rats which had received AV3V lesions 3 days earlier with that of control rats which had drinking water available and control rats from which water had been withheld for 3 days. Degenerating axons and axon terminals were present in paraventricular nuclei of lesioned rats. The degenerating terminals were in axodendritic and less often in axosomatic synapses. Both symmetric and asymmetric synapses contained degenerating elements. Morphometric evaluation revealed that neurosecretory cells did respond to the dehydrated state of the adipsic lesioned animals, as evidenced by increases in cell and nuclear size and cytoplasmic changes. However, their response was significantly attenuated compared to that which occurred in control rats deprived of water for 3 days. It appears that AV3V lesions damage afferent connections to paraventricular as well as supraoptic nuclei. However, although neurosecretory cells of the paraventricular nuclei do not undergo normal hypertrophy in response to dehydration, their response is not completely prevented by AV3V lesions during the adipsic period following AV3V lesioning, as it has been shown to be in cells of the supraoptic nuclei. Supported in part by USDA PL95-1113 Section 1433 and NIH HL-14388.

29.4 EFFECTS OF HYPOTHALAMIC PERIVENTRICULAR (PV) LESIONS ON SOMATOSTATIN (SRIF) RELEASE FROM PERIFUSED BLOCKS OF PREOPTIC AREA-HYPOTHALAMUS (POA-H). L. W. Kaler*, A. Dyke* and V. Critchlow. Reproductive Biology and Behavior, Oregon Regional Primate Research Center, Beaverton, OR 97006.

As shown previously, lesions in the hypothalamic PV nucleus reduce median eminence (ME) SRIF content by ≈80% without affecting nonstress plasma growth hormone (GH) levels or the GH response to stress. Our aim was to study the effects of PV lesions on SRIF release during POA-H perifusion. A large deficit in such release would suggest that most SRIF is PV-derived and available in excess for control of GH secretion; no deficit would suggest major extra-PV sources of SRIF. Female rats received anterior or posterior PV lesions; sham-lesioned and intact rats served as controls. At 2, 4, and 16 weeks after surgery, nonstress and stress plasma GH levels were similar in all groups. The rats were killed at 18 weeks and each POA-H was perifused with a Krebs-Ringer-Hepes-Bicarbonate medium saturated with 95% O_2:5% CO_2 (pH 7.4; 37°C) at 0.1 ml/min; effluents were collected at 10 min intervals. The POA-Hs of the PV- and sham-lesioned rats released similar amounts of SRIF (5.9 ± 0.5 pg/ml), and these were higher ($P<0.001$) than from POA-Hs of intact rats (0.9 ± 0.4 pg/ml). All POA-Hs showed similar increases ($P<0.05$) in SRIF release after 56 mM K^+. Two rats were chosen randomly from each group to assess ME SRIF content. PV lesions caused 80% depletion; sham lesions did not. These results confirm that most SRIF neurons in the PV nucleus and 80% of ME SRIF content are not essential for apparently normal control of GH secretion under nonstress and stress conditions and indicate that PV or sham lesions in the rostral forebrain enhance in vitro SRIF release; this SRIF may stem from neurons located outside of the PV nucleus. Supported by Grants RR00163 and AM32442 from the NIH.

29.5 DISTRIBUTION OF PUTATIVE NICOTINIC CHOLINERGIC RECEPTORS WITHIN THE SUPRAOPTIC NUCLEUS OF THE RAT HYPOTHALAMUS.
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Mechanisms for nicotinic cholinergic (nACh) stimulation of vasopressin (VP) release are present in the basal hypothalamus (HYP) (Sladek, 1983). The specific anatomical site (s) of action of nACh agonists in HYP are unknown. Based on our recent experiments utilizing ^{125}I-αbungarotoxin (^{125}I-αBTX) as a probe for the nicotinic cholinergic receptors (nAChR), we have verified the presence of both high (K_D = 0.5- 1.6 X 10^{-10} M) and low (K_D = 5.5 X 10^{-9} M) affinity binding sites in the rat HYP (Meeker et al, In prep). However, only the former exhibits an affinity similar to the purified nAChR. In order to anatomically localize these putative nAChR, we have mapped the distribution of high affinity ^{125}I-αBTX binding sites within the rat HYP. Our new technique for quantitative assessment of binding to 0.5% paraformaldehyde fixed tissue sections allowed pharmacological characterization of the receptor, autoradiographic localization and histochemical staining on the same section. Concentrations of ^{125}I-αBTX which almost exclusively label the high affinity sites (10-20 pM; K_D = 0.2 X 10^{-10}M) result in accumulation of silver grains over the entire supraoptic nucleus (NSO) with particularly high density in the ventral cell-free zone and over the magnocellular (MgC) neurons along the lateral border of the optic chiasm. The distribution of grains within the remainder of the NSO is over cell bodies, around the edges of cells and appears to follow the NSO-hypophysial tract axons over the optic chiasm. No other HYP regions accumulate significant label at these low concentrations. At 30-60 pM ^{125}I-αBTX the intensity of NSO label increases and an additional diffuse distribution of silver grains begins to appear across the basal HYP with regions of slightly higher density apparent in the nucleus circularis, scattered dorsal to NSO, dorso- and ventro-lateral to the suprachiasmatic nucleus and in the lateral region of the paraventricular nucleus. These data provide the first evidence for high affinity ^{125}I-αBTX binding to putative nAChR on MgC and fibers of the NSO in rat HYP. These data suggest that nACh stimulation of VP release is complex due to direct stimulation of nAChR at several sites on the membranes of MgC neurons within the NSO of rat HYP.

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29.6 VASOPRESSIN RELEASE FROM ORGAN-CULTURED HYPOTHALAMO-NEUROHYPOPHYSEAL EXPLANTS FROM SPONTANEOUSLY HYPERTENSIVE RATS: RESPONSE TO ANGIOTENSIN II.
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Organ-cultured explants of the hypothalamo-neurohypophyseal system (HNS) obtained from young spontaneously hypertensive rats (SHRs) release significantly greater amounts of VP in response to acetylcholine than explants obtained from normotensive Wistar-Kyoto (WKYs) rats (Fed. Proc. 42:1117, 1983). This hyperresponsiveness disappears in explants from 18 week old SHRs. Angiotensin II (AII) is another potent stimulus for VP release. Several investigators have suggested a role for the brain isorenin-angiotensin system in the development of hypertension in SHRs. Therefore, we have investigated the effect of AII on VP release from HNS explants obtained from SHR and WKY donors at 8 and 18 weeks of age.

Blood pressure was significantly greater in the SHR donors. At 8 weeks it was 132 $\pm$ 2, n=21 in the SHR and 95 $\pm$ 5, n=23 in the WKY ($p<.001$); at 18 weeks it was 183 $\pm$ 3, n=8 in SHR and 129 $\pm$ 3, n=8 in WKY ($p<.001$). Explants were maintained in culture as previously described (Endocrinology 101:411, 1977). On the 4th day of culture, AII at 10^{-8}, 10^{-6}, and 10^{-5}M increased VP release from all explants obtained from 8 week old donors of both strains, but the response to the two lower concentrations was significantly greater in the explants from SHRs ($p<.05$): At 10^{-8}M, SHR: 321 $\pm$ 70% of basal release, n=5; WKY: 131 $\pm$ 3%, n=4; at 10^{-6}M, SHR: 382 $\pm$ 80%, n=5; WKY: 173 $\pm$ 26%, n=5; at 10^{-5}M, SHR: 389 $\pm$ 93%, n=5; WKY: 294 $\pm$ 106%, n=6. The response to 10^{-6}M AII was comparable in explants from 18 week old SHR and WKY donors (SHR:335 $\pm$ 77% basal release, n=6; WKY:300 $\pm$ 54%, n=6). Basal VP release was not significantly different between explants from the two strains at either age.

These data indicate an enhanced sensitivity of the HNS to AII in young SHRs, but not in chronically hypertensive SHRs. Thus, it is similar to the hyperresponsiveness observed to acetylcholine and may be partially responsible for the exaggerated VP response to decreased plasma volume in young SHRs (Neurosci. Abst.9:546, 1983).

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29.7 NEUROHYPOPHYSEAL SECRETION IN RESPONSE TO NAUSEA-PRODUCING STIMULI ASSOCIATED WITH LEARNED TASTE AVERSIONS.
J.G. Verbalis, T.W. Gardiner,* C.M. McHale* & E.M. Stricker. Depts. of Medicine & Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15261.

Chemical agents producing learned taste aversions in rats have been assumed to cause nausea as the unconditioned stimulus. Because nausea stimulates vasopressin (AVP) secretion in man, secretion of AVP and oxytocin (OT) was studied in rats after administration of agents known to produce learned taste aversions. Adult rats (200-250 g) were given ip injections of lithium chloride (LiCl), copper sulfate ($CuSO_4$) or apomorphine (APO) followed by collection of trunk blood at 15-20 min. Plasma levels of OT and AVP (uU/ml) were measured by specific radioimmunoassays with <1% crossreactivity. Each agent produced dose dependent increases in OT rather than AVP:

LiCl (mEq/kg)	AVP	OT	$CuSO_4$ (mg/kg)	AVP	OT	APO (ug/kg)	AVP	OT
0.75	2±1	10±1	2.5	3±1	7±2	100	4±1	8±1
1.50	3±1	22±1	5.0	3±1	45±10	200	2±1	7±4
3.00	3±2	44±4	10.0	18±9	67±16	400	4±2	22±7

Similar results were obtained using water expanded hyponatremic rats, confirming that OT secretion was not caused by hypovolemia or hyperosmolality. In addition, ip injection of 2.0 M NaCl (which also produces learned taste aversions in rats) resulted in stimulation of OT (94±12 uU/ml) and AVP (24±13 uU/ml) despite maintenance of plasma [Na+] significantly below the osmotic threshold for neurohypophyseal secretion. In contrast to results in rats, studies in primates confirmed the robust AVP response reported in humans, but without significant elevation of OT.

These studies demonstrate preferential stimulation of OT secretion after administration of agents causing learned taste aversion, and presumably nausea, in rats. While the significance of neurohypophyseal secretion during nausea is not known, the opposite responses of AVP and OT in higher animals suggests an evolutionary reversal in the pattern of neurohypophyseal hormone secretion in response to nausea.

29.8 OXYTOCIN SECRETION AFTER NAUSEA-PRODUCING STIMULI: CHARACTERIZATION OF STIMULATORY INPUTS.
C.M. McHale,* J.G. Verbalis, T.W. Gardiner* & E.M. Stricker (SPON: R.W. Keller). Depts. of Medicine and Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

We have found that oxytocin (OT) is secreted in response to chemical agents causing learned taste aversions in rats, probably representing neurohypophyseal stimulation by nausea. Some agents are thought to act centrally (LiCl), while others cause gastrointestinal irritation ($CuSO_4$). These studies were undertaken to ascertain whether the OT response to these two agents could be similarly characterized. Adult rats (250 g) were given injections of 0.15 M LiCl (3 mEq/kg) or $CuSO_4$ in 0.9% NaCl (5 mg/kg) both intraperitoneally (ip) and intravenously (iv) on successive days. Blood samples were obtained via venous catheters 15 min after injection, and plasma levels of OT (uU/ml) were measured by specific radioimmunoassay. LiCl caused equivalent stimulation of OT whether given ip or iv (62±7 vs 56±13, NS), but $CuSO_4$ resulted in a 4-5 fold greater stimulation when given ip rather than iv (94±15 vs 23±6, $p<0.01$). In agreement with learned taste aversion studies, LiCl appeared to act centrally whereas $CuSO_4$ had a more pronounced visceral effect. To further examine the afferent pathways mediating $CuSO_4$ stimulation of OT, studies were repeated in rats two weeks after subdiaphragmatic vagotomy; completeness of vagotomy was verified by absent gastric acid secretion to 0.5 U/kg insulin. These animals also had a 4-5 fold greater secretion of OT with $CuSO_4$ given ip than iv, and OT stimulation was not significantly inhibited compared to weight-matched controls. This may be contrasted to reports that vagotomy significantly attenuates the learned taste aversions produced by ip $CuSO_4$. These studies demonstrate that neurohypophyseal stimulation by nausea-producing agents in rats may occur either via central or gastrointestinal stimulation, but suggest that non-vagal afferent pathways or humoral factors may be involved in the latter response.

29.9 OSMOTIC INHIBITION OF VOLUME-STIMULATED VASOPRESSIN AND OXYTOCIN SECRETION IN RATS. E.M. Stricker & J.G. Verbalis. Depts. of Biological Sciences and Medicine, University of Pittsburgh, Pittsburgh, PA 15260.

Secretion of the antidiuretic hormone, arginine vasopressin (AVP), is stimulated both by increases in the effective osmotic pressure of body fluids and decreases in plasma volume. Osmotic dehydration and hypovolemia also are independent stimuli for water intake. Thirst is abolished, however, when hypovolemia occurs in association with osmotic dilution. These experiments examined the effects of osmotic dilution on the AVP response to hypovolemia. Plasma AVP and oxytocin (OT) levels were determined by radioimmunoassays with less than 1 percent cross reactivity.

Adult male rats (250-300 g) were injected subcutaneously with 5 ml or 10 ml of 30% polyethylene glycol (PEG) solution, and then were denied access to drinking water. The colloid causes a progressive isosmotic leaching of plasma fluid into a local edema. After 7 hr, plasma volume deficits ranged from 20-35%; AVP levels increased linearly from basal values of 3-7 uU/ml up to 80 uU/ml. Intragastric loads of 10 ml water lowered plasma sodium concentrations to 132-137 mEq/liter and abolished the increase in AVP despite continued hypovolemia. Similarly, rats consumed and retained 8-14 ml when allowed free access to drinking water after PEG treatment, and AVP levels again were comparable to basal values. Identical results were obtained for plasma OT, determined 7 hr after PEG treatment; values increased linearly from 2-7 uU/ml up to 80 uU/ml when water was withheld, but showed little change despite severe hypovolemia in the presence of osmotic dilution. By 24 hr after PEG treatment, however, AVP levels still were elevated in proportion to continuing plasma volume deficits if drinking water was absent, whereas OT levels had returned to basal values.

These results demonstrate that hypovolemia stimulates the initial secretion of both neurohypophyseal hormones, although only AVP release is sustained when plasma volume deficits are chronic. The thresholds for the two responses are comparable at about 20-25% volume losses, approximately the level of hypovolemia at which arterial hypotension begins and well above the 4-10% threshold for thirst in rats. When allowed to drink, release of the two hormones can be suppressed by merely a 3-6% dilution of body fluids. This modest dilution not only inhibits further consumption of water, it also may limit the contribution of AVP to water conservation in urine.

29.10 EFFECTS OF LITHIUM ON VASOPRESSIN SECRETION FROM THE ISOLATED RAT NEUROHYPOPHYSIS. G. Clifton*, R. Baricos*, W. Eggert* and J. D. Wallin*. (SPON: F. Domer). Section of Nephrology, Tulane Medical School, New Orleans, LA 70112.

Lithium is an effective drug used in the treatment of various psychiatric disorders. In man and rats chronic lithium therapy produces a diabetes-insipidus characterized by impaired urinary concentrating ability and by elevated plasma vasopressin (AVP) levels. Recent in vivo studies in man indicate that lithium causes an increased hypothalamic osmoreceptor sensitivity which may be responsible for the observed elevated AVP levels.

In the present in vitro studies we show that lithium, at low concentrations, stimulates AVP secretion from the isolated rat neurohypophysis in a dose-related manner. This increase in AVP secretion cannot be attributed to an osmoreceptor response since the neural lobe has been separated from the hypothalamus. During sequential 10 minute incubation periods, lithium, 0.01 mM or 0.1 mM, gives a 2-3 fold increase in AVP release with a potassium (K^+) concentration of 6 mM and a calcium (Ca^{+2}) concentration of 2.2 mM. In

Lithium	AVP Secretion (pg/ml/10 min. ± S.E.M.)			
Concentration	6mM K^+	60mM K^+	6mM K^+	60mM K^+
0	121± 9	418±40	232±21	362±24
0.01 mM	229± 7*	513±18	418±18*	420± 8
0.1 mM	365±18*	529±62	436±37#	387± 8

* $p<0.001$ # $p<0.005$

contrast, AVP secretion in the presence of lithium, either 0.01 or 0.1 mM, and 60 mM K^+ is not significantly increased compared to respective controls. Also, in a separate set of experiments conducted with similar conditions we have shown that net uptake of radioactive Ca^{+2} for groups of neurohypophyses exposed to lithium, either 0.01 or 0.1 mM, is not significantly increased compared to Ca^{+2} uptake for control neurohypophyses.

These results indicate that lithium has a direct stimulatory effect on AVP release from the isolated neurohypophysis in the presence of 6 mM K^+. The stimulation with lithium is not additive to that achieved through depolarization with 60 mM K^+. This increase in AVP secretion occurs without a net positive Ca^{+2} influx suggesting that lithium is influencing intracellular processes responsible for hormone release.

29.11 DOSE-DEPENDENT ACTIONS OF NORADRENALINE ON THE ACTIVITY OF PUTATIVE VASOPRESSIN NEURONS OF THE SUPRAOPTIC NUCLEUS. T.A. Day, J.C.R. Randle and L.P. Renaud, Neurosciences Unit, Montreal General Hospital and McGill University, Montreal, Quebec, Canada.

Magnocellular vasopressin (VP) neurons of the paraventricular (PVN) and supraoptic (SON) nuclei are surrounded by a dense noradrenergic terminal plexus thought to originate from the A1 cell group of the ventrolateral medulla. Despite reports that iontophoresis of noradrenaline (NA) inhibits PVN and SON neurosecretory cells, we have recently demonstrated that stimulation of the A1 cell group excites putative VP-secreting neurons of both nuclei. Due to the inherent uncertainty as to the quantity of neurotransmitter delivered by iontophoretic application methods, we have re-examined the effects of NA on the activity of SON VP cells using pressure application.

In rats anaesthetized with sodium pentobarbital, the ventral surface of the hypothalamus was surgically exposed and extracellular recordings obtained from neurosecretory SON cells identified by antidromic invasion from the neurohypophysis. Cells either displaying a phasic pattern of firing, or which were continuously active but inhibited by baroreceptor activation, were classified as putative VP neurons. Drug solutions were applied by pressure ejection (1-40 psi) from a 3-barrel micropipette rigidly attached to the recording electrode; tip separation between recording electrode and ejection pipette ranged from 10-50 µm.

The effects of exogenously applied NA were tested on a total of 76 putative VP neurons. Low concentrations of NA (50-100 µM), n tested = 16) were either excitatory (62.5%) or had no effect; intermediate concentrations (150-500 µM, n tested = 52) excited the majority of cells (65.4%), although some were inhibited (17.3%); high concentrations of NA (1-100 mM, n tested = 8) were primarily inhibitory (62.5%). The excitatory effects of low and intermediate concentrations of NA were mimicked by the α-adrenergic agonist methoxamine (300-400 µM) and blocked by administration of the α-1 antagonist prazosin (100 nM). The inhibitory actions of high concentrations of NA were blocked by the β-adrenergic antagonist timolol (20 µM).

These observations indicate that the effects of exogenously applied NA on the activity of SON VP neurons are dose-dependent. The excitatory effects of low and intermediate concentrations (50-500 µM) appear to be mediated via α-1 adrenergic receptors, while the inhibitory effects of higher concentrations involve a β-adrenergic mechanism. Supported by MRC.

29.12 ONTOGENY OF OPIATE INHIBITION OF VASOPRESSIN AND OXYTOCIN RELEASE IN RESPONSE TO OSMOTIC STIMULATION. R. Hartman*, S. Emmert*, L. Rosella-Dampman*, J. Summy-Long. Dept. Pharm., Penna. State Univ. Col. of Med., Hershey, PA 17033.

During dehydration and hemorrhage, endogenous opioid peptides(EOP) promote the preferential release of vasopressin (VP) from the hypothalamo-neurohypophysial system(HNS) by inhibiting oxytocin(OT) release. Hemorrhage produces hypovolemia whereas dehydration causes both osmotic and hypovolemic stimulation of VP release. Our aims were 1) to find out if EOP inhibit OT secretion during an osmotic stimulus alone, and 2) to study the ontogeny of opiate inhibition of OT and VP release. Effects of EOP on the HNS were determined using the antagonist naloxone(N). Release of VP and OT was stimulated by increasing plasma osmolality 15±2 mosm/kg H_2O with hypertonic saline(HS). Adult male rats were injected with saline(S) (1 ml/kg) or N(5 mg/kg) s.c. 5 min before S or HS(1M; 15 ml/kg, s.c.). After 170 min a second injection of S or N was given. Rats were decapitated 10 min later. Male and female rats at 8 and 21 days of age received S(5 ml/kg) or N(5 mg/kg) i.p. 5 min before S or HS(2.5%; 20 ml/kg, i.p.). 15 min later pups were decapitated. VP and OT in pituitary and plasma extracts were measured by RIA. Plasma data, (pg/ml±SEM; n 8-20), were analyzed by ANOVA. There was a decrease ($p<0.05$) with age in plasma [VP] (8d>21d>Adult: 8±1>4±0.3>2±0.3) and [OT] (8d>21d>Adult: 39±4>27±2>11±1). This may reflect a decrease in basal secretion of these hormones from the HNS, as pituitary stores (ng/gland) of VP(8d<21d<Adult: 29±5<158±19< 2320±160) and OT(8d<21d<Adult: 4±0.5<78±11<2030±160) simultaneously increased ($p<0.01$, Student's t) with age. Osmotic stimulation elevated ($p<0.01$) plasma [VP] to 53±3(8d), 28±2(21d), and 50±5(Adult) and [OT] to 71±5(8d), 204±16(21d), and 50±5(Adult). Blocking the action of EOP with N increased ($p<0.01$) plasma [OT] in HS-treated animals of all ages (8d: 71±5 vs 178±13; 21d: 204±16 vs 681±52; Adult: 50±5 vs 93±12) and plasma [VP] in 8d(53±3 vs 95±9) and 21d(28±2 vs 37±3) pups, but not in adults. We conclude that EOP(s) interact with naloxone-sensitive receptors to inhibit OT release by osmotic stimulation, thus promoting preferential secretion of VP at all ages studied. In immature rats, however, release of VP by HS is also inhibited. Thus, an opiate system (EOP, receptors) inhibiting the HNS develops in the rat by 8 days of age, and may function to conserve pituitary stores of both OT and VP during osmotic stimulation in the immature animal. (Supported by HL32826).

29.13 DEXAMETHASONE DIFFERENTIALLY ALTERS NALTREXONE EFFECTS ON PLASMA VASOPRESSIN AND OXYTOCIN CONCENTRATIONS ELEVATED BY TAIL ELECTROSHOCK IN RATS. Lillian M. Rosella-Dampman* and Joan Y. Summy-Long (SPON: R.W. Lehman). Dept. of Pharm., Penn. State Univ., Coll. of Med., Hershey, PA. 17033.

Endogenous opioid peptides (EOP) inhibit vasopressin (VP) and oxytocin (OT) release from the hypothalamo-neurohypophysial system after various stimuli including tail electroshock. The origin of EOP which modulate release of VP and OT is unknown. Two possible sources of EOP are the anterior (AP) and the neurointermediate (NIL) lobes of the pituitary. We tested if EOP derived from the AP inhibit secretion of VP but not OT during tail electroshock. Dexamethasone (DEX) suppresses the release of endorphins with ACTH from the AP. Thus, we evaluated the effects of blocking the action of EOP with naltrexone (N) on the rise in plasma [VP] and [OT] after tail electroshock in rats given DEX. Male S.D. rats received a daily s.c. injection of saline (S; 3.2ml/kg) or DEX (0.2mg/kg). Thirty min. after S or DEX was given on day 17, animals were injected s.c. with S (1ml/kg) or N(1mg/kg). After 15 min rats received tail electroshock (41V; 30 sec) and were decapitated 15 sec later. Control animals were treated similarly but not shocked. VP and OT were extracted from plasma and quantified by RIA. Data are expressed as pg/ml ±SEM (n 4-18).

		NO SHOCK		SHOCK	
Injections		VP	OT	VP[a]	OT[a]
S	S	4±1	13±1	359±58	65±12
	N	5±1	14±1	658±61[b]	257±56[b]
DEX	S	6±1	13±1	1325±137[b]	210±46[b]
	N	6±1	16±3	1553±177	765±218[c]

[a]$p<0.05$ vs No Shock; [b]$p<0.05$ vs S-S-Shock; [c]$p<0.05$ vs DEX-S-Shock; based on 1 and 2-way ANOVA.

Plasma [VP] and [OT] were elevated in all rats given tail electroshock. This release of VP and OT by shock was greater in DEX-treated rats. In animals given S for 17 days then shocked, greater increases were measured in both plasma [VP] and [OT] after N. In DEX-treated rats, however, N elevated only plasma [OT] and not [VP] after shock. Plasma [VP] and [OT] of controls receiving S or DEX were unaltered by N. Since DEX prevented the rise in plasma [VP] after N, we propose that EOP derived from the AP suppress release of VP by tail electroshock. In contrast, the increase in plasma [OT] during shock is partly reduced by EOP from sites, such as the NIL, that are unaffected by DEX. (Supported by Grant HL 32826).

29.14 EXCITATION BY HISTAMINE OF SUPRAOPTIC NEURONS IN VITRO. W. E. Armstrong and C. D. Sladek. Depts. Neurology and Anatomy, Univ. of Rochester Med. Sch., Rochester, NY 14642.

Histamine was tested for its ability to modify the electrophysiological activity of rat supraoptic neurons recorded in vitro from acutely-prepared hypothalamo-neurohypophysial explants. Antidromically-identified extracellular potentials were monitored from the retrochiasmatic portion of the supraoptic nucleus, which lies superficially along the tuber cinereum and which contains a marked majority of vasopressin-immunoreactive neurons.

Of 148 tested neurons, ~70% exhibited slow/silent activity while ~30% exhibited phasic bursting. Histamine applied briefly in the perifusate excited ~1/3 of the slow/silent neurons and ~2/3 of the phasic neurons. Three phasic neurons appeared inhibited by histamine. Most cells were sensitive only to high concentrations (10^{-3}-10^{-5} M) but occasional concentration-dependent activity was observed between 10^{-3} and 10^{-9} M histamine. Excitations in slow/silent neurons most often consisted of a single burst. In phasic neurons either an "extra" burst was produced, or continuous firing developed during the application.

In an additional 17 silent neurons, a single burst could be triggered by antidromic stimuli. In 11 of these cells, histamine elongated the evoked burst in concentration-dependent fashion, while having no ability to generate a burst in the absence of antidromic stimuli.

Neurons sensitive to histamine in concentrations lower than 10^{-4} M were also excited by the relatively selective but less potent H_1-receptor agonists, 2-pyridylethylamine and 2-thiazolethylamine but not the selective H_2-agonists dimaprit and impromidine. Histamine-induced excitations were blocked by the H_1-antagonist promethazine (10^{-6}-10^{-8} M) but not by cimetidine (10^{-5} M), an H_2-antagonist.

Thus histamine's ability to effect antidiuresis and vasopressin release in situ appears due to the activation of vasopressin-secreting neurons via an H_1 receptor. Recent studies showing histamine-immunoreactive nerve fibers and H_1 binding sites within the supraoptic nucleus suggest a physiological role for histamine in controlling vasopressin release. Whether the receptor lies on the vasopressin neuron itself or on a presynaptic element remains to be determined.

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29.15 EFFECT OF 17β-ESTRADIOL ON PHOSPHOLIPIDS METHYLATION OF RAT PITUITARY MEMBRANES. S.V.Drouva, P.Leblanc, E.Laplante, A. L'Heritier and C.Kordon.U-159 INSERM, 2ter rue d'Alesia 75014 Paris France.

Estradiol($17\beta E_2$) affects the sensitivity of pituitary cellsto several neurohormones, as LHRH,TRH ordopamine,presumably by modulating receptor coupling mechanisms. We attempted to precise the membrane processes underlying this modulation and studied the effect of E_2 on pituitary membrane phospholipid methylation.Anterior pituitary membranes prepared from ovariectomized (OVX) or OVX-E_2 implanted rats were assayed for phospholipid methylation according to Hirata et al (PNAS 75,1718,1978). Methylated phospholipids were separated by thin layer chromatography.Incorporation of ^{3}H-methyl radicals into phospholipids increased with membrane concentration and incubation time with S-adenosyl-L(methyl-^{3}H)methionine;it was not Mg^{++} dependent and was inhibited in a dose dependent manner by S-adenosyl-L-homocysteine,a methyltransferase inhibitor. Formation of phosphatidylcholine, phosphatidyl-monomethylethanolamine and phosphatidyl-dimethylethanolamine, was markedly stimulated by treatment with E_2. The effect increased progressively when animals were sacrificed 15 hours to 5 days after E_2 implantation. The response involved a shift in the Vmax of methylating enzymes(403-4 pmoles/mg prot.,as compared to 97-2 for untreated OVX rats). Administration of 17α-E_2,an inactive stereoisomer of 17β-E_2, was ineffective, pointing out to a stereospecific interaction. After differential centrifugation of pituitary membranes (DeDuve et al Biochem.J.60,604,1955),the highest specific methyltransferase activity was found in light mitochondrial (L)and microsomal(P) fractions and the lowest in purified nuclei(N_1) and heavy mitochondrial(M) fractions.After sucrose density gradient centrifugation, methylated phospholipids were preferentially recovered from fractions corresponding to the endoplasmic reticulum. E_2treatment for 5 days did not modify the subcellular distribution of methyltransfe rase activity but stimulated it in all fractions;in contrast it did not modify the activity of the other enzymes measured as fraction markers. Under the same experimental conditions, phospholipid methylation in membranes prepared from cortex, anterior and mediobasal hypothalamic structures was not affected by the steroid, with the exception of a slight increment of ^{3}Hmethyl-incorporation into mediobasal hypothalamic membrane phospholipids after 5 days of E_2treatment. These results indicate that E_2 induced changes in pituitary responsiveness are concomitant with selective effects of the steroid on specific membrane enzymatic activities.

29.16 EFFECTS OF THYROXINE ON THE CONTROL OF GONADOTROPIN SECRETION FROM THE ANTERIOR PITUITARY OF THE CASTRATE MALE RAT. J.L. Cameron* (SPON: A.A. Lamperti). Dept. of Obstetrics & Gynecology, Univ. of Pennsylvania, Philadelphia PA 19104.

Elevations in circulating thyroid hormone concentrations depress circulating luteinizing hormone (LH) levels in castrate rats without altering LH clearance rate. The purpose of this investigation was to determine if thyroxine (T_4) treatment, in vivo, alters pituitary responsiveness to the neuropeptide, LHRH, or modifies LHRH stimulation of the anterior pituitary (AP). Adult, male Sprague-Dawley rats were castrated and 7 days later started on daily treatment regimens of T_4 (20 ug/100 gr body weight, s.c.) or saline, for 1, 2 or 4 weeks. Twenty hours after the last injection, rats were decapitated, trunk blood collected, and the AP rapidly removed, washed and bisected. Hemipituitaries (HP) were incubated for 3 hr. at 37°C in media with or without LHRH (10^{-8}M). After incubation, the HP were homogenized in 10 mM Tris-HCl, the homogenate centrifuged at 3000 RPM for 15 min., and the supernatant assayed for protein content. Additional fresh HP were collected and similarly processed for determination of LH content. T_4 concentrations in serum and LH concentrations in serum, media, and pituitary extracts were measured by RIA. Serum concentrations of T_4 were elevated to comparable levels (15 ± 0.84 ug/dl in T_4-treated rats vs. 4.03 ± 0.12 ug/dl in saline-treated rats) after 1, 2 and 4 weeks of T_4 treatment. Serum LH concentrations were similar in T_4- and saline-treated rats at 1 week, but were significantly depressed, $p<0.05$, in rats treated with T_4 at 2 (19% lower than control rats) and 4 (31% lower than control rats) weeks. Pituitary content of LH was comparable in saline- and T_4-treated rats at 2 weeks (465 ± 32 and 495 ± 62 ng/ug protein, respectively), but was depressed, $p<0.025$, in T_4-treated rats at 4 weeks (524 ± 31 ng/ug protein) compared to saline-treated controls (887 ± 90 ng/ug protein). At no time was there a difference in the amount of LH secreted into the media by HP from saline- versus T_4-treated rats in the presence or absence of LHRH. In summary, (1) T_4 treatment, in vivo, did not alter the ability of the pituitary to secrete LH during short-term incubation, in vitro, or affect the responsiveness of the pituitary to LHRH, and (2) depression of serum LH concentrations in T_4-treated rats occurred prior to a detectable reduction in pituitary LH content. These findings suggest that T_4 depresses LH secretion, in vivo, by modifying LHRH stimulation of the AP, rather than by altering AP sensitivity to LHRH or the ability of the AP to secrete LH.

29.17 CHARACTERIZATION OF THE RELATIONSHIP BETWEEN THE RAPID "TONIC" AND THE DELAYED "INDUCTION" COMPONENTS OF THE PROLACTIN-INDUCED ACTIVATION OF TUBEROINFUNDIBULAR DOPAMINE NEURONS. K.T. Demarest, G.D. Riegle* and K.E. Moore. Depts. of Pharmacol./Toxicol. and Physiol., Michigan State Univ., East Lansing, MI 48824.

Results of previous studies (Demarest et al., Neuroendocrinology, in press) demonstrated that there are two components to the activation of tuberoinfundibular dopaminergic (TIDA) neurons by prolactin (PRL): a "tonic" component, which is responsive to acute changes in [PRL], and an "induction" component, which is activated by long-term changes in [PRL]. The present sudies were undertaken to examine time course and dose-response relationships for the action of PRL on TIDA neurons. The activity of these neurons was estimated by the rate of DOPA accumulation in the median eminence 30 min after injection of NSD 1015 (100 mg/kg, i.p.). Rats were pretreated with bromocriptine to decrease circulating [PRL] which, in turn, decreases TIDA neuronal activity. Using this model, the intracerebroventricular administration of PRL (10 µg) stimulated TIDA neurons by 2 h. The continuous i.v. infusion of PRL (1 µg/min; which maintained circulating [PRL] at 500-700 ng/ml) also increased TIDA neuronal activity by 2 h. There was no clear dose-response relationship between the changes in trunk blood [PRL] obtained by varying concentration of the PRL infusion, 0.03-3 µg/min; which yielded [PRL] of 27 to 1106 ng/ml) and the stimulation of TIDA neuronal activity; all doses were effective. Further studies examined the effect of i.v. PRL infusion in rats in which the "induction" component of the feedback mechanism was activated by pretreatment with haloperidol (2.5 mg/kg x 3 d) to elevate serum concentrations of PRL. Haloperidol pretreatment increased the responsiveness of TIDA neurons to PRL as evidenced by an increase in activity of these neurons; this increase was blocked by decreasing [PRL] with bromocriptine 4 h prior to sacrifice. In this model, the increased capacity to respond to PRL could be demonstrated by i.v. PRL infusion 2 h prior to sacrifice. The increased responsiveness of TIDA neurons to PRL (i.e., the induction component) induced by haloperidol pretreatment was not characterized by a shortened onset of action of, or an increase in the sensitivity of TIDA neurons to the action of PRL. Instead, an increase in the magnitude of response of these neurons to PRL was observed. These results suggest that the "tonic" component of the PRL feedback mechanism increases TIDA neuronal activity in response to short-term changes in circulating PRL concentrations and that the magnitude of response of TIDA neurons to these short-term increases in PRL is determined by the preceding "history" of the secretion of this hormone via the induction component. (Supported by USPHS grants AG02644 and NS09174.)

29.18 VASOACTIVE INTESTINAL PEPTIDE (VIP) MAY STIMULATE PROLACTIN RELEASE BY AN ACTION ON THE HYPOTHALAMUS. T.L. Garthwaite* (SPON: A. Bloom). Department of Medicine, Medical College of Wisconsin, Wood VA Medical Center, Milwaukee, WI 53193.

VIP is a putative prolactin releasing factor (PRF) because: 1) it is found in the hypothalamus and hypophysial stalk plasma, 2) it releases prolactin (PRL) in vivo and in vitro, 3) VIP receptors are found on lactotrope membranes, 4) anti-VIP antiserum attenuates suckling and serotonin (5HT)-stimulated prolactin secretion. However, the physiologic role of VIP as a PRF is in question since: 1) neurons which contain VIP do not terminate near the portal capillary plexus, 2) large doses of VIP are needed to stimulate PRL during suckling, 3) the in vitro potency of VIP is widely variable among laboratories. PHI, a peptide which is structurally homologous to VIP, has recently been proposed as a PRF.

To assess the site and time course of action of VIP and PHI on PRL secretion, quartered rat anterior pituitaries were perifused with (H/PITS) and without (PITS) an upstream chamber containing rat hypothalamic fragments. Fractions (2 ml) were collected every 5 minutes for RIA of PRL. VIP (10^{-6} M), PHI (10^{-6} M), or 5HT (10^{-8}, 10^{-6} M) were administered in 15 minute pulses.

VIP (n=12) and PHI (n=4) consistently stimulated PRL release from PITS. However, this release was modest and delayed (40-80% increase over pre-stimulated rate, peak effect 20-25 minutes after initial VIP exposure). Low dose 5HT caused a rapid stimulation (70% increase, n=8) but the high dose had minimal effect (<20% increase, n=8). In contrast, VIP caused a 200-260% increase in the rate of PRL secretion from H/PITS (n=12) with the peak effect in the initial fraction (0-5 minutes of VIP). PHI did not affect PRL release from H/PITS (n=4). 5HT had a minor effect only at the high dose.

Conclusions: 1) VIP-stimulated PRL release at the pituitary level is modest and delayed. 2) VIP stimulation of PRL in the presence of hypothalamic tissue is rapid and marked. 3) The major effect of VIP on prolactin secretion may be at the hypothalamus to release a PRF, although these data are also consistent with the tonic or VIP-stimulated release of a hypothalamic factor which is synergistic with VIP at the pituitary.

29.19 NEUROTENSIN RAPIDLY AND SELECTIVELY STIMULATES PHOSPHATIDYLINOSITOL (PI) BREAKDOWN AND ARACHIDONIC ACID RELEASE IN RAT ANTERIOR PITUITARY GLANDS IN VITRO. P.L. Canonico, M.A. Sortino*, C. Speciale* and U. Scapagnini*. Dept. Pharmacol., Univ. of Catania Sch. of Med., Catania, Italy.

Neurotensin (NT) stimulates prolactin release in vitro. Its action involves stimulation of PI turnover in several tissues and stimulation of Ca^{2+} uptake at pituitary level. An increased PI turnover occurs in the pituitary following the addition of prolactin-stimulating factors such as TRH and bombesin. We investigated the effect of NT on PI breakdown in rat anterior pituitary glands in vitro. Hemipituitary glands were preincubated for 150 min in Medium 199 containing 5-10 uCi/ml ^{3}H myo-inositol. The glands were then washed and reincubated for 30 min or other times in fresh Medium 199 with or without NT and/or other drugs. NT stimulated in a concentration-dependent manner PI hydrolysis. The effect was already significant at 100 nM and maximal at 1-10 uM; it was rapid, maximal (41%) at 2.5 min and still present after 30 min. To determine the specificity of NT action, other proteins and peptides were tested. Fraction V bovine serum albumine (BSA) and somatostatin did not modify PI hydrolysis, while TRH produced changes in PI turnover similar to NT. The increased PI breakdown produced by NT did not appear to be protein synthesis dependent since 100 uM cycloheximide did not modify NT-induced PI hydrolysis.

The PI response is often accompanied by release and oxidation of arachidonate by specific lipases. To determine whether NT caused changes in the level of free or unesterified arachidonic acid, pituitary glands were incubated for 150 min with 1 uCi/ml ^{3}H-arachidonic acid before NT addition. NT caused a rapid and dose-dependent increase in the level of free arachidonic acid. A specific diacylglycerol (DG) lipase which cleaves arachidonate directly from DG was recently decribed in several tissues. We investigated the possible involvement of this pathway in the mechanism(s) governing NT-stimulated prolactin release using the selective inhibitor of DG lipase activity RHC 80267. 70 uM RHC 80267 prevented NT effect on prolactin release. These results indicate that DG lipase pathway may link NT-PI effect to arachidonate production from membrane phospholipids.

29.20 PROLACTIN (PRL) SUPPRESSION OF LH RESPONSES TO LHRH IN PERIFUSED RAT ANTERIOR PITUITARY CELLS. S. Moore* and C.Y. Cheung (SPON: J. Willey). Division of Perinatal Biology, Loma Linda University, Loma Linda, CA 92350.

Pathological and experimentally-induced hyperprolactinemia is the frequent cause of amenorrhea and suppressed gonadotropic function where the positive feedback response to estrogen is absent. Little is known regarding the mechanisms involved in the antigonadotropic effects of hyperprolactinemia. We have recently shown that PRL directly suppressed basal and LHRH-stimulated LH release from rat anterior pituitaries in vitro. The purpose of the present study was to investigate the direct effect of PRL on LHRH responsiveness, and the modulation of this effect by estrogen in anterior pituitary cells in vitro. Pituitaries were obtained from rats 5 days after ovariectomy (ovx) or ovariectomy and subcutaneous implantation of a 5 mm silastic capsule containing estradiol (E_2). The anterior pituitaries were dispersed into single cells using 0.3% collagenase, mixed with Bio-gel P-2, loaded onto a column and perifused in Krebs-Ringer bicarbonate buffer containing 0.5% BSA. Five min (1.5 ml) fractions were collected for LH measurements using the standard NIADDK radioimmunoassay. To test the effect of PRL, the cells were perifused with control buffer or buffer containing 40 ug/ml PRL and challenged with LHRH for 10 min or continuously for 60 min. LH response determined by integrating the area under the response curve was expressed as a percentage of the basal level. In ovx cells perifused with control buffer, the LH responses to acute 10 min challenges of 10^{-9}, 10^{-7} or 10^{-6}M LHRH were 106%, 182% and 265% resp. However in ovx cells perifused with PRL, the LH responses were 69%, 82% and 50% resp., significantly lower than that observed in the control. Continuous perifusion of ovx cells with 10^{-7} or 10^{-6}M LHRH stimulated LH release by 112% and 254% resp. and these responses were not affected by PRL treatment. E_2 cells showed significant enhancement in LH response to LHRH stimulation where the responses to 10 or 60 min challenges of 10^{-7}M LHRH were 1793% and 3307% resp. PRL significantly reduced these responses to 500% and 728% resp. Basal release of LH from ovx and E_2 cells were not altered 1 h after PRL treatment, while E_2 cells released 84% less LH than ovx cells. The results suggest that 1) PRL suppressed pituitary responsiveness to acute but not continuous LHRH challenge, 2) E_2 pretreatment enhanced pituitary responsiveness to acute and continuous LHRH challenges, and 3) PRL suppressed both LH responses in E_2 pretreated anterior pituitary cells.

29.21 CALCIUM IS NECESSARY FOR TRH-INDUCED INCREASES IN PROLACTIN AND ARACHIDONATE RELEASE FROM ANTERIOR PITUITARY CELLS. K. Koike*, A.M. Judd*, T. Yasumoto* and R.M. MacLeod (SPON: G. Hanna. Dept. of Internal Medicine, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908.

Recently we found that TRH-induced prolactin (PRL) release is associated with the release of arachidonic acid (AA). To determine whether the release of AA is specific for TRH-induced PRL release, primary cultures of anterior pituitary cells were preincubated for 90 min with ^{3}H-AA to incorporate ^{3}H-AA into esterified lipids. After an extensive wash with ^{3}H-AA free medium the cells were incubated for 30 min with test drugs. The lipids that were released into the medium were extracted and applied to silica gel TLC plates and the dpm in the ^{3}H-AA band determined. TRH significantly increased both PRL and ^{3}H-AA release while VIP and neurotensin significantly increased only PRL release. Since TRH is well-recognized to mobilize Ca^{2+}, we determined whether TRH-induced PRL and AA release is associated with Ca^{2+} mobilization. The calcium channel activator maitotoxin (MTX) significantly ($P < 0.01$) increased ^{3}H-AA and PRl release. Although basal PRL release was decreased by low Ca^{2+} medium or 2.5 mM cobalt, a Ca^{2+} channel blocker, basal ^{3}H-AA release was not affected by either agent. However, TRH-induced ^{3}H-AA and PRL release was reduced by both cobalt and low Ca^{2+} medium. Similarly, 1 uM penfluridol, an agent that binds to and inactivates several calcium binding proteins including calmodulin, decreased basal PRL but had no effect on basal ^{3}H-AA release. Penfluridol significantly reduced both PRL and ^{3}H-AA release stimulated by TRH. Medium containing 2 mM EGTA to chelate free calcium increased basal ^{3}H-AA and PRL release but reduced TRH-induced ^{3}H-AA and PRL release.

In conclusion, the release of prolactin induced by TRH, but not that induced by VIP or neurotensin, appears to be associated with a release of AA. The effects of TRH on PRL and ^{3}H-AA release require calcium and increasing cellular calcium via maitotoxin increases PRL and ^{3}H-AA release. Therefore it appears that calcium mobilization, AA release, and PRL release may be closely coupled events during TRH stimulation of pituitary cells.

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29.22 INHIBITION OF GnRH STIMULATED LH RELEASE BY HYPOTHALAMIC EXTRACT. J.C. Hwan* and Marc E. Freeman* (SPON: J.Elam). Dept. Biological Science, Florida State University, Tallahassee, Florida 32306.

This study was performed to determine if the rat hypothalamus contains a substance which inhibits LH secretion from enzymatically dispersed anterior pituitary cells (APC) in vitro. In response to crude acid extract of rat hypothalamus (HEx), APC affected release of LH in a biphasic dose-response manner. Amounts of HEx up to 2 hypothalamic equivalents (HE) caused a significant dose-dependent increase in LH release, while greater than 2 HE caused attenuated stimulatory responses. Prolactin release was unaffected. This biphasic LH secretory response was not observed from APC treated with equivalent amounts of cortical extract. To obviate the possibility that the decrease in LH release was due to desensitization caused by increasing amounts of GnRH in the HEx, experiments were performed to separate an LH inhibitory activity from native GnRH. The first step involved application of boiled HEx to a Sephadex G-25 column (1.6 x 80 cm, 0.1M NH_4OAC, pH 5.5). Elution of 3.5 ml fractions (frx) resolved 2 distinct activities: one stimulated LH release from APC and was shown to coelute with authentic GnRH. A second, while unable to suppress LH below untreated controls, did significantly suppress GnRH-stimulated LH release. These findings indicate that HEx contains a factor distinct from GnRH which is capable of desensitizing the gonadotroph to maximal stimulation by GnRH. Frxs containing this desensitizing activity were next pooled and applied to a carboxymethyl cellulose column (3 x 20 cm) and eluted over a 0.03-0.3M NH_4OAC (pH 4.6-7.0) gradient. One frx was identified which completely inhibited GnRH stimulated LH release. In fact, this frx inhibited LH release below that of non-stimulated control cells. These data taken together suggest that the rat hypothalamus contains a non-proteolytic activity distinct from GnRH which can suppress LH release from APC in vitro. (Supported by NSF PCB 81-20408 and The Ford Foundation)

29.23 ESTROGEN STIMULATES IN VIVO GnRH RELEASE IN OVARIECTOMIZED RATS. J. E. Levine, D. R. Bangsberg* and H. G. Spies. Reproductive Biology and Behavior, Oregon Regional Primate Research Center, Beaverton, OR 97006.

Estrogen (E_2) activates a daily neuronal signal in the female rodent which directs the luteinizing hormone (LH) surge. Evidence indicates that this E_2-dependent signal is composed of an increase in hypothalamic gonadotropin-releasing hormone (GnRH) release. Although it has been shown that progesterone can stimulate GnRH release after low-dose E_2-priming (Levine and Ramirez, *Endo.*, 107:1782), we have not described GnRH release following E_2 stimuli that elicit LH surges in the rat. The aim of this study was to determine if GnRH release is changed during the E_2-induced LH surge in conscious, freely-moving rats. Female rats were ovariectomized and after more than 5 days later they were stereotaxically implanted with push-pull cannulae (PPC) in the mediobasal hypothalamus (MBH). Two to 3 days later, each animal received a blank (CTL, $N = 5$) or estradiol-17β-filled (E_2, $N = 6$) s.c. Silastic capsule implant. On day 2, 3, or 4 following capsule implantation, animals underwent push-pull perfusion (PPP) of the MBH for more than 5 hr between 1000 hr-1900 hr. A blood sample was obtained from each animal before (1000 hr-1200 hr) and after (1800 hr-2000 hr) PPP by venipuncture. GnRH and LH levels in push-pull perfusates and serum, respectively, were determined by RIA. GnRH release rates in CTL rats were 0.1-0.5 pg/12 min, and mean levels did not vary throughout CTL PPP experiments. In all E_2-treated rats, GnRH release was significantly higher at 1500 hr-1800 hr vs. prior intervals or corresponding CTL values. E_2-stimulated GnRH release was typically composed of large pulses reaching 1-11 pg/12 min in amplitude. LH levels in CTL rats were < 250 ng/ml before and after PPP. In E_2-treated rats LH levels at 1800 hr (772 ± 304 ng/ml) were significantly higher than 1000 hr-1200 hr values and were not different from those observed at 1800 hr in E_2-rats that did not receive PPC implants (866 ± 186 ng/ml). These data demonstrate that in E_2-treated rats, afternoon GnRH release is activated prior to or during the E_2-induced LH surge. Our observations are consistent with the hypothesis that an E_2-sensitive neuronal oscillator governs the daily release of GnRH which, in turn, directs the LH surge. (Supported by NIH grants RR-00163 and HD-16631).

29.24 STRESS-INDUCED CHANGES IN THE SECRETION OF α-MSH. O. Khorram*, J.C. Bedran de Castro*, and S.M. McCann (SPON: S. Kiser). Department of Physiology, Univ of Texas Hlth Sci Ctr, Dallas, Texas 75235.

The aim of this study was to analyze the changes in the secretion of α-MSH during immobilization stress, and to correlate these changes with plasma Prl and LH. Long-term ovariectomized (OVX) rats bearing jugular vein cannulae were used. After the removal of a baseline blood sample animals were tied to a restraining board. Blood samples were withdrawn 5, 15, 30 and 60 minutes after the onset of stress. After the last blood sample animals were sacrificed, and the content of α-MSH in the anterior (AL) and posterior lobe (PL) of the pituitary gland, the median eminence (ME), and mediobasal hypothalamus (MBH) were determined by a specific RIA. Five minutes after the onset of stress plasma levels of Prl and α-MSH were significantly ($p<.025$) elevated compared to baseline levels, with no apparent changes in plasma levels of LH at this time. By 30 min a maximal elevation in plasma α-MSH and Prl ($p<.001$) had occurred, whereas plasma levels of LH were significantly ($p<.001$) depressed. Sixty min after the onset of stress plasma Prl and α-MSH began to decline, whereas plasma LH levels had declined maximally by this time.

Analysis of tissue levels of α-MSH indicated that stress induced a significant ($p<.05$) increase in the content of α-MSH in the AL and PL. The total content of α-MSH in the MBH of the stressed rats was significantly lower than that of the non-stressed rats; however, no statistically significant differences existed between the two groups when the α-MSH content was expressed in terms of tissue protein. The most significant change occurred in the ME, where the levels of α-MSH were 10 fold higher in the stressed rats as compared to the non-stressed controls.

These results indicate that stress induces a marked increase in the synthesis and release of α-MSH from the pituitary. The marked stress-induced increase in the ME content of α-MSH, as postulated earlier (Neuroendo 34:433), could stimulate the activity of the dopaminergic neurons, thereby leading to a lowering of plasma Prl and LH that occurred 1 hr after the onset of stress. Supported by NIH grants HD-09988 and AM-10073.

29.25 IMMUNOHISTOCHEMICAL EVALUATION OF THE ORGANIZATION OF CHEMICALLY SPECIFIC SYSTEMS IN THE ARCUATE NUCLEUS AND MEDIAN EMINENCE FOLLOWING NEONATAL ADMINISTRATION OF MONOSODIUM GLUTAMATE. R.Y. Moore & J.P. Card (SPON. A. Rosen). Dept. of Neurology, SUNY @ Stony Brook, Stony Brook, N.Y. 11794.

Numerous studies have demonstrated that neonatal administration of monosodium glutamate (MSG) induces neuronal degeneration in the arcuate nucleus (AN) which is accompanied by severe disturbances in endocrine function. As a result, MSG treatment has been routinely used as a model to study the role of the AN in neuroendocrine regulation. However, Seress (Neurosci. 7: 2207, '82) has demonstrated that neurons in the caudal half of the AN are resistent to the toxic effects of MSG. In the present study we have analyzed the localization of dopamine- and B-endorphin (B-END)-containing neurons in the AN and the organization of their axonal projections in the median eminence (ME) of rats treated neonatally with MSG. Paired litter mates of both sexes received subcutaneous injections of either MSG (4mg/g body wt) or vehicle on alternate days during the first 10 days postpartum. After 6-12 months survival, animals were sacrificed and processed for immunohistochemistry with antibodies generated against tyrosine hydroxylase (TH; to demonstrate dopamine cells and axons) and B-END. These studies demonstrated differential sensitivity of both TH- and B-END-containing neurons in the AN to the toxic effects of MSG. In control animals, TH immunoreactivity was observed in two populations of neurons. Large, multipolar neurons were situated along the lateral border of the nucleus and a tightly packed group of smaller, bipolar neurons were observed in the dorsal periventricular aspect of the AN. MSG treatment induced selective degeneration of large neurons in the rostral half of the nucleus. B-END immunoreactivity was also localized in two populations of arcuate neurons of control rats. Small bipolar neurons confined to the limits of the AN were present throughout the rostrocaudal axis. Larger multipolar cells were also present at all levels of the AN and extended laterally into the mediobasal hypothalamus. MSG treatment lesioned both cell groups in the rostral AN but did not affect neurons in the caudal half of the nucleus. MSG treatment reduced the dorsoventral height of the ME by approximately one third but did not alter the organization of axons displaying either TH or B-END immunoreactivity. These findings support Seress' contention that MSG has a regional effect on arcuate neurons that should be considered when employing this neurotoxin in studies on the role of the AN in neuroendocrine regulation of pituitary function. (Supported by NS-16304 & NS-19714)

STRESS, HORMONES, AND THE AUTONOMIC NERVOUS SYSTEM

30.1 INFLUENCE OF PRENATAL MATERNAL STRESS ON THE MATURATION OF COMPONENTS OF THE IMMUNE RESPONSE IN RATS. S. K. Sobrian, V. T. Vaughn* and E. F. Bloch*. Dept. of Pharmacology. Howard Univ. Col. of Med., Washington, D.C. 20059.

A variety of environmental manipulations (stressors) can modify immune processes in animals. Generally, the effects of stress are immunosuppressive. This decrease may indirectly affect behavior via CNS mechanisms. Prenatal maternal stress (PMS) can alter both adult and developing behavioral patterns as well as neurochemical ontogeny. We therefore determined if environmental and psychological PMS could influence the development of the immune response in the offspring, or modify their hormonal and immunological response to postnatal stress. Timed-pregnant Sprague-Dawley rats were exposed daily for 30 min. on gestational days 15-21 to either environmental stress (ES) [15 unsignalled, inescapable electric foot shocks (0.5 mA for 0.5 sec.) session], psychological stress (PS) [pregnant rats were placed in a non-electrified section of the apparatus and allowed to see, hear and smell a non-pregnant partner that was being environmentally stressed], or a non-shocked apparatus control (C). Females delivered naturally and pups were not cross-fostered. Serum corticosterone (CCS) and IgG levels were monitored every 7 days from birth to postnatal day (PND) 28. From PND 29-33, offspring were environmentally stressed for 15 min. [8 shocks at 0.20 mA for 0.5 sec]; hormonal and immune responses were determined on PND 34.

Indices of maternal pup interaction, i.e., latency to nest build or retrieve pups, nursing and grooming, were not significantly different among the groups. The development of the humoral immune response, as measured by serum IgG, was unaffected by PMS. In offspring from all groups, IgG levels increased during the first and second postnatal weeks, peaked between PND 14 and 21, and then declined to adult levels at PND 28. In contrast, IgG levels were reduced in offspring of ES and PS female on PND 0, and in ES offspring on PND 7, and again on PND 28. These changes in IgG levels were not related to differences in serum CCS. Moreover, nutritional factors were ruled out due to unaltered body weights. However, at PND 28, organ-body weight ratios showed enlarged spleens in the ES offspring; adrenal, thymus and brain weights were unaffected. Exposure of PMS pups to postnatal shock or handling did not alter IgG or CCS levels when compared with controls. However, in ES rats PND 34 IgG levels increased from those of PND 28, suggesting that mild or moderate environmental stress might improve immunocompetence in animals with a deficient response.

30.2 STRESS-INDUCED SECRETION OF CORTICOTROPIN RELEASING FACTOR IMMUNOREACTIVITY IN RAT CEREBROSPINAL FLUID. K.T.Britton*, M.Lyon*, W.Vale and G.F.Koob (SPON:D.Segal). Univ. of California, San Diego and VA Hospital, La Jolla, CA 92093; Peptide Biology Lab., The Salk Inst., San Diego, CA 92138; Div. of Preclinical Neuroscience and Endocrinology, Scripps Clinic and Research Foundation, La Jolla, CA 92037.

Corticotropin releasing factor (CRF) is a 41-residue polypeptide which stimulates the release of ACTH and β-endorphin and has been proposed to play a role in regulating the endocrine, autonomic and behavioral responses to stressful stimuli. The current study was undertaken to test this hypothesis by establishing, first, whether CRF release is altered by footshock stress and, second, whether the response of CRF to stressful stimuli can be attenuated by the antianxiety drug diazepam.

Rats were each implanted with a chronic cannula aimed at the cisterna magna. On the 6th postoperative day, 100 µl cerebrospinal fluid (CSF) were withdrawn by gravity to determine baseline CRF levels. The following day rats were placed in a chamber and thirty 0.3 mA footshocks were administered on a variable interval (30 sec) reinforcement schedule over 15 minutes. Control rats were placed in the shock chamber for 15 minutes without footshock. Immediately following this procedure, CSF was withdrawn and CRF-like immunoreactivity was determined. Additional animals were tested as above after receiving a pretreatment of diazepam (3.0 mg/kg s.c.).

Cerebrospinal fluid CRF immunoreactivity concentration rose 2-fold from an initial level of 6.24 $\pm$ 1.0 to 13.96 $\pm$ 1.99 pg/100 µl. No significant changes were observed in CRF immunoreactivity in control animals. Pretreatment with diazepam (3.0 mg/kg) attenuated the footshock-induced increase in CRF immunoreactivity. These observations support the hypothesis that CRF plays a role in mediating an organism's response to stressful stimuli.

30.3 DYNAMIC CHANGES IN HIPPOCAMPAL CHOLINERGIC SYNAPTIC MECHANISMS AFTER STRESS OR CORTICOSTERONE TREATMENT. G.M. Gilad, Y. Finkelstein*, B. Koffler* and J.M. Rabey*, Center for Neurosciences and Behavioral Research, and Dept. of Isotope Research, The Weizmann Institute of Science, Rehovot Israel.

We have recently demonstrated that the septo-hippocampal cholinergic system is actively involved in the response to stress (Gilad, G.M. et al., Brain Res. 267:171, 1983). This is expressed by a reduction in high affinity choline uptake and increased muscarinic binding capacity in rat hippocampus following chronic intermittent immobilization stress. In the present study we sought to characterize changes in high affinity [^{3}H]choline uptake, newly synthesized [^{3}H]acetylcholine (ACh) release, and [^{3}H]quinuclidinyl benzilate (QNB) binding in rat hippocampal synaptosomal preparations after: 1) different intervals of stress; 2) 10 min of corticosterone injection (25 mg/Kg in peanut oil, i.p.), and 3) at different times after chronic intermittent (2hx5d) stress. Choline uptake was increased to 125% of unhandled controls after 10 min of stress, after 2h it returned to control levels and after chronic stress uptake was reduced to 75% of control. ACh release was enhanced after all stress intervals. Maximal muscarinic (QNB) binding capacity (Bmax) was increased (to 145% of control), only after chronic stress, with no change in K_D values. After chronic stress the changes observed in cholinergic synaptic mechanisms all persist for up to 2d. Recovery occurred only by the 7th post-stress day. Acute corticosterone treatment resulted in similar increases in choline uptake and ACh release as after acute stress. Corticosterone also activated these presynaptic mechanisms directly, in vitro, in a concentration dependent manner. We conclude: 1) presynaptic hippocampal cholinergic terminals are rapidly activated by stessful stimuli and this is expressed by an increase in choline uptake and newly synthesized ACh release; 2) after prolonged periods of stress adaptive changes in the cholinergic terminals are expressed by a reduction in choline uptake and an elevation in the number of muscarinic binding sites, and 3) corticosterone can directly activate hippocampal cholinergic terminals. The results imply that: 1) after acute stress elevation in circulating corticosterone levels may directly activate the cholinergic terminals, and 2) a reduction in the high affinity choline uptake system is a key compensatory mechanism of the cholinergic synapse during adaptation to lengthy periods of neuronal activity induced by stress.

30.4 NEONATAL STRESS ALTERS SUBSEQUENT STRESS RESPONSIBILITY OF THE ADRENAL MEDULLA: SEX DIFFERENCES. A. Pylypiw* and L.L. Ross (SPON: D. Stoff). Dept. of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

We have shown that the adrenomedullary system can be permanently altered by stress during the first 10 days of life and that males and females respond differently to neonatal stress depending on the type of stress and the age administered. To examine the ability of the altered system to respond to stress administered after the neonatal period, we performed the following experiment.

From postnatal days 2 to 14, rat pups of both sexes were subjected to immobilization stress for 10 days for one hour each day. The animals were then reared together with their unstressed littermates to 40 days of age. At this time, both prestressed and unstressed rats were subjected to one hour of immobilization and sacrificed by decapitation. An additional matched control group consisted of animals who were sacrificed by decapitation. Adrenal glands were analyzed for catecholamine and serotonin levels and tyrosine hydroxylase activity. Blood plasma was analyzed for catecholamines. All amine assays were performed by HPLC/EC. Enzyme assays were done by the Waymire method.

The adrenal levels of norepinephrine and epinephrine and the adrenal tyrosine hydroxylase activity were significantly lower in neonatally stressed males when compared with both control groups of the same sex. In the neonatally stressed females, adrenal serotonin levels were greater than controls while plasma norepinephrine levels were lower. Males showed no differences from controls in plasma epinephrine or norepinephrine levels.

These data indicate that neonatal stress does alter the responsiveness of the adrenomedullary system to a subsequent stress and that male and female rats respond differently in this regard.

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30.5 EFFECTS OF CHRONIC TAIL SHOCK ON PLASMA HORMONE RESPONSE IN RATS. B.N. Bunnell, T.E. Orr, W.D. Hills, E.H. Mougey, L.L. Pennington and J.L. Meyerhoff. Dept. Psychology, Univ. of Georgia, Athens, GA 30602 and Dept. Med. Neurosciences, Walter Reed Army Inst. of Research, Wash., DC 20307

The results from experiments on chronic stress using electric footshock reveal that some neuroendocrine responses habituate to daily repetitions of exposures to stress. This habituation may be due, in part, to the rats' ability to reduce the shock received by assuming postures which minimize their contact with the shock grids. Attaching electrodes to the tail, however, might allow the assessment of the chronic effects of stress on neuroendocrine mechanisms without the complication of such behavioral habitutation. In the present study, plasma prolactin (PRL) and corticosterone (CS) were assayed after 1, 3, and 5 days of tail shock to study the habituation of the hormonal response.

After adaptation to handling, 24 adult male hooded rats were placed in small, triangular shaped boxes with their tails protruding through an opening at the rear of the chamber. A pair of cylindrical electrodes were taped to the rats' tails. This imposed a degree of restraint on the rats. One group of rats received .017 watts of shock, delivered through the tail electrodes, on a variable time 1 shock/min schedule for 15 min each day. A second group of 12 was similarly restrained by their tails in the boxes, but received no shock, and a third group of 6 rats served as home cage controls. A set of animals was sacrificed from each group after 1, 3, or 5 days of treatment. PRL and CS were measured by radioimmunoassay.

Both tail shock and restraint in the shock chambers without shock produced significant elevations in PRL and CS in comparison with home cage controls. Plasma PRL was higher in the shock group than in the restraint group, and neither group showed habituation on this measure over five days. The CS response was similar in the shock and restraint groups for the first three days. On day five, the shock group was elevated, while the restraint group exhibited significant habituation. These results suggest that tailshock may be a useful method for minimizing behavioral habituation when using electric shock in experiments investigating neuroendocrine responses to chronic stressors. Mean values (+/- SEM) were:

	PRL (ng/al)			CS (ug/100ml)		
DAYS	1	3	5	1	3	5
TAILSHOCK:	479.0	218.0	374.0	23.9	24.3	33.7
(+/-)	(179.3)	(47.1)	(43.4)	(6.8)	(0.6)	(2.7)
RESTRAIT:	94.5	191.0	114.3	21.9	27.9	13.4
(+/-)	(32.5)	(38.8)	(25.3)	(2.4)	(2.1)	(2.1)
HOMECAGE:		8.5	(1.7)	3.4 (1.2)		

30.6 Inhibition of stress-induced cortisol secretion by dietary tyrosine in rats.
H. Lehnert, D. K. Reinstein*, I. Richardson* and R. J. Wurtman
Medizinische Universitätspoliklinik Münster, West-Germany and Laboratory of Neuroendocrine Regulation, Massachusetts Institute of Technology, Cambridge USA.

We have previously shown that acute and dietary administration of tyrosine prevents stress-induced neurochemical and behavioral changes, such as depletion of regional brain levels of norepinephrine (NE) and depression of active motor behavior. Since it has been suggetsed that hypothalamic NE may inhibit the release of ACTH, we now investigated whether dietary tyrosine administration could modify the stress-induced cortisol secretion.

The stress procedure consisted of electric tail shock given for 5 sec every 30 sec at 20 V intensity (AC, about 2 mA) over a period of 60 minutes. Tyrosine was administered in a high tyrosine diet (in which the casein was supplemented with four times as much free tyrosine) consumed for three days. Following the stress, behavioral activity was measured using an open field / hole poke apparatus that was unfamiliar to the animal and rated by an independent observer. Tyrosine and corticosterone were determined fluorometrically, NE by HPLC and ACTH by radioimmunoassay.

Hypothalamic tyrosine was doubled in both the stressed and unstressed group receiving tyrosine when compared to both control groups. The stress procedure significantly reduced the concentration of hypothalamic NE in control animals receiving no tyrosine, while the high tyrosine diet restored NE levels in stressed rats almost to normal. Stress alone significantly depressed locomotion and exploratory behavior, while the group receiving stress and tyrosine was not different from controls. Pituitary and plasma levels of ACTH tended to increase in the group exposed to stress alone. Plasma corticosterone concentrations were significantly elevated in the stressed animals receiving a normal diet; the high tyrosine diet was capable of markedly attenuating the stress-induced increase in cortisol secretion. In no case had tyrosine alone (i.e. without stress) any effect.

Our results suggest that cortisol secretion may depend on hypothalamic NE levels and that by supplementing activated noradrenergic neurons with additional tyrosine as their circulating precursor the stress-induced increase in ACTH release and subsequent cortisol secretion may be prevented.

30.7 SOCIAL INFLUENCES ON CONDITIONED CORTISOL SECRETION IN THE SQUIRREL MONKEY. Seymour Levine, Mark E. Stanton, and Jeffrey M. Patterson.* Dept. Psychiatry and Behavioral Sciences, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Social variables can have a major influence on an individual's response to stressful life events. In human populations, the incidence of stress-related illness is inversely correlated with factors indicative of "social support" (marital status, church membership, etc.). In an attempt to model this phenomenon of "social buffering" in primates, our laboratory has found that squirrel monkeys show elevated cortisol when exposed to an unconditioned fear stimulus (the sight of a snake) if they are tested individually but not if they are tested in groups. The present study sought to generalize this finding to a fear-evoking conditioned stimulus (CS).

A between(conditioning)-within(social conditions) experimental design was used. Adult male squirrel monkeys were assigned to two groups. Group-paired (n=6) received pairings of the CS (a 20-sec flashing light) with foot-shock (1 sec, 4 mA, scrambled). Group Control (n=6) received CS presentations without shock. All animals were tested under 3 social-housing conditions in 4 successive phases of the experiment: individual-, dyad-, group-, and individual-housing. In each phase, animals received 3 daily conditioning sessions (10 trials per day, 90 sec intertrial interval, in a standard conditioning chamber) followed 24 hr later by a (basal) blood sample and, 48 hr after that, by another (test) blood sample, taken 20 min after 10 presentations of the CS without shock. These CS presentations took place in the animal's home cage. The home cage contained single animals in the individual housing condition, one Paired and one Control animal in the dyad condition, and 3 Paired and 3 Control animals in the group condition.

Neither group showed a cortisol response to the CS prior to training. During training, all Paired animals showed significant ($p<.001$) levels of agitated behavior to the CS, whereas none of the Control animals did. Following training, CS-evoked elevations of cortisol were found ($p<.05$) only in Group Paired, only in the individual-housing condition.

These results replicate and extend our previous finding that the presence of conspecifics can ameliorate the neuroendocrine response to psychological stressors in squirrel monkeys. The biological and neural analysis of social buffering may have important implications for the management of stress pathology in humans.

30.8 CORTICOID VARIATIONS ASSOCIATED WITH LABORATORY AND LIFE STRESSORS. Angela Corradini* and Hymie Anisman. Dept. Psychology, Carleton University, Ottawa, Canada.

Life-stress events may be associated with the induction or exacerbation of various psychological and physical pathologies, as well as alterations in the concentrations of plasma corticoids and norepinephrine (NE). Likewise, stressors applied in a laboratory setting (e.g., insolvable problems) have been shown to influence plasma corticoids. These alterations were correlated with experiential factors (e.g., the day-to-day annoyances subjects had recently experienced), as well as personality variables (e.g., coronary prone behavior patterns). Thus, both stressors applied in a laboratory setting and life-event changes may influence endogenous corticoids. However, it is not known whether an individual will respond similarly to different stressors (i.e., individuals differing with respect to reactivity) or whether the nature of the stressor interacts with individual characteristics in determining corticoid and NE variations. It was observed that relative to control subjects and individuals that received solvable problems, an increase of plasma corticoids and NE was evident in subjects that received insolvable problems in a laboratory situation. Likewise, relative to baseline levels, a rise of corticoid and NE concentrations was evident 1 hr prior to an academic examination. However, the changes in corticoids and NE during the laboratory stress session were not predictive of the changes associated with examination stress. Evidently, those events which are stressful to one individual may be perceived as less threatening to another individual. Accordingly, the variations in endogenous corticoids and NE will vary with the particular stressor employed. In assessing the contribution of stressful events to pathology, the interindividual response to stressors should be considered. In addition, the results of the present investigation also suggested that personality characteristics and previous life-events may influence the corticoid response to stressors and might also contribute to affective state.

30.9 NEUROHUMORAL RESPONSES TO BRAIN INJURY. P.D. Woolf, J.V. McDonald, M. Kelly, L. Lee, R.W. Hamill. Monroe Community Hospital/University of Rochester Medical Center, Rochester NY 14603

Activation of the sympathoadrenomedullary (SA) axis and the hypothalamic-pituitary-adrenocortical (HPA) system is probably the major neurochemical response to stress. Previous clinical studies of central nervous system injury indicate that heightened autonomic responses attend brain injury and may reflect the extent of brain injury (BI), and/or exert deleterious effects. The present investigations were designed to extend these studies and characterize the effects of BI on the activation profile of SA and HPA axes by utilizing plasma levels of norepinephrine (NE), epinephrine (E), dopamine (DA), and cortisol (C); comparison of these neurochemical indexes with the extent of BI as reflected by the Glascow Coma Scale (GCS) permits clinical correlation. In general, within 48 hrs of injury, severe traumatic BI (GCS 3/4) resulted in a 4-5 fold elevation of NE (1306 ± 202 pg/ml) and E (240 ± 48 pg/ml) whereas plasma DA (126 ± 37 pg/ml) only slightly increased. Plasma cortisol also increased (37 ± 3 ug/dl) and reached levels greater than 60 ug/dl. In contrast, patients with relatively mild brain injury (GCS > 11) exhibited plasma levels of catecholamines which were only slightly elevated or within the normal range (NE: 458 ± 44, E: 9 ± 33; DA: 87 ± 18). Patients with marked (GCS 5,6,7) and moderate (GCS 8,9,10) brain injury exhibited indices between those aforementioned values; suggesting that a gradient exists and that peripheral neurohumoral markers may reflect the extent of BI. For example, NE values exhibited the following profile: GCS 3/4 - 1306 ± 202 pg/ml; GCS 5/6/7 - 682 ± 104 pg/ml; GCS 8/9/10 - 576 ± 114; GCS > 11 - 458 ± 44. In order to determine whether plasma NE values have any prognostic value, initial NE levels in patients with GCS 3/4 on entry were compared with their GCS status at one week. Patients who were CGS 3/4 at one week had markedly elevated initial NE levels (2176 ± 531 pg/ml) whereas patients who were GCS > 11 at one week had entry NE levels which were only slightly elevated (544 ± 89 pg/ml). Apparently, markedly elevated NE levels reflect the extent of BI and may have prognostic value. Conversely, a GCS of 3/4 is not necessarily associated with activation of the SA/HPA axes.

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30.10 SYMPATHO-ADRENAL ACTIVATION DURING DEFENSIVE BEHAVIOR IN THE CAT. S. L. Stoddard-Apter, V. Bergdall*, and B. E. Levin. Dept. of Anatomy, Indiana U. Med. Sch., Ft. Wayne, IN 46805 and Dept. of Neurosciences, N.J. Med. Sch., Newark, NJ 07103.

These studies were designed to describe the components of sympatho-adrenal (SA) activation which are integral to defensive behavior in the cat. Cannulae were placed in the atrial appendage through the left external jugular vein for the withdrawal of peripheral blood samples, and in the aortic arch through the left internal carotid artery for the continuous monitoring of mean arterial blood pressure (MAP) and heart rate (HR). Defensive behavior was elicited by exposure to three stimuli: 1) a barking dog, 2) a cat with a hypothalamic electrode implanted to evoke affective aggressive behavior, and 3) a male cat that spontaneously attacked other cats. Three baseline blood samples were withdrawn prior to defensive behavior, and at 0.25, 0.5, 0.75, 1, 2, 3, and 5 min following exposure to the behavioral stimulus. Levels of plasma norepinephrine (NE) and epinephrine (E), indices of SA activation, were determined by radioenzymatic assay. Data were evaluated by determining the percent change from baseline average for each parameter. Preliminary data indicate that both inter- and intraspecific defensive behaviors were accompanied by increases in plasma E as well as plasma NE. In each cat both the greatest increase in E (range: 104 - 6567%) and the greatest tachycardia (range HR ↑ : 10-57%) was seen following confrontation with a barking dog. In most trials the peaks of NE and E following the behavioral stimulus were coincident, suggesting that the initial increase in plasma NE was contributed by the adrenal medulla. However, plasma levels of NE frequently remained elevated after E had returned to baseline, suggesting either continued activation of the sympathetic adrenergic nerves or a differential secretion of NE by the adrenal medulla.

30.11 REGIONAL CHANGES OF MONOAMINES IN THE HYPOTHALAMUS AFTER ACTIVITY-STRESS. D.H. Hellhammer, M. Bell*and M.A.Rea. Dept. Clinical Psychology and Max-Planck-Clinical Research Unit for Reproductive Medicine, University of Münster, D-4400 Münster, FRG

We have recently shown that activity-stress alters levels of biogenic amines in the whole hypothalamus and serum hormone levels (Hellhammer et al., Neuroscience,7,1982:92; Psychosomatic Med.,45,1983:115). These data suggest that a stress-induced increase in noradrenergic activity and a decrease in serotonergic activity modulates the hypophyseal release of ACTH and gonadotropins. In this study, we investigated levels of norepinephrine (NE), 3-methoxy-4-hydroxy-pheylglykol (MHPG), 5-hydroxyindoleacetic acid (5-HIAA), serotonin (5-HT), dopamine (DA), and 3,4-dihydroxyphenyl-acetic acid (DOPAC) by HPLC/EC in the following five micro-punched regions of the hypothalamus: medial preoptic area, mammilary bodies, mediobasal, ventromedial, and ventricular portion.

Activity-stress resulted in an increase of MHPG (+20%) and a decrease of 5-HIAA (-22%) in the medial preoptic area. DA levels in the mediobasal hypothalamus were tendencially lower in activity stressed rats, and significantly reduced in food restricted control animals (-56%), suggesting that a reduced food intake results in a depletion of DA in this hypothalamic portion. No other significant changes were found for any of the amines in one of these hypothalamic regions.

In summary, our study allows the conclusion that activity stress affects serotonin and norepinephrine turnover in the medial preoptic area, and may cause disturbance of the modulatory role of this brain part on the hypophyseal release of gonadotropins and ACTH.

30.12 A NONDETERGENT HPLC METHOD FOR PLASMA CATECHOLAMINES APPLIED TO HUMAN STRESS STUDIES. K.H. Tachiki*, B.D. Naliboff*, K. Spidell*, M.J. Cohen* and A.S. Kling (SPON:M.K. Menon). V.A. Med. Center, Sepulveda, CA 91343 and UCLA School of Med. Los Angeles, CA 90024.

A number of methods have been described for the quantitative assay of endogenous levels of norepinephrine (NE), epinephrine (E) and dopamine (DA) which employ high performance liquid chromatographic (HPLC) techniques and electrochemical detection. In cases where a reverse-phase HPLC column is used for the chromatographic separation of the catecholamines, a detergent counter ion often forms an integral component of the mobile phase. A number of major drawbacks are associated with this use of detergents, including prolonged column equilibration time, shifts in peak retention times during the course of the experiment, and a restriction to the use of isochratic solvent systems.

We have developed a simple dilute sodium phosphate/acetonitrile (45:1, v/v) solvent system which gives baseline separations of NE, E and DA within a total chromatographic time of 12 minutes. The peak retention time for NE was 5.5 min., 7.1 min. for E and 10.1 min. for DA. The separations were acheived using a Waters Associates Radial-Pak C8 column and Z-Module system. The flow rate was 1.0 ml/min. An ESA (Bedford, Mass) model 5100A coulochem dual electrode electrochemical detector was employed for quantitation. Electrode #1 was set at an oxidizing voltage of +0.5 v and Electrode #2 was set at a reducing voltage of -0.35 v. Recordings from the reduction electrode showed no peaks interferring with catecholamines extracted from plasma samples. The catecholamines were concentrated and extracted from plasma employing microcolumns containing 30 mg of Sepralyte SCX resin equilibrated with phosphate buffer, pH 6.5. The catecholamines were eluted from the column resin with 200 ul of perchloric acid with yeilds of greater than 90% recovery.

This method was used to analyze plasma samples collected from 10 healthy subjects during periods of quiet relaxation and periods of stressful vigilance. A detection limit in the low picogram range permitted the simultaneous determination of NE, E and DA. This non-detergent method greatly reduced the time required for the analytical assays. Sensitivity limits and chromatographic kurtosis were similar to those odtained through the use of solvent systems containing detergent counter ions.

Support for this research was provided by the Veterns Administration.

30.13 IN VITRO ASSESSMENT OF STRESS-INDUCED CHANGES IN NORADRENOCEPTOR SENSITIVITY. D.M. Bronstein*, K. Haak*, P.L. Garvey*, and L.D. Lytle (SPON: L. Wilson). Laboratory of Psychopharmacology, Department of Psychology, University of California, Santa Barbara, CA 93106.

Acute exposures to different stressors cause an activation of the sympathetic nervous system and a concomitant release of norepinephrine (NE) neurotransmitter molecules from postganglionic nerve terminals. However, chronic exposure to stressors may result in compensatory changes in receptor sensitivity. For example, repeated restraint stress has been shown to decrease catecholamine receptor binding sites [E.A. Stone & J.E. Platt, *Brain Res. 237: 405 (1982)*]. Unfortunately, little is known about the possible functional consequences of stress-induced alterations in adrenoceptor binding sites. In the present experiments we determined whether chronic immobilization stress might change noradrenoceptor mechanisms important for the synthesis of the pineal gland hormone, melatonin. The activity of the enzyme, N-acetyltransferase (NAT), which rate-limits the overall synthesis of melatonin, is controlled primarily by stimulation of pinealocyte β-noradrenoceptors by NE released from postganglionic sympathetic neurons innervating the pineal gland.

Male albino rats, weighing approximately 220-250 g, were left undisturbed with *ad libitum* access to laboratory chow and water, or were immobilized for 24-48 hr without food or water. All animals were exposed to a 12:12 hr light:dark cycle (lights on at 0700 hr) and were killed during the middle of the light phase of the cycle. Pineal glands were rapidly removed and incubated in a culture medium containing a 10^{-3} N HCl vehicle or the β-noradrenoceptor agonist drug, isoproterenol (10^{-8}, 10^{-6}, or 10^{-4} M) for a 4 hr period and were then assayed for changes in NAT activity [A. Altar, T.P. Motroni & L.D. Lytle, *J. Neural Trans. 58: 231 (1983)*]. There were no differences in the basal pineal gland NAT activity in control or stressed animals. Isoproterenol produced concentration dependent increases in the activities of the enzyme in either the control or restraint stressed animals, but these increases were significantly less in stressed animals at each of the drug concentrations tested. The neurochemical mechanisms causing the compensatory stress-induced receptor changes remain to be elucidated. Nevertheless, our data and the results of others suggest that receptor subsensitivity might be associated with stress-induced elevations in synaptic catecholamines. (Supported in part by an NIMH grant MH-31134 to L.D.L. and by a Canadian NSERC fellowship award to D.M.B.).

30.14 PHYSIOLOGICAL RESPONSE TO PENTYLENETETRAZOL IN RATS: FURTHER EVIDENCE FOR ITS CLASSIFICATION AS AN ANXIOGENIC DRUG. H. Lal, C.M. Harris, S. Yaden* and S. Greene.* Dept. Pharmacology, Texas Coll. Osteopathic Med. Fort Worth, TX 76107.

Pentylenetetrazol (PTZ) produces intense anxiety in human subjects, but physiological evidence for its aversive action in animals is not established. If PTZ is an anxiogenic drug, it should produce overt signs normally associated with aversive stimuli or stress-provoking conditions in rats. We therefore measured PTZ effects on blood pressure, excretion of feces and urination. A dose-dependent increase in blood pressure (5-20% above baseline) was produced over the dose range between 5 and 20 mg/kg. This effect was increased in rats chronically treated (2-3 times a week) with PTZ, such that the same doses elevated blood pressure by 10% - 40%. Chronic treatment also sensitized the rats to other hypertensive treatments such that methoxamine, 1.25 mg/kg, which raised blood pressure by 35% in naive rats, increased it by 55% after chronic PTZ. In rats habituated to handling by sham injections, and food-deprived for 24 hours, PTZ (20 mg/kg ip) produced a greater incidence of defecation (63%) compared to that produced by saline (6%). The average number of fecal boli was greater after PTZ (2.06) than after saline (0.11). The excretion of fecal boli was blocked by pretreatment with diazepam, 5 mg/kg ip. The incidence of urination was 43% after saline and 77% after PTZ. Diazepam blocked the urination produced by saline and reduced the incidence of urination after PTZ to 13%. Over the first four exposures to PTZ, the incidence of defecation was reduced to 40%, the number of fecal boli diminished from 2.06 to 0.7 and the incidence of urination dropped from 77% to 25%. These data indicate that autonomic responses to PTZ are similar to those which occur in rats exposed to other noxious stimuli. Other findings which support the classification of PTZ as an anxiogenic drug are 1) the generalization of other anxiogenic drugs to the discriminative stimuli produced by PTZ and their blockade by anxiolytics (for review see Lal and Emmett-Oglesby, Neuropharmacology 22:1423,1983), 2) generalization of the stress induced by fighting to the PTZ stimulus (Spencer, personal communication), and 3) a pro-conflict effect of PTZ in a conflict test (Corda et al., Proc. Natl. Acad. Sci. USA. 80:2072, 1983). (Supp. by TCOM Faculty Res. Grant, NIH Biomedical Res. Support Grant 1SO7RR05879-1 and HHS Grant NHLBI-T32-HL07465)

30.15 THE EFFECT OF FORNIX TRANSECTION ON TESTOSTERONE MEDIATED PERSISTENCE DURING APPETITIVELY MOTIVATED EXTINCTION BEHAVIOR IN RATS. R. J. Horta* and B. Osborne (Spon: T. Root). Dept. of Psychology, Middlebury College, Middlebury, VT 05753

The extinction of learned behavior has been reported to be affected by alterations of testosterone levels and the changes are similar to those seen after alterations in the functioning of the hippocampus. Since the hippocampus is an uptake site for testosterone and testosterone metabolites, the present experiment examined the possible interaction of these two effects. The behavior of normal male rats, castrated males, males with fornix transection and castrated males with fornix transection was examined during ten days of acquisition and three days of extinction of an appetitively motivated lever press response. Castration resulted in lower response rates during acquisition. Following the transition to extinction, rats with fornix transection maintained food related behavior longer than rats without fornix transection but showed less response variation and less exploratory behavior. On all measures, castration resulted in mild effects, that were in each case, opposite that of fornix transection, and these effects were also present in rats with fornix transection. The results are interpreted to mean that there are effects of circulating testosterone of appetitive extinction but that these effects must be mediated extra-hippocampal structures.

30.PO UPTAKE OF TYROSINE IS DIFFERENTIALLY AFFECTED BY A PREDICTABLE AS OPPOSED TO UNPREDICTABLE STRESSOR. J. M. Weiss, Dept. of Psychiatry, Duke University Medical Center, Durham, NC 27710; L. Pohorecky, Center for Alcohol Studies, Rutgers University, New Brunswick, NJ 08903.

In stressful situations psychological and behavioral factors profoundly influence metabolism of many biochemical constituents. The present study examined how predictability vs. unpredictability of a stressor would affect the uptake of tyrosine in brain, heart and stomach.

Matched triplets (3 subjects) of rats were placed into plexiglas cages with electrodes attached to their tails, and each of the three cages was then placed into a separate soundproof chamber. Two of the three subjects in a triplet were given exactly the same electric shocks (2.0mA intensity, 2sec duration, through the tail electrodes wired in series), the shocks being delivered at varying intervals with an average interval between shocks of 120sec. For one subject, shock was preceded by a pulsating (or "beeping") tone signal that commenced 7sec before each shock was given. Thus, the shock was signalled, or predictable, for this subject. The second shocked subject in the triplet received a similar tone but it was presented randomly with respect to shock. Thus, the shock was unsignalled, or unpredictable, for this subject. The third subject of the triplet received the tone without shocks. After the animals were exposed to these conditions for two hours, the three subjects of a triplet were injected subcutaneously with 100μcuries of ^{3}H tyrosine and returned to their respective stress conditions. Triplets were removed and sacrificed either 10, 30, or 60min later.

Analysis of ^{3}H tyrosine content of brain, heart and stomach revealed marked differences in the rate of uptake of tyrosine in the three experimental conditions. Ten minutes after injection of ^{3}H tyrosine, specific activity of ^{3}H tyrosine (SAty) in the Unpredictable-Shock condition was significantly higher in stomach and brain (cortex and hypothalamus) than it was in the Predictable-Shock or No-Shock conditions. In stomach, SAty in the Unpredictable-Shock group had in fact reached its peak by 10min after injection and declined thereafter, whereas SAty did not reach its peak until 60min post-injection in the other two conditions. Tyrosine uptake in heart showed a similar trend as was seen in stomach and brain but effects were much less pronounced in this tissue.

These results indicate that the psychological/behavioral variable of predictability in a stressful situation can exert considerable influence on the uptake of tyrosine, and the effects appear to be somewhat tissue-specific.

PAIN MODULATION I

31.1 INHIBITION OF THE SPINAL NOCICEPTIVE TAIL-FLICK (TF) REFLEX FROM THE LATERAL RETICULAR NUCLEUS (LRN) IS MEDIATED BY SPINAL α_2 ADRENERGIC RECEPTORS. G.F. Gebhart and M.H. Ossipov, Dept. of Pharmacology, University of Iowa, Iowa City, IA 52242.

Recent studies suggest that the medullary LRN is involved in tonic descending inhibition; stimulation in the LRN also inhibits spinal dorsal horn unit responses to noxious stimuli. In the present experiments, electrode tracks were made mediolaterally and rostrocaudally through the caudal medulla of lightly pentobarbital-anesthetized rats. Constant current cathodal stimulation (100 Hz, 100 μsec, 6.25-200 μA) was started 10 sec before and continued during radiant heating of the tail. The intensity of LRN stimulation (LRNS) was increased stepwise until the TF reflex was inhibited (TF latency > 7 sec) or to a maximum of 200 μA. Only stimulation sites near or in the LRN inhibited the TF reflex at low intensities of stimulation (≤ 25 μA). Monosodium glutamate (50 mM, 100 mM, and 200 mM) microinjected into the LRN produced a dose-related, short-acting inhibition of the TF reflex. Glutamate microinjected outside of the LRN did not affect the TF reflex. LRNS strength-duration curves yielded chronaxies of 92 and 195 μsec for small and larger diameter electrodes, respectively. The intrathecal administration of naloxone (10 and 20 μg), propranolol (10 μg), haloperidol (10 μg) and atropine (4 μg) did not affect either the TF latency or thresholds of LRNS. Methysergide administered intrathecally (15 and 30 μg) did not alter TF latency; the latter dose elevated significantly the LRNS threshold 37 ± 14.1% from a mean threshold of 22.5 ± 2.7 μA. Intrathecally administered phentolamine (30 μg) decreased significantly the TF latency by 20 ± 8.4% from 2.79 ± .24 sec and elevated significantly the LRNS threshold 59 ± 12.6% from 23.1 ± 2.6 μA. The α_2 antagonist yohimbine (15 and 30 μg) decreased TF latency and elevated the LRNS threshold. The latter dose decreased the TF latency 22 ± 7.4% from 2.11 ± .17 sec and elevated significantly the LRNS threshold 94 ± 26% from 25.0 ± 3.9 μA. In contrast, administration of the α_1 antagonist prazosin (15 and 30 μg) did not change either the TF latency or LRNS threshold. These data establish that stimulation in the LRN inhibits the spinal nociceptive TF reflex, and that this descending inhibition is mediated by spinal α_2 adrenergic receptors. Supported by DA 02879 and NS 19912.

31.2 OPIOID, CHOLINERGIC, AND α ADRENERGIC INPUT MODULATION OF DESCENDING INHIBITION FROM THE LATERAL RETICULAR NUCLEUS (LRN). M.H. Ossipov and G.F. Gebhart, Department of Pharmacology, University of Iowa, Iowa City, IA 52242.

Involvement of the LRN in tonic descending inhibition and in inhibition of the nociceptive tail flick (TF) reflex has been described. The neurotransmitter input to the LRN mediating the descending inhibition has not been studied. Male Sprague-Dawley rats were anesthetized and a guide cannula (26 ga) implanted dorsal to the LRN. While lightly anesthetized, a site where cathodal electrical stimulation (100 Hz, 100 μsec) in the LRN (LRNS) inhibited the TF reflex was identified; artificial CSF, norepinephrine (NE), or morphine sulfate (MS) was microinjected into that site and the LRNS threshold redetermined. CSF did not alter the TF latency. Microinjection of NE (2 μg) decreased significantly the TF latency by 19% and elevated the LRNS threshold by 74% relative to CSF control. MS (5 μg) slightly decreased the TF latency and elevated the LRNS threshold 120% In other, awake animals drugs were microinjected into the LRN and the rats tested on the TF and hot-plate (HP; 55°C) tests:

	% Change TF	% Change HP
MS, 5 μg	85 ± 27*	100 ± 30*
MS, 10 μg	155 ± 35*	209 ± 71*
Carbachol, 5 μg	213 ± 39*	178 ± 101*
Clonidine, 10 μg	6 ± 20	-62 ± 5*
Naloxone, 1 μg	-24 ± 3	-35 ± 7*
Naloxone, 2 μg	34 ± 34	-10 ± 8
Phenylephine, 10 μg	46 ± 28	-12 ± 15

* Significant change from pretreatment control.

The antinociceptive effect of carbachol was significantly attenuated in both tests by pretreatment (15 min) with atropine (5 μg in the LRN); the effects of MS were similarly antagonized by naloxone. Neither a cholinergic component of opiate-induced (atropine pretreatment), nor an opiate component of cholinergic-induced (naloxone pretreatment), antinociception was detected. These data suggest that opioid and cholinergic inputs to the LRN independently elicit an antinociception, while noradrenergic input produces a hyperalgesia mediated by α_2 adrenoceptors in the LRN. Supported by DA 02879 and NS 19912.

31.3 ADRENERGIC MEDIATION OF THE DESCENDING INHIBITION OF THE NOCICEPTIVE TAIL FLICK (TF) REFLEX FROM THE LOCUS COERULEUS/SUBCOERULEUS. S.L. Jones and G.F. Gebhart, Dept. of Pharmacology, University of Iowa, Iowa City, Iowa 52242.

Electrical stimulation in the locus coeruleus/subcoeruleus (LC/SC) suggests the involvement of spinopetal efferents in descending inhibition of spinal dorsal horn nociceptors. The purposes of this study were to systematically examine and characterize (1) the descending inhibition of the TF reflex produced by stimulation in the dorsolateral pons and (2) the spinal neurotransmitters mediating the inhibition. Rats were anesthetized with pentobarbital (45 mg/kg) for craniotomy and cannulation of the femoral artery and vein. The rats were subsequently maintained in a lightly anesthetized state (corneal and flexion reflexes present) with an iv infusion of pentobarbital (3-6 mg/kg/hr). Monopolar, cathodal constant current stimulation (100 Hz, 100 µs, 12.5-200 µA) was begun 10s prior to the application of heat to the tail to determine those sites in the pons producing inhibition of the TF reflex. Inhibition of the TF reflex (TF latency > 7s) was produced throughout a large portion of the dorsolateral pons with varying intensities of stimulation (12.5 - 100 µA); however, the lowest intensities of stimulation (< 25 µA) to inhibit the TF reflex were in the LC/SC region. Microinjections of monosodium L-glutamate (100 mM, 0.5 µl) into the LC/SC inhibited the TF reflex, indicating that the stimulation of cell bodies produced the inhibition. Strength-duration characterization of stimulation in the LC/SC resulted in chronaxies of approx. 200 µs. Stimulation at sites outside the LC/SC resulted in chronaxies of approx. 125 µs. To neurochemically characterize the descending inhibition, intrathecal injections of pharmacologic antagonists were made at the level of the lumbar enlargement. The administration of naloxone (5 & 10 µg), methysergide (15 & 30 µg) phentolamine (15 & 30 µg), prazosin (15 & 30 µg) and yohimbine (15 & 30 µg) revealed that only phentolamine and yohimbine reliably increased the threshold of stimulation in the LC/SC for inhibition of the TF reflex. This indicates that spinal α_2 adrenoceptors mediate the descending inhibitory effects of stimulation in the LC/SC. Supported by DA 02879 and NS 19912.

31.4 SPINAL ALPHA-TWO NORADRENERGIC RECEPTORS MEDIATE THE ANTINOCICEPTION PRODUCED BY MICROINJECTION OF CARBACHOL INTO THE NUCLEUS RAPHE MAGNUS. H.K. Proudfit and M.S. Brodie. Dept. Pharmacology, University Ill. Coll. Med., Chicago, IL 60612.

Electrical stimulation of the nucleus raphe magnus (NRM) has been repeatedly demonstrated to produce antinociception. Recently, we have shown that activation of NRM neurons by local injection of carbachol also produces antinociception which appears to be mediated by bulbospinal noradrenergic neurons, but not by raphe-spinal serotonergic neurons. The present study was designed to extend these observations and examine the noradrenergic receptor subtypes in the spinal cord which are involved in mediating the antinociception produced by injecting carbachol into the NRM.

Sprague-Dawley derived rats were implanted with guide cannulae directed toward the NRM as well as intrathecal catheters projecting to the subarachnoid space of the lumbar spinal cord. One week after surgery, the nociceptive threshold of each animal was assessed using the hot plate and tail flick test. Carbachol (2.5 µg in 0.5 µl) was then microinjected into the NRM. Ten minutes later, nociceptive threshold was assessed and one of the following solutions was given by intrathecal injection: saline (15 µl), yohimbine (40 µg in 15 µl saline), WB4101 (37 µg in 15 µl saline), DMSO:water (1:1, v:v) vehicle (15 µl), or prazosin (38 µg in 15 µl DMSO:water vehicle). Nociceptive threshold was assessed at various times after the intrathecal injection.

Microinjection of carbachol into the NRM elevated the tail flick latency 9-12 seconds above baseline scores; an elevation of 20-30 sec was seen on the hot plate test after carbachol administration. The alpha-two antagonist yohimbine completely reversed the elevation in tail flick latency produced by carbachol microinjection. Conversely, the alpha-one antagonists WB4101 and prazosin produced a statistically significant ($P<0.01$) elevation of tail flick latency above that of their respective controls. When the antinociceptive actions of carbachol were assessed using the hot plate test, none of the noradrenergic antagonists produced a significant reversal.

These data lead to the suggestion that the carbachol-induced antinociception is mediated by norepinephrine acting at spinal alpha-2 noradrenergic receptors. Furthermore, the failure of intrathecal antagonists to alter the antinociceptive actions of carbachol on the hot plate test suggests that supraspinal sites may be involved in mediating these actions of carbachol. (Supported by USPHS Grant NS-18636)

31.5 DESTRUCTION OF SPINAL NOREPINEPHRINE TERMINALS REDUCES ANTINOCICEPTION DUE TO MICROINJECTION OF CARBACHOL INTO THE NUCLEUS RAPHE MAGNUS. M.S. Brodie and H.K. Proudfit. Dept. Pharmacology, Univ. Ill. Coll. Med., Chicago, IL 60612.

Microinjection of carbachol, a cholinergic agonist, into the nucleus raphe magnus (NRM) produces analgesia which is probably due to interruption of pain transmission at the spinal level. An earlier report from this laboratory using pharmacological antagonists indicated that spinal norepinephrine (NE), but not spinal serotonin (5HT), is involved in the production of analgesia by microinjection of carbachol into the NRM. In the present study, specific neurotoxins for 5HT or NE were used to further define the role of these two neurotransmitter systems in mediating carbachol-induced hypoalgesia.

Rats were implanted with guide cannulae directed toward the NRM as well as intrathecal catheters projecting to the subarachnoid space of the lumbar spinal cord. One week after surgery, each animal was tested using the hot plate and tail flick tests and then given an intrathecal injection of one of the following: vehicle (0.2% ascorbic acid in saline), 6-hydroxydopamine (6-OHDA, 20 µg in 15 µl vehicle) to deplete NE, or 5,7 dihydroxytryptamine (5,7 DHT, 20 µg in 15 µl vehicle) to deplete both NE AND 5HT. An additional group of rats was given 5,7 DHT after pretreatment with desmethylimipramine (DMI) to prevent destruction of NE terminals. Nociceptive thresholds of these rats were assessed at weekly intervals. Three weeks after neurotoxin treatment, rats were tested and then carbachol (2.5 µg in 0.5 µl saline) was microinjected into the NRM. Tail flick and hot plate latencies were measured at intervals after carbachol microinjection. Carbachol significantly ($P< 0.01$) elevated the nociceptive thresholds of control rats on both tests. Depletion of 5HT did not alter the time course or the magnitude of the hypoalgesia produced by the microinjection of carbachol into the NRM ($P>0.05$). Depletion of NE, however, significantly ($P< 0.01$) reduced both the time course and magnitude of this hypoalgesia on both tests. These data support our previous studies which suggest that the hypoalgesia produced by the local injection of carbachol in the NRM is mediated by bulbospinal NE neurons, but not raphe-spinal serotonergic neurons. (Supported by USPHS Grant NS-18636)

31.6 THE ROLE OF THE A5 CATECHOLAMINE NUCLEUS IN RAPHE-SPINAL PAIN MODULATION. J. Sagen and H.K. Proudfit. Dept. of Anatomy and Pharmacology, Univ. Ill. Coll. Med., Chicago, IL 60612.

Previous reports from this laboratory have suggested that neurons in the nucleus raphe magnus (NRM) are tonically inhibited by brainstem noradrenergic (NA) neurons. Blockade of this inhibitory NA input by the microinjection of NA antagonists into the NRM decreases sensitivity to noxious stimuli. This hypoalgesia appears to be mediated by spinally-projecting serotonergic and NA neurons, since it is attenuated by the intrathecal injection of serotonergic and NA antagonists. Furthermore, histochemical studies reveal that NA terminals in the NRM originate primarily from the A5 catecholamine group. The purpose of the present study was to investigate the role of the A5 nuclei in the modulation of nociception.

In the first study, the effect of A5 lesions on pain sensitivity was assessed. Following determination of baseline tail flick latencies (TFL), rats received either bilateral, unilateral, or sham electrolytic lesions of the A5. TFL's were again assessed at 1, 7, 14, and 21 days following the lesion. Animals receiving either sham lesions or lesions outside of the A5 region exhibited no alteration in TFLs at anytime following the lesion. In contrast, animals with unilateral, or bilateral A5 lesions showed significant elevation in TFLs (hypoalgesia) 1 day after the lesions. Animals with unilateral A5 lesions recovered by day 7, while TFLs of bilaterally-lesioned animals remained elevated, at day 7 and 14, and recovered toward baseline by 21 days.

In the second study, animals received bilateral A5 lesions and were implanted with intrathecal catheters. Animals with elevated TFLs one day following the lesions received an intrathecal injection of either the NA antagonist phentolamine (10 µg), the serotonin antagonist methysergide (10 µg), or saline. The injection of saline did not alter the hypoalgesia induced by lesions in the A5 region. In contrast, the elevation in TFL produced by A5 lesions was significantly attenuated by the intrathecal injection of either phentolamine or methysergide. TFLs were reduced to pre-lesion baseline values within 5 min after the injection of phentolamine and remained depressed at 30 minutes following the injection. The intrathecal injection of methysergide also reduced TFLs within 5 min although not to pre-injection control values.

These results suggest that the A5 CA group plays an important role in the modulation of nociception. These results support the hypothesis that NA neurons in the A5 region regulate nociceptive threshold by tonically inhibiting NRM neurons. Furthermore, the analgesia induced blocking this inhibitory input appears to be mediated by spinally-projecting NA and serotonergic neurons. Supported by USPHS Grant NS 18636)

31.7 MEDULLO-SPINAL INHIBITION OF CAT DORSAL HORN NEURONS. S. Pretel, M.J. Guinan & E. Carstens. Dept. of Animal Physiology, Univ. of California, Davis, CA 95616.

Electrical stimulation in the medullary nucleus raphe magnus (NRM) and adjacent reticular areas produces analgesia and inhibits spinal nociceptive neurons. We wished to investigate the extent of inhibitory medullary areas and to compare effects of medial and lateral medullary stimulation on spinal nociceptive transmission.

Responses of single lumbar dorsal horn units to noxious radiant heating (eg., 50°C, 10 s) of glabrous footpad skin were recorded in cats anesthetized with sodium pentobarbital and ventilated with 70% N_2O. Effects of stimulation (100 ms trains at 100 Hz; 3/s; up to 600 μA mono- or bipolar) at a variety of medullary sites on unit heat-evoked responses (response during medullary stimulation expressed as % of control response without stimulation) were tested using an array of electrodes medio-laterally spaced 2 mm apart and advanced in 1-2 mm steps into the medulla.

Responses of over 60 units tested to date were reduced during stimulation at one or more medullary sites. Powerful inhibition (to 0-50% of control) was typically produced by stimulation in widespread areas spanning NRM and gigantoand magnocellular reticular nuclei bilaterally, confirming several recent reports. Unit responses generally increased linearly with graded stimulus temperature increases from threshold (39-46°C) to 52°C. Slopes of these temperature-response lines were reduced (to 29-65%) with variable change in firing threshold (-0.1 to +1.9°C) in 10 of 12 units during medial medullary stimulation; 2 units showed smaller slope changes (71,101%) and larger threshold increases. During stimulation at lateral medullary sites, 5 units showed small slope changes (76-104%) accompanied by threshold increases (0.6-2.3°C), while 6 units showed slope reductions (28-59%) with or without threshold changes (-0.4 to +3.1°C). These results do not indicate that the differential inhibitory effects observed with medial and lateral midbrain stimulation (J. Neurophysiol. 43:332, 1980) are preserved at the medullary level.

Naloxone (1 mg/kg i.v.) had no effect on medullo-spinal inhibition in each of 7 units tested. The serotonin antagonist methysergide (1-2 mg/kg i.v.) slightly reduced inhibition evoked by medial medullary stimulation in 6 of 7 units; inhibition evoked by lateral medullary stimulation in the 2 units tested was unaffected by methysergide. Serotonin, but not opiates, may thus be partly involved in raphe-spinal inhibition. Supported by N.I.H. grant NS19330-02.

31.8 RESPONSE OF SEROTONERGIC NEURONS IN N. RAPHE MAGNUS TO PHASIC AND TONIC NOXIOUS STIMULI AND RESTRAINT STRESS. C. Fornal, S. Auerbach, and B.L. Jacobs, Prog. Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544.

Serotonergic neurons in the nucleus raphe magnus (NRM) are implicated in the inhibition of nociception. Recently we examined the activity of these neurons in freely moving cats in response to systemic morphine and to mildly painful pinch and radiant heating of the tail (Soc. Neurosci. Abstr. 9:553, 1983). These data indicate that opiate-induced analgesia is not dependent upon activation of serotonergic neurons in the NRM. However, activation of serotonergic neurons in response to noxious stimuli could be important in stress-induced analgesia. Thus, we examined the effects of other painful and stressful stimuli on the activity of these neurons in freely moving cats, using methods previously described (Soc. Neurosci. Abstr. 9:553, 1983). All units in the area of the NRM displaying slow and regular discharge patterns, suppression of activity during REM sleep, and a decrease in discharge in response to a specific serotonin agonist, 5-methoxy-N,N-dimethyltryptamine (250 μg/kg, i.m.), were considered to be serotonergic. Unit activity in the NRM was studied before and after: 1) subcutaneous injection of 0.1 ml of 5% formalin into the main pad of the forepaw; 2) electrical stimulation of the alveolar nerve; and 3) physical restraint produced by the experimenter or by placing the animal in a canvas bag.

The spontaneous discharge rate of serotonergic cells recorded in the NRM during waking was 3.9 ± 0.7 spikes/s (mean ± SE). Unit activity increased in response to phasic and tonic noxious stimuli. The degree of activation is correlated with the level of behavioral arousal associated with these noxious stimuli rather than their painful nature per se. Thus, the increase in unit discharge (∿ 30%) observed with the formalin test was transient and did not correlate temporally with objective behavioral ratings of pain. Restraint produced an immediate and sustained increase in serotonergic unit activity (10 - 50%) that did not outlast the period of immobilization. This is of interest given previous evidence that both painful and non-painful stressors induce analgesia. However, activation of serotonergic neurons is observed during any period of behavioral arousal, whether or not arousal is due to aversive treatment of the animal. Therefore, serotonergic inhibition of nociception may be engaged by all forms of behavioral arousal, including those that are non-stressful. (Supported by NIMH grant MH 23433).

31.9 DIFFERENTIAL EFFECT OF MICROINJECTION OF MORPHINE INTO THE PERIAQUEDUCTAL GRAY ON ACTIVITY OF TWO CLASSES OF NEURONS IN THE ROSTRAL VENTROMEDIAL MEDULLA. M.M. Heinricher, Z.F. Cheng* and H.L. Fields. Depts. of Physiology and Neurology, University of California, San Francisco, CA 94143

The effects of periaqueductal gray (PAG) morphine microinjections on activity of tail flick (TF)-related neurons of the rostral ventromedial medulla (RVM) were studied. Neurons whose activity either decreased (off-cells) or increased (on-cells) immediately prior to occurrence of the TF were examined.

A guide cannula, aimed for the PAG, was implanted in pentobarbital-anesthetized rats (60 mg/kg, i.p.). Animals were maintained in a lightly anesthetized state by a constant infusion of methohexital (15 to 30 mg/kg/ hour, i.v.) in order to maintain a stable baseline TF latency (4-5 s). Activity of off- and on-cells was recorded with a Pt-plated stainless steel electrode. Measures of spontaneous and TF-related activity (peristimulus time histogram plotted as a function of TF or of tail temperature) were obtained. Morphine sulfate (5μg in 0.3μl) was infused over a period of 90s through a 31 gauge injector.

The PAG microinjection increased TF latency (to 10 s cut-off) and differentially affected the spontaneous activity of off- and on-cells. Prior to morphine, both cell types exhibited an irregularly cyclic activity ranging from 0 to 60 Hz. Within 5 to 10 min after the morphine microinjection was complete, off-cell discharge accelerated and shifted from periodic to continuous firing, at a rate equal to or greater than the peak pre-morphine rate. In all cases, on-cell activity ceased following the microinjection. TF-related activity of both cell types was greatly reduced or eliminated after morphine. These effects were reversed within seconds by systemic naloxone (0.25 to 1.0 mg/kg).

The effects of PAG morphine microinjections mimic those of systemic morphine (Fields, et al., Nature,306:684): off-cell activity increases, on-cell activity decreases, and TF-related activity is blocked. This differential effect of opiates is specific, and contrasts with that of electrical stimulation in the PAG region which uniformly excites both off- and on-cells. These results provide further evidence that opiates produce analgesia through an action on RVM neurons which control nociceptive transmission and nocifensive reflexes at the level of the spinal cord (Fields, et al., J. Neurosci,3:2545).

31.10 STIMULATION OF THE PERIAQUEDUCTAL GRAY COUNTERACTS SENSITIZED PAIN DURING NARCOTIC WITHDRAWAL IN MORPHINE-DEPENDENT RATS. R. Emmers, Dept. of Physiol., Coll. of P&S, Columbia U., New York, N. Y. 10032.

Previous work (Exp. Neurol. 83:118, 1984) has revealed that during narcotic withdrawal the nociceptive system becomes sensitized in morphine-dependent rats; innocuous stimuli can induce pain. Since this is caused mainly by a progressive deactivation of the descending antinociceptive system, a question was raised whether stimulation of the periaqueductal gray (PAG) would have any electroanalgetic effect. Rats were adapted to morphine by daily injections. The initial dose (10mg/kg/day) was raised on alternate days by 10mg/kg to reach 100mg/kg/d. 16 hrs after the last injection, electrophysiological experiments were performed on individual animals under chloralose-urethane anesthesia. Single electrical pulses (0.5 ms, 9V) were applied to the sciatic nerve (sc.n.) at 2sec intervals to evoke responses from individual neurons of the nucleus VPL of the thalamus. Nociceptive neurons were identified by the unique spacing of spike potentials accumulated in poststimulus time histograms. The initial burst of short-latency spikes was followed by a period (130-140 ms) of suppressed activity, and then, by refiring of the neuron at about 80 ms intervals (Emmers, R. Pain. Raven Press, 1981.) In morphine-naive rats, stimulation of the PAG with 0.5 ms pulses at 70/sec for 400 ms prior to each sc.n. stimulation reorganized the late refiring of the thalamic nociceptive neuron: after the short-latency spikes, a single late activity peak occurred at a 250 ms delay. This reorganization of spikes is typical of electroanalgesia (ibid). In morphine-dependent rats, PAG stimulation had little effect on the refiring of the thalamic neuron, unless the stimulus frequency was raised from 70 to above 100/sec. Only then the refired spikes began to form a single late peak. Increasing the stimulus frequency (200-300/sec) was particularly necessary after intracarotid infusion of naloxone (0.1 mg/kg), when refiring of the neuron occurred at much lower (2.5V) intensity of sc.n. stimulation. Apparently, electroanalgesia can be induced via a neural pathway that ascends from the PAG to the nVPL (Brain Res. 130:335, 1977) without participation of the descending antinociceptive system. (Aided by grant DA-03292 from NIDA).

31.11 A QUANTITATIVE ULTRASTRUCTURAL ANALYSIS OF SEROTONIN-LIKE IMMUNOREACTIVITY IN THE MIDBRAIN PERIAQUEDUCTAL GRAY.
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The goal of this study was to quantify serotonin(5-HT) immunoreactivity in the midbrain periaqueductal gray (PAG) of the rat stereologically. Volume fractions of 5-HT immunoreactivity were compared within four PAG subdivisions within rostral, middle and caudal levels of the PAG. Midbrain sections from 5 adult, Sprague-Dawley rats were cut on a vibratome, stained immunohistochemically for serotonin using the PAP procedure of Sternberger (1979) and processed for viewing with a Zeiss 10 electron microscope. The volume of 5-HT immunoreactive processes per unit volume of tissue was estimated using point counting. Light microscopic analysis revealed 5-HT axons throughout the PAG while immunoreactive cell bodies and dendrites, belonging to the B-7 group of Dahlstrom and Fuxe, were confined to the caudal ventrolateral quadrant of the PAG. The volume-fraction percent (Vv%) of 5-HT immunoreactive processes was 4X greater in the caudal PAG compared to the rostral portion of this midbrain region. With regard to PAG subdivisions the dorsal division contained significantly less 5-HT immunoreactivity than the other 3 divisions. A split-plot analysis of variance showed no significant difference between the medial and lateral PAG subdivisions but differences were detected between the dorsal and ventral halves of the central gray. The Vv% of 5-HT in the ventromedial PAG, for example, was 6.5X greater than the dorsomedial PAG at inferior collicular levels and 4X greater at superior collicular levels. Very few synapses were found between 5-HT axon terminals and other elements of the neuropil suggesting that nonjunctional 5-HT terminals are a prominent feature of the 5-HT innervation of this midbrain region. The dendrites of 5-HT neurons in the ventrocaudal PAG receive synaptic input from axon terminals containing predominantly round or a combination of round and ovoid synaptic vesicles. More synapses were found on the dendritic shafts of 5-HT neurons located ventromedially than ventrolaterally. The data obtained in this investigation are currently being compared to the distribution of 5-HT receptors in the PAG to ascertain any similarities or differences. We thank Dr. R. Elde for providing the 5-HT antiserum. Supported by NSF BNS 83-11214.

31.12 INTRACELLULAR ELECTROPHYSIOGICAL AND GOLGI ANALYSIS OF THE MIDBRAIN PERIAQUEDUCTAL GREY (PAG) OF THE RAT.
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Previous studies have shown that cytochemical and cytoarchitectural subdivisions can be recognized in the midbrain PAG. The functional organization of the PAG is apparent from the non-homogenous distribution of pure analgesia-producing sites. In this study we assessed the anatomical and functional heterogeneity at the single cell level, using intracellular peroxidase and Golgi techniques.

Intracellular recordings were made in the ventrolateral, caudal PAG and in the dorsal raphe of barbiturate-anesthetized rats. Cells were characterized with natural and electrical stimuli, labelled with HRP and reconstructed. The majority of cells had a low spontaneous activity; receptive fields were large and bilateral. A variety of noxious stimuli either excited or inhibited the neurons. Six categories of nociceptive neurons were found: 1) phasic excitation, lasting a few seconds 2) phasic inhibition 3) maintained excitation for the stimulus duration 4) maintained inhibition 5) prolonged excitation, lasting minutes after stimulus removal 6) prolonged inhibition.

Reconstructed cells were generally small (10-20um diameter), spindle-shaped, polygonal or round. Dendritic arbors were simple. Two to four primary, aspiny dendrites typically gave off an additional 1 to 3 branches. Similar morphological types were seen in Golgi preparations. As yet no correlation between physiological and morphological cell type has been seen. EM analysis of the inputs (peptidergic, etc.) to intracellulary characterized and Golgi-impregnated cells is in progress.

These data provide a first step towards the analysis of circuits involved in the initiation of complex, descending pain control systems.

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31.13 DORSAL COLUMN (DC) INPUT INTO PERIAQUEDUCTAL GRAY (PAG) IN DECEREBRATE DECEREBELLATE CATS.
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The PAG receives a wide variety of descending and ascending inputs and has been implicated in stimulation and chemically produced analgesia (reviewed in Fields & Basbaum, '78). Anatomical evidence suggests that part of the ascending input comes directly from the DC nuclei (Schroeder & Jane, '71; Hazlett et al., '72; Beitz, '82). The purpose of this report is to present electrophysiological evidence for a DC input into the PAG and its functional significance.

PAG neurons (stereotaxic coordinates: A 0 to 1, L 1 to 1.5 and V 3 to -0.5) were recorded in supracollicular decerebrate (or decorticate) and decerebellate cats with one of two types of spinal lesions effected at both C_1 and C_3 levels. One type of lesion involved transection of the dorsal half of the spinal cord (d-cuts), thus leaving the ventral tracts intact. The other type of lesion transected all the spinal cord except for the DC's (v-cuts). In addition, the first three dorsal roots were transected bilaterally in all cats. When combined with localized peripheral and central stimuli the above cuts allowed separation and interaction of DC and anterolateral column (ALC) inputs into the PAG.

Searching stimuli consisted of either peripheral shocks (in v-cuts) or DC shocks rostral to C_1 (in d-cuts). Most of the 27 neurons driven by the DC input (latency range 1.5-10 msec) were also activated by the ALC's (with longer latencies). In an earlier study, reversing the stimulation and recording positions showed that some cuneate neurons can be antidromically activated by PAG stimulation (Jundi et al., '82). PAG neurons (in v-cuts) responded to touch, pressure or joint movements and had relatively small receptive fields.

Using similar preparations, DC stimulation rostral to DC cuts has been shown to modulate activities of dorsal horn neurons and spinal reflexes evoked by nociceptive stimuli (Saadé et al., '84). This indicates the importance of a DC-brain stem-spinal loop in explaining the antinociceptive effects of DC stimulation in man and animals. The present work gives further evidence for physiological triggering of the PAG by the DC's.

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31.14 MODULATION OF CUTANEOUS NOCICEPTOR FUNCTION BY ELECTRICAL STIMULATION IN THE BRAIN STEM OF THE CAT.
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Electrical stimulation at brain stem sites is known to inhibit the excitation of dorsal horn neurones by noxious cutaneous stimuli, probably via brain stem-spinal pathways. However, this reduced responsiveness could also reflect decreased discharges in nociceptive afferent fibers. Therefore, we have studied whether and to what extent the inhibition of dorsal horn neurones is due to reduced nociceptor excitation.

In 6 cats anaesthetized by pentobarbitone (35 mg/kg, maintenance: N2O, pentobarbitone), spikes were recorded from single dorsal horn neurones with glass microelectrodes, and from single afferent nociceptive A delta- and C-fibers dissected from the posterior tibial nerve, in response to noxious skin heating (skin temperature 50°C, 10 s in duration). Stimulation (100 Hz, 100 ms trains, 3 Hz) at 4 brain stem sites (midbrain periaqueductal gray and lateral reticular formation, medullary raphe and reticular formation) during heating reduced responses of 7 dorsal horn neurones to 32% of control (mean of 29 brain stimulations). In contrast, such stimulation had small and variable effects upon heat-evoked activity in afferent fibers: increases (114% of control, mean of 16 brain stimulations) and decreases (80% of control, mean of 15 brain stimulations) were observed in 8 filaments, each containing between 1-4 A-delta and C-fibers responding to skin heating. Brain stimulation produced transient blood pressure changes which could be a cause of modulation of nociceptor responsiveness. It is concluded that brain stem stimulation influences cutaneous nociceptors but the inhibitory effect onto nociceptor inflow is too small to explain the powerful inhibition of dorsal horn neurones from supraspinal stimulation.

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'on leave from University of Delaware.

31.15 STIMULATION OF KOLLIKER-FUSE AND SUBCOERULEUS NUCLEI INHIBIT DORSAL HORN CELL RESPONSES. C.J. Hodge, A.V. Apkarian, B. Hanson and R.T. Stevens. Department of Neurosurgery, Upstate Medical Center, Syracuse, NY 13210.

The Kolliker-Fuse nucleus (KF) and subcoeruleus nucleus (SC) of the dorsolateral pons have been shown to project to the lumbar spinal cord. This area of the pons has been implicated in descending pain modulating systems. This study was undertaken to determine if electrical stimulation of these nuclei causes changes in responsiveness of dorsal horn cells to innocuous and noxious skin stimulation.

Single unit extracellular recordings were made from 63 dorsal horn neurons in 12 chloralose anesthetized cats. Unit responses to innocuous skin stimulation with an electromagnetically driven probe and/or noxious stimulation with a Peltier contact thermode were recorded. The effects of stimulating either KF or SC on the dorsal horn cell responses were then determined. All brain stem stimulation sites were verified histologically and the recording sites of 40 of the dorsal horn cells were determined.

Stimulation of both KF and SC resulted in potent inhibition of the responses of dorsal horn cells to skin stimulation. The thresholds for inhibiting responses to noxious skin stimulation (29.2 uA for KF, 35.6 uA for SC) were significantly lower than the thresholds for inhibiting responses to innocuous skin stimulation (58.2 uA for KF, 49.2 uA for SC). The sites where stimulation was effective were quite specific, with movement of the brain stem electrode by as little as 0.5 mm, frequently resulting in loss of the inhibitory effect. In two cats pretreated with reserpine(1 mg/kg) no inhibitory effect could be found when KF was stimulated with current strengths up to 100 uA.

These studies, together with prior evidence indicating the inhibitory effect of locus coeruleus (LC) on dorsal horn cells, suggest that inhibitory sensory effects, some of which are likely noradrenalin dependent, can be elicited from widespread areas of the dorsolateral pons. Whether there is a functional difference in the normal physiologic roles played by KF, SC, or LC remains unclear at present.

31.16 STIMULATION-PRODUCED ANALGESIA FROM PONTINE VENTROLATRAL TEGMENTUM. J.F. Miller and H.K. Proudfit. Dept. Pharmacology, University of Illinios College of Medicine, Chicago, IL 60612.

Intrathecal injection of noradrenergic (NA) alpha-agonists has been reported to produce analgesia in the rat (Reddy et al., J. Pharmacol. Exp. Ther. 1980, 213 525). Recent anatomical evidence suggests that the NA innervation of the spinal cord is derived from the pontine NA cell groups, including the A7 group of the ventrolateral pontine tegmentum (VLPT) (Westlund et al., Brain Res. 1983, 263, 15). The possible role of the VLPT region in modulation of nociception was investigated in the present study.

Adult female Sprague-Dawley rats were implanted with a unilateral twisted-wire bipolar stimulating electrode into the VLPT. Following a recovery period of 2-8 days, baseline tail flick latencies (TFL) were determined and the effect of electrical stimulation of VLPT sites on TFL was assessed. For many of the VLPT placements, stimulation (0.1 msec square wave pulses, 60 Hz, 30-200 μA) produced a potent analgesia on this test. Stimulation of some VLPT sites , however, elicited motor responses which precluded testing. Paw withdrawal thresholds to pinch were also assessed in several rats in which VLPT stimulation elevated TFL. Stimulation of VLPT sites in these rats significatnly elevated withdrawal thresholds for both hindpaws, suggesting a bilateral descending system may be involved.

Other rats were implanted with both a unilateral bipolar sitmulating electrode into the VLPT and an intrathecal catheter terminating in the lumbar subarachnoid space. After a recovery period of 2-11 days, basline TFLs were determined and the rats were screened for analgesic VLPT placements. Rats in which VLPT stimulation elevated TFL then received intrathecal micorinjections of either phentolamine, yohimbine, or saline, and the effectiveness of VLPT stimulation in elevating TFL was again assessed 15, 30, or 60 min after injection. Intrathecal microinjection of alpha antagonists was found to markedly attenuate the stimulation-produced analgesia from VLPT sites.

In a preliminary study, it was found that microinjection of monosodium glutamate (10 μg) unilaterally into the VLPT also produces a moderate-to-potent analgesia on the tail flick test. The analgesic effect of glutamate microinjection was maximal at 2.5-5 min after injection. The analgesic action of glutamate microinjection inot the VLPT suggests that the effect is mediated by activation of perikarya in the VLPT, rather than fibers of passage.

These data suggest that a descending NA spinal projection system from the VLPT A7 group may participate in modulation of pain transmission. (Supported by USPHS Grant 18636.

31.17 EVIDENCE FOR AN OVERLAPPING DISTRIBUTION OF NEURONS IN MIDBRAIN AND BRAINSTEM NUCLEI WHICH PROJECT TO AMYGDALA AND SPINAL CORD: A DOUBLE LABELING STUDY IN THE RAT. B.B. Sandrew,* D.L. Edwards, C.E. Poletti* and W.E. Foote. Departments of Psychiatry and Neurosurgery, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

Various limbic structures have been implicated in the modulation of nociception. In particular, the amygdala can produce analgesia as well as inhibition of primary afferent dorsal horn neurons following intra-amygdalar stimulation or microinjection of opiates. However, the afferents which are capable of activating such antinociceptive functions in the amygdala remain unclear. We examined possible pathways through which afferent nociceptive information is transmitted to amygdala by looking at midbrain and brainstem structures known to send descending inhibitory projections to the spinal cord.

Ten rats were injected unilaterally in amygdala with either True Blue (TB) or fluorescent latex microspheres (Beads)(L.C. Katz et al, 1984, submitted for publication) and bilaterally in lumbar spinal cord with either Beads or Diamidino Yellow Dihydrochloride (DY HCl). Rats were perfused 4-6 days post-injection and 40um histological sections were examined with a fluorescence microscope.

Direct projections to amygdala from ventral periaqueductal grey (PAG), n. cuneiformis, pontomedullary raphe nuclei, n. gigantocellularis, n. paragigantocellularis and n. paragigantocellularis lateralis were demonstrated using TB or Beads. Many of these nuclei have been implicated in the modulation of nociception through descending projections to spinal cord. We found no evidence that neurons send collaterals to amygdala and spinal cord. However, neurons retrogradely labeled from amygdala were consistently found in close association with cells labeled from spinal cord within the same nuclei suggesting that interactions, possibly through interneurons, may be likely. The potential for simultaneous activation of descending spinal and ascending limbic afferents may have significant implications toward a comprehensive understanding of pain and endogenous analgesia.

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31.18 ELECTRICAL STIMULATION OF THE RAT HABENULAR COMPLEX INDUCES A NALOXONE REVERSIBLE ANALGESIA. A.L. Benabid and G.Mahieux.* LMCEC, UER de Médecine Grenoble, 38700 La Tronche, France.

During a previous study of the Nucleus Parafascicularis(Pf) (Benabid et al., Brain Res., 280 : 217-231, 1983) cells were recorded in the Lateral Habenula (HbL) which exhibited response patterns to peripheral noxious stimuli (NS) similar to those recorded in Pf . In order to study the possible role of the Habenular Complex (Hb) in pain processing, we investigated the effect of electrical stimulation of Hb on the tail flick latency. For each series of experiments, 15 male rats were implanted unilaterally, either on right or left side, into Hb with bipolar electrodes. A week after, the animals were submitted to measurements of tail-flick latency, every 10 minutes, for a period of 3 hours. The amount of analgesia was estimated by the percentage of increase in latency. Four intensities of current (50, 100, 200, 300 and 400 μA) were used for stimulation during 60 seconds, at 50 Hz, 0.5 msec pulse width. A group was given Naloxone IP 1 mg/kg 40 min after Hb stimulation to study the reversibility of the analgesia. A group of animals had their Hb destroyed by coagulation and the effect on tail flick latency was checked once a week for 3 weeks. The results of these experiments clearly demonstrate an Hb stimulation induced analgesia, the maximum of which occurs 60 to 80 minutes after stimulation and then decreases slowly. The maximal amount of analgesia increases with the intensity of current up to 200μA, without any behavioral side effect. At 300μA, the analgesia is not significantly different from the one induced with 200 μA. At 400μA, behavioral side effects (fear, escape) appear and the analgesia is weaker. 200μA appears to be the most efficient current intensity, and induces an average 80 % increase in tail flick latency. The group which was given Naloxone exhibited a dramatic and complete reversal of analgesia.

The group which had Hb destroyed did not show any difference with the sham operated group a week after surgery. During the following weeks, both lesioned animals and controls exhibited an habituation-like analgesia, which reached 75 % for the lesioned rats and 57 % for the controls, and which was not naloxone reversible. Review of the litterature does not provide explanation for Hb induced analgesia. Medial Habenula (HbM) which projects mainly on the interpeduncular nucleus has a very high content in pain related transmitters as Sub P and Enkephalins. HbL projects on the dorsal raphe, the stimulation of which is known to induce a Naloxone reversible analgesia. This hypothesis concerning the mechanism of action must be investigated by further experiments.

31.19 ANALGESIA ELICITED BY PREFRONTAL STIMULATION. S.G.P. Hardy. Dept. of Anat., Univ. of Miss. Med. Ctr., Jackson, MS 39216.

It has been determined that stimulus-produced analgesia (SPA) may be elicited from various regions of the midbrain. In rats, SPA is most easily evoked from the periaqueductal gray (PAG), the reticular formation lying ventrolateral to the PAG, and the deep layers of the superior colliculus. In further support of the notion that these three midbrain regions are involved in analgesic mechanisms, it has been determined that each of these regions share the following features: (1) Each receives somatosensory input, (2) Each contains neurons which project to the nucleus raphe magnus, (3) Each contains enkephalinergic neurons. Recently it has also been determined that the prefrontal cortex (PFC) projects directly upon these same three midbrain sites and alters the firing rates of nocisponsive neurons within these sites. Accordingly, it has been suggested that the PFC may influence the analgesic functions ascribed to these midbrain sites. The purpose of the present study was to determine whether PFC stimulation could alter noci-evoked response latencies of rats, as tested with hot plate and tail-flick techniques.

In chloral hydrate anesthetized rats, bipolar stimulating electrodes were placed into the medial PFC or control sites (occipital or cerebellar cortices) and secured with dental acrylic. Following a 2-4 week recovery period, preliminary tests were performed to determine the maximum sub-seizure current (i.e. the maximum current that could be administered without evoking a tonic-clonic seizure) which could be tolerated by each rat. In subsequent testing either the maximum sub-seizure current or the minimum current necessary to elicit analgesia (whichever was less) was used. The current was administered via a train of 1ms square waves at 10Hz.

When the hot plate and tail-flick studies were performed it was determined that PFC stimulation produced a highly significant ($p<.001$; 2-tailed, paired t-test) elevation of response latencies. Stimulation in the control sites did not produce an increase in the response latencies. Furthermore, it was observed that rats receiving PFC stimulation were able to respond normally to various innocuous stimuli. This suggested that PFC stimulation did not disorient the animals nor inhibit their voluntary motor activity.

The results of this study seem to indicate that the PFC is a site from which SPA may be elicited. Furthermore, it seems likely that this effect may be mediated via analgesia-related areas of the midbrain. This project was supported in part by NIH Grant 5 S07 RR05386.

PAIN MODULATION II

32.1 SELECTIVE REDUCTIONS IN ENVIRONMENTAL ANALGESIC RESPONSES IN RATS BY SCOPOLAMINE AND METHYLSCOPOLAMINE. E. Sperber, J. Schulman* and R.J. Bodnar. Depts. of Psychology and Chemistry, Queens College, CUNY, Flushing, NY 11367.

Neural and hormonal mechanisms appear to mediate the analgesia in rats following acute exposure to several environmental stressors. In this regard, blockade of central muscarinic cholinergic receptors with scopolamine reduces some, but not all forms of foot shock analgesia. The present study evaluated the role of central and peripheral muscarinic cholinergic receptors in analgesia following cold-water swims (CWS) and 2-deoxy-D-glucose (2DG) glucoprivation. Separate groups of rats received either scopolamine (SCOP: 0.01- 10.0 mg/kg), methylscopolamine (MSCOP: 1- 10 mg/kg) or vehicle 5 min before CWS (20C, 3.5 min) with jump thresholds, tail-flick latencies and core body temperatures assessed 30, 60 and 120 min later. CWS analgesia on the jump test was reduced at 30 min and eliminated at 60 and 120 min after the swim by both SCOP and MSCOP. CWS analgesia on the tail-flick test and CWS hypothermia were potentiated marginally by SCOP and MSCOP. In a second experiment, groups were treated identically except that 2DG (600 mg/kg) was administered. While SCOP and MSCOP failed to alter 2DG analgesia on the jump or tail-flick tests, both suppressed 2DG hyperphagia in a dose-dependent manner. These data indicate that SCOP and MSCOP reduce CWS analgesia as a function of the pain test employed and that changes in one stress response do not necessarily covary with others induced by a given stressor. The reductions in CWS analgesia on the jump test by SCOP and MSCOP suggest an important role for peripheral cholinergic receptors and will be discussed in terms of modulation of CWS analgesia by the hypothalamo-pituitary-adrenal axis. (Supported by PSC/CUNY Grant 6-63210).

32.2 THE EFFECTS OF ADRENAL INTEGRITY ON STIMULATION PRODUCED ANALGESIA: A BEHAVIORAL ANALYSIS OF NEUROMODULATION. R.L. Bailey* and B.E. Thorn. Dept. of Psychology, The Ohio State University, Columbus, OH 43210.

It has been observed that bilateral ablation of the adrenal glands potentiates the analgesic effects induced by parenteral administration of morphine. Moreover, it has been shown that opioid compounds (i.e., Beta-endorphin) are present in equimolar concentration with ACTH in CNS tissue following electrical brain stimulation, and that both β-endorphin and ACTH arise from a common precursor. It thus seems feasible to investigate the neuromodulatory role of adrenal secretions on the endogenous pain-inhibitory system.

Male albino rats of the Holtzman strain were implanted with a stainless steel bipolar electrode terminating in the ventral aspect of the periaqueductal gray region (PAG). Biphasic trains of stimulation consisting of rectangular pulse pairs were applied via a Grass stimulator with a frequency of 50 Hz and pulse duration of 1 ms. The intensity of stimulation was increased in a step-wise fashion at intervals of 10 μa beginning with a minimum of 10 μa increasing to a maximum of 100 μa in order to determine the minimum intensity required to elicit analgesic tail-flick responses. After establishing a minimum intensity threshold and monitoring the duration of resulting analgesia, each animal was randomly assigned to an adrenalectomy group, in which case a bilateral adrenalectomy was performed, or a sham adrenalectomy group. Following a recovery period of 10 to 14 days, animals were again tested for stimulation-produced analgesia. After adrenalectomy, most animals failed to regain analgesia at the same or higher levels of stimulation (up to 600 μa). Control animals demonstrated stimulation-produced analgesia at similar levels following sham adrenalectomy. These results implicate adrenal hormonal secretions as a neuroregulatory substrate of the endogenous pain-inhibitory system activated by periaqueductal gray stimulation.

32.3 **A STRESS-INDUCED INCREASE IN BRAIN TRYPTOPHAN UPTAKE: A GENERAL MECHANISM FOR THE EFFECT OF STRESS ON PAIN SENSITIVITY.** S. J. Kelly and K. B. J. Franklin. Department of Psychology, McGill University, Montreal, Quebec, H3A 1B1.

Part of the physiological response to stress by a rat is an increase in brain tryptophan uptake which results in an increase in serotonin availability. We have previously shown that morphine analgesia in the tail flick test is potentiated by this mechanism (Kelly and Franklin, Neurosci. Lett. 44: 305, 1984). We now present further evidence that an increase in brain tryptophan uptake may be part of a general mechanism for the modification of pain sensitivity by stress.

The role of stress-induced changes in tryptophan uptake on pain sensitivity in the tail flick test was examined in three situations: potentiation of morphine analgesia by restraint stress, potentiation of morphine analgesia by the stress of a novel environment, and direct induction of analgesia by a severe form of restraint stress in which the animal was tied to grid for 3 hours.

In all three situations, analgesia was reduced by loading animals with L-valine (200 mg/kg) which competes with L-tryptophan for uptake to the brain and prevents the stress-induced increase in brain serotonin availability (Kennett and Joseph, Neuropsychopharmacol. 20: 39, 1981). Stress potentiation of analgesia was restored if L-tryptophan (100 mg/kg) was administered along with L-valine but not if L-tyrosine (100 mg/kg) was combined with L-valine. This manipulation confirms that the effect of L-valine was due to its interference with serotonin synthesis and not to interference with catecholamine synthesis. L-valine had no effect on pain sensitivity when animals were not subjected to the stressful manipulation.

These results show that pain sensitivity can be reduced by a variety of mild and severe stressful treatments and that the reduction in pain sensitivity is prevented when the increase in brain tryptophan uptake induced by the stressful treatments is blocked. We suggest that an increase in brain tryptophan uptake may be a general mechanism for the stress modulation of pain sensitivity which involves serotonin. (Supported by NSERC Canada Grant A6303).

32.4 DIFFUSE INHIBITION OF FLEXION REFLEX BY TRANSCUTANEOUS ELECTRICAL NERVE STIMULATION (TENS) IN MAN. H.Tsang* and C.W.Y. Chan (SPON:D.Baxter). School of Physical and Occupational Therapy, McGill Univ., Montreal, PQ, Canada H3G 1Y5.

There is some evidence that acupunctural analgesia involves the release of endorphin, whereas conventional (low intensity, high frequency) TENS evokes immediate onset and offset e.g. of subjective pain relief. We therefore carried out a comparative study on the differential effects of the 2 types of TENS-applied either segmentally (L5) or heterosegmentally (C6)- on the flexion reflex (FR).

With the hip, knee and ankle joints fixated, FR from 11 normal subjects was elicited by stimulating the median arch of the right foot with a 200 Hz train of 6x(1 msec square pulses) over 30 msec, with an inter-stimulus interval varying between 10 to 20 sec. Surface EMGs from hip flexors (HF), biceps femoris (BF) and tibialis anterior (TA) were recorded. Using averaging techniques (n=20), the amplitude and area values of the FR of each muscle were computed at 10 min intervals prior to, during and for 60 minutes after the application of conventional- or acupuncture-TENS at the low back (L5) or the contralateral wrist (C6). Thus each subject was tested on 4 separate but randomly ordered occasions.

In contrast to placebo-TENS application which resulted in no significant change of the FR in all the muscles studied, the following findings were observed in 60-100% of the subjects tested:

1) Both conventional- and acupuncture-TENS caused a significant inhibition ($p<.001$) of the amplitude and area of the FR of all the lower limb flexors.

2) Both types of TENS resulted in an inhibition of the FR that has a slow onset, reaching peak inhibition in 10-30 min after the start of TENS; as well as a gradual return to control values (at least 10 min) after TENS.

3) Both the amount and time occurrence of maximal inhibition of the FR did not differ ($p>.01$) significantly during the two types of TENS.

4) Furthermore, heterosegmental stimulation evoked similar inhibitory effects on the FR as segmental stimulation.

5) Sometimes the proximal limb flexors (HF and BF) were more inhibited than distal ones (TA).

These findings suggested that TENS appears to evoke an inhibitory system that has diffuse inputs (regardless of the type or origin of afferent fibers) and outputs (directed to all three lower limb flexors). Furthermore, a gradual onset and offset of this inhibitory influence implicates the possible involvement of a humoral-like substance.

32.5 TOBACCO SMOKE AND NICOTINE ALTER PAIN SENSITIVITY IN RATS. S. Mousa*, V.J. Aloyo and G.R. Van Loon. VA Medical Center and Department of Medicine, University of Kentucky, Lexington, KY 40511.

Acute nicotine produces a potent antinociceptive response in both mice and rats. We have confirmed, using tail-flick latency as an index of pain sensitivity, that acute administration of nicotine (1 mg/kg sc) produces analgesia in adult male Sprague-Dawley rats. Many of the behavioral effects of tobacco smoke are attributed to the nicotine content of the smoke. Thus, we examined the effects of acute and chronic tobacco smoke on tail-flick latency in similar groups of rats. Tobacco smoke was administered by inhalation for 10 min once daily by the standard protocol of the University of Kentucky Tobacco and Health Research Institute using a peristaltic pump and the 2RI reference cigarette.

Acute exposure to tobacco smoke produced analgesia similar to that seen with acute sc nicotine. This smoking procedure involves restraining the animals during smoke exposure. This acute restraint stress procedure produced analgesia, but of significantly lesser degree than that seen with acute tobacco smoke exposure. Both repeated restraint stress and repeated smoke exposure resulted in the rapid development of tolerance to stress- or smoke-induced analgesia, respectively. In chronically smoke-exposed rats, the tail-flick latency measured immediately before the daily smoke exposure (after 24 hr withdrawal) was increased relative to that in either the control or chronically stressed rats. Thus, at this time, chronically smoke-exposed rats appear to demonstrate analgesia. Acute smoke exposure in these chronically smoke-exposed rats, decreases the tail-flick latency to that of controls. The development of cross-tolerance is suggested since neither acute smoke exposure in chronically stressed rats nor acute restraint in chronically smoke-exposed rats altered tail-flick latency.

These data suggest that restraint stress, tobacco smoke and nicotine may produce analgesia through a common mechanism.

(Supported by the University of Kentucky Tobacco and Health Research Institute and the Veterans Administration)

32.6 STUDIES ON THE ANALGESIC EFFECTS OF N-ACETYLSEROTONIN IN THE MEDIATION OF PAIN RESPONSES IN THE CNS. S. Psarakis*, G.M. Brown and L.J. Grota. Department of Neurosciences, McMaster University, Hamilton, Ont., Canada, L8N 3Z5 and Dept. of Psychiatry, University of Rochester, N.Y., U.S.A. (L.J.G.).

We assessed the antinociceptive activities of various substances injected intracerebroventricularly (ICV) into male Wistar rats (Woodlyn Laboratories, Guelph, Canada) maintained on a 12:12 light:dark cycle prior to and after surgery. Animals were implanted with stainless steel guide cannulae (19 g) stereotaxically placed in the lateral ventricle under sodium pentobarbital anesthesia. Each ICV injection was administered using a 10 microliter volume over a period of several minutes under light ether anesthesia. Pain sensitivity was measured with the tailflick apparatus calibrated to give quantitative measurements with a baseline tailflick latency of 4 to 6 seconds. At least 5 trials separated by 30 second intervals were obtained for each animal for each time of testing. At least 5 animals were used for each experiment along with appropriate controls. Tailflick latencies were determined 0.5 hours before treatment and every hour thereafter for at least 4 hours.

The responses to N-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan amide, N-acetylserotonin (NAS), serotonin (5-HT), antisera to NAS were tested as compared to controls. In the first experiment, the synthetic dipeptide N-acetyl-5HTP-5HTP amide (gift from Dr. Tamir) was given in a 10 nmol amount dissolved in 0.9% saline. An increase in pain threshold was observed lasting several hours. This confirmed the observation of Tamir (Tamir, H., et al., Life Sciences, 25:655, 1979). Pain response after 5-HT (Sigma) administration (10 nmol) was not significantly different from response to saline. Animals receiving 10 nmol NAS (Sigma) however demonstrated an increase in pain threshold comparable to the analgesia observed with the dipeptide. In both situations, latencies had returned to baseline 24 hours after treatment. In the last experiment, animals given undiluted antisera to NAS experienced hyperalgesia lasting several hours. This effect was significantly different from the controls, normal rabbit serum (undiluted) and 0.9% saline.

The present data support a role for NAS in the pain system. Its mechanism of action however remains to be elucidated.

32.7 ANALGESIC PROPERTIES OF FLUPIRTINE. R. Gordon*, T.L. Walter*, W. Diamantis* and R.D. Sofia. Wallace Laboratories, Div. of Carter-Wallace, Inc., Cranbury, NJ 08512.

Flupirtine is an orally effective analgesic compound with antipyretic but no anti-inflammatory properties. The analgesic action is not antagonized by naloxone or levallorphan, and there is no apparent addiction liability associated with flupirtine. No ulcerogenic activity has been detected at doses in excess of the analgesic range. We have evaluated the antinociceptive properties of flupirtine in mice employing two models for analgesia: hot plate and acetic acid writhing tests. In addition, central antinociceptive properties were evaluated by measuring the elevation of tooth-pulp nociceptive thresholds following electrical stimulation in the conscious rabbit. The potency of flupirtine was compared with the following analgesic drugs: zomepirac, codeine, meptazinol and ciramadol.

In the hot plate test flupirtine was more potent than codeine, meptazinol and ciramadol (oral ED_{50}s = 66, 125, 131 and 147 mg/kg, respectively). No ED_{50} value was obtained for zomepirac. In the writhing test flupirtine was less potent than zomepirac and codeine but more potent than the agonist-antagonist drugs, meptazinol and ciramadol (oral ED_{50}values = 32, 0.58, 6.4, 45 and 66 mg/kg, respectively).

Tooth-pulp nociceptive thresholds were significantly elevated at least 40 to 55% by flupirtine (6 and 10 mg/kg, i.v.). Codeine (1, 3 and 6 mg/kg, i.v.) also produced a significant increase in nociceptive thresholds, whereas zomepirac was ineffective. Ciramadol (1, 3 and 6 mg/kg, i.v.) but not meptazinol (1, 3, 6 and 10 mg/kg, i.v.) significantly elevated pain thresholds.

The potent analgesic properties of flupirtine have been demonstrated in several animal models. In addition, the analgesic effects of flupirtine, at least in part, have been shown to be centrally mediated since the tooth-pulp assay does not detect peripherally-acting analgesic drugs (Skingle and Tyers, 1979).

32.8 SPINAL PURINERGIC MODULATION OF MORPHINE-INDUCED ANTINOCICEPTION. T.K. Chatterjee*, P. Chatterjee* and G.F. Gebhart. (SPON: W.J. Steele). Dept. of Pharmacology, Univ. of Iowa, Iowa City, Iowa 52242.

Recent studies suggest a neurotransmitter/modulatory role for purines in the CNS. We investigated the interaction between putative adenosine receptor agonists and an antagonist on morphine-induced antinociception in tail flick (TF) and hot plate (HP; 55°C) tests when these agents were co-administered with morphine (MOR) in the spinal subarachnoid space. An intrathecal catheter inserted 7.5 cm down the spinal subarachnoid space to the rostral aspect of the lumbar enlargement was permanently implanted in rats one week before drug administration. The systemic (ip) administration of (5-10 µmol/kg) of the selective adenosine A_1 receptor agonists N^6-L-phenylisopropyladenosine (L-PIA) and 2-chloroadenosine (2-CA) increased significantly HP and TF latencies. In contrast, intrathecal administration of L-PIA, its D stereoisomer D-PIA, and 2-CA did not affect control HP (5.4-6.6 s) or TF (1.8-2.1 s) latencies. However, intrathecally administered L-PIA (0.2 nmol) attenuated significantly by a factor of 3 the antinociceptive dose (AD50) of MOR both in the HP (MOR AD50 = 1.82 µg) and TF (MOR AD50 = 1.66 µg) tests whereas at a higher 2 nmol dose, L-PIA potentiated (~2.5 times) the antinociceptive effect of MOR. 2-CA and D-PIA at a 2 nmol dose attenuated MOR's effect, comparable to the attenuation produced by 0.2 nmol of L-PIA. A high 20 nmol dose of 2-CA, however, potentiated MOR's effect. The adenosine receptor antagonist 8-phenyltheophylline (8-PT, 0.05-2 nmol) attenuated MOR's antinociceptive effect dose-dependently; the maximum effect (~2.5 times) was observed at a dose of 0.5 nmol. The calculated slope of the derived Schild plot of 8-PT antagonism of MOR significantly differed from -1.

These results suggest the interaction of a spinal purinergic system in the modulation of the antinociceptive effects of MOR at the spinal level. These results are also consistent with the proposed existence of high and low affinity A_1 adenosine receptor sites; adenosine agonists at a low dose may attenuate MOR's antinociceptive effect by an interaction at a high affinity site whereas at a high dose, these agents affect both high and low affinity A_1 sites and potentiate MOR's antinociceptive effect. (Supported by DA 02879).

32.9 FRONT PAW (FP) AND HIND PAW (HP) FOOTSHOCK INDUCED ANALGESIA (FSIA) ARE NEUROCHEMICALLY DISTINCT, INDEPENDENT OF CURRENT INTENSITY. I.B. Kinscheck†, L.R. Watkins & D.J. Mayer, Dept. of Physiol. & Biophys., Med. Coll. of VA, Richmond, VA 23298

We have shown (Br. Res., 242:299) that 90 seconds (s) of 1.6 mA rms, 60 Hz, sinusoidal, constant current shock produces naloxone-reversible (opiate) analgesia when the FPs are shocked and non-opiate analgesia when the HPs are shocked. Since HP FSIA induced by these parameters is much more potent than FP FSIA, Cannon et al. (pers. commun.) tested the hypothesis that opiate analgesia may be produced by relatively less intense shock, while non-opiate analgesia may result from relatively more intense shock. They found that either FP or HP FSIA could be opiate or non-opiate depending upon the current levels used. Since several variables, as well as the dose-response curves obtained, were significantly different from our original report, we tested the hypothesis using our original paradigm.

Rats were shocked for 90 s on either the FPs (1.7, 2.0, 2.3 or, in a later experiment, 3.5 mA) or the HPs (0.7, 0.57, 0.53 or 0.39 mA). One group at each shock level received 10 mg/kg/ml naloxone 10 min pre-shock; a second group received saline. Rats were tested for analgesia using a blind procedure via the tail flick test at 0, 1, 2 and every 2 min through 14 min post-shock.

The degree of FP FSIA increased with increasing shock intensity up to 2.3 mA, but did not appear to increase further at 3.5 mA. Naloxone attenuated analgesia at each shock level.

In contrast, potent analgesia was produced by HP shock in response to currents as low as 0.57 mA. A quantal attenuation in analgesia occurred at 0.53 mA. In no case was HP FSIA attenuated by naloxone. In a separate study, potent analgesia was seen after 45 s of 0.6 mA shock, but was markedly decreased in response to either 30 s, 0.6 mA or 45 s, 0.57 mA shock.

In contrast to Cannon et al. who found both FP and HP FSIA to be graded responses (1.6-3.5 mA), both being opiate at low and non-opiate at high currents, our data indicate that FP and HP FSIA are qualitatively, not just quantitatively, distinct. We found that: 1) FP and HP FSIA dose-response curves were not parallel; 2) Equivalent levels of analgesia were seen over different current ranges; and 3) At no shock intensity was FP FSIA non-opiate or HP FSIA opiate. It appears that small changes in paradigms can cause major differences in the neurochemical substrates activated. Supported by PHS grant DA 00576 to DJM.

32.10 EFFECTS OF INTRATHECAL (IT) THYROTROPIN RELEASING HORMONE (TRH) & ARGININE VASOPRESSIN (VAS) ON PAIN & ANALGESIA. L. R. Watkins, S. N. Suberg,& C. L. Thurston*. Dept. of Animal Physiology, Univ. California, Davis, CA 95616, U.S.A.

A variety of peptides, first identified as hormones, are now considered to be neurotransmitters in the brain & spinal cord. Their presence in the spinal cord dorsal horn suggests that they may modulate pain either by directly altering pain sensitivity or by influencing the expression of opiate analgesia.

The effects of IT administration of 2 such neuropeptides, TRH & VAS, were examined on pain sensitivity & on IT morphine analgesia (MA). Using the tail flick (TF) test, rats were assessed for baseline pain sensitivity and then, using a blind procedure, for the effect of either TRH (log doses of .25 ng to 2.5 µg in .5 µl saline) or VAS (log doses of .0025 ng to .25 µg in .5 µl saline) in combination with either morphine (3 µg in .5 µl saline) or saline vehicle (.5 µl). For each dose of TRH & VAS, the drug was delivered 10 min & again just prior to either morphine or saline. The morphine dose was chosen to produce submaximal analgesia in order to allow either potentiation or attenuation of MA by TRH or VAS to be observed. After completion of drug delivery, TF latencies were recorded each 5 min for 40 min.

TRH, while exerting no effect alone, markedly altered MA in a dose-dependent manner. An inverted U-shaped dose-effect function was observed with .25 ng & 2.5 µg TRH completely inhibiting MA, 250 ng & 25 ng TRH partially antagonizing MA & 2.5 ng TRH potentiating MA.

In contrast, VAS neither potentiated nor inhibited MA at any dose tested. VAS did, however, produce analgesia at higher doses. Doses below 25 ng produced small, transient increases in TF latencies. At 25 ng VAS, potent, prolonged analgesia was seen which was not associated with any observable deficits in motor function, muscle tone or righting/grasping reflexes. Neither 2.5 ng nor 2.5 µg TRH produced marked effects on VAS (25 ng) analgesia. "Paralysis" and/or myoclonic twitches were observed in some animals at 250 ng VAS. Inhibition of the TF was not correlated with "paralysis" or myoclonic twitches. Neither analgesia nor "paralysis" was reversed by 10 mg/kg i.p. naloxone.

The fact that TRH was observed to act primarily as an opiate antagonist whereas VAS induced non-opiate analgesia emphasizes that neuropeptides can exert diverse effects on pain modulation at the level of the spinal cord.

32.11 MORPHINE-INDUCED SUPPRESSION OF SPINAL TRANSMISSION IS POTENTIATED BY PROGLUMIDE IN A NALOXONE REVERSIBLE MANNER. S.N. Suberg, L.R. Watkins, E.S. Culhane* & E. Carstens. Dept. Animal Physiol., U. California, Davis, CA 95616, USA.

Behavioral studies using noxious radiant heat showed that intrathecal (IT) administration of the putative cholecystokinin (CCK) antagonist, proglumide (PR), potentiated morphine (MOR) analgesia (Watkins et al., Science, 224: 395). The aim of this study was to electrophysiologically examine the interaction of PR & MOR on a cellular level.

An agar pool was constructed around the lumbosacral enlargement to allow drugs to be placed onto the spinal cord in a fashion similar to that of IT administration. Tungsten microelectrodes were used to record the responses of single dorsal horn cells to noxious heat (50° & 52°C, 10 sec/2 min) applied to the hind footpad of rats receiving continuous infusion of sodium pentobarbital to maintain a constant level of anesthesia. The femoral vein was cannulated for administration of naloxone. Recordings of heat responsive dorsal horn cells were made, over time, to the application of MOR (.1 ug), PR (.04 ng) & MOR + PR. MOR remained on the cord until heat-evoked responses were 50% of baseline or for 30 min if no suppression occurred. PR remained on the cord for 40 min. Morphine + PR remained on the cord between 30-60 min, dependent on the cell's prior response to MOR. Between each drug application the cord was rinsed with saline & recording was continued until responses returned to baseline.

All cells studied to date (N=5), in response to PR, either remained at baseline levels or showed slight excitation (<10%). In cells that showed a MOR-induced suppression (N=3), combined application of MOR + PR produced a far greater inhibition than MOR alone; specifically an enhancement of the onset and degree of inhibition. In cells which showed no MOR-induced suppression (N=2), there was marked inhibition of the cells' responses with MOR + PR. In all cells the inhibition observed following MOR + PR was reversed by i.v. naloxone (10 mg/kg) within 1-5 min. Naloxone alone either had no effect on (N=4) or suppressed (n=2) the heat responsiveness of dorsal horn neurons.

For all cells studied to date, proglumide potentiated morphine-induced suppression of spinal nociceptive transmission. These data provide the first electrophysiological evidence that endogenous CCK may modulate the pain suppressive effects of opiates. Supported by a gift from A.H. Robins.

32.12 EFFECTS OF SPINAL SUFENTANIL. L. M. Kitahata, M. Aoki,* M. Senami,* and J.G. Collins. Dept. of Anesthesiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

Introduction. Sufentanil has a higher lipid solubility than morphine (n-octanol/water partition coefficient = 3.95 for sufentanil and 0.36 for morphine) and thus may be less likely to produce respiratory depression following spinal opioid analgesia. This study therefore examined the ability of spinally administered sufentanil to block noxiously evoked activity of neurons in the dorsal horn of cat spinal cord.

Methods. Cats were anesthetized with a halothane, nitrous oxide, oxygen mixture for surgical procedures. Following decerebration, anesthesia was discontinued, and animals were ventilated with F_IO_2 = 0.21. A tungsten microelectrode with a 10 megohm impedance was advanced into the spinal cord to record extracellular neuronal activity from single wide dynamic range (WDR) neurons. WDR receptive fields on the footpads of the hindpaw were stimulated by noxious radiant heat (51°C) which was focused on the center of the receptive field. Following control studies, the normal saline which had been bathing the spinal cord was removed, and sufentanil, 2.5 and 5.0 ug, dissolved in 0.5 ml of physiologic saline, was applied gently to the spinal cord. I.V. naloxone (0.1 mg) was administered 30 minutes later.

Results. The noxiously evoked activity of all WDR neurons studied was significantly suppressed by spinally administered sufentanil. Suppression was seen within 3 minutes of drug administration, and maximum suppression occurred approximately 30 minutes after administration of sufentanil (activity was suppressed to 55% and 20% of control values by 2.5 ug and 5 ug doses, respectively). Naloxone reversal following intravenous administration (0.1 mg) was demonstrated.

Discussion. The results of the present study have demonstrated a dose-dependent naloxone reversible suppression of WDR neurons following spinal administration of sufentanil. Sufentanil's relatively high lipophilicity should result in less drug remaining in the CSF and thus reduce the potential for respiratory depression. The duration of action was relatively long, probably due to its strong receptor affinity. This study suggests that in light of the significant suppression of noxiously evoked activity by a relatively lipid soluble, long acting drug, sufentanil may be a desirable drug of choice for spinal opioid analgesia. (Supported by NIH Grant NS-09871)

32.13 D-BACLOFEN IS AN AGONIST/ANTAGONIST AT BACLOFEN RECEPTORS MEDIATING ANTINOCICEPTION IN THE SPINAL CORD. J.Sawynok and C. Dickson*, Dept. of Pharmacology, Dalhousie University, Halifax, Nova Scotia B3H 4H7

Baclofen (β-p-chlorophenyl GABA) is an agonist at recently characterized $GABA_B$ receptors (TIPS (182) 3:400). Some pharmacological effects of baclofen may be due to an interaction with this receptor, but direct testing of this hypothesis has been hampered by the lack of a specific antagonist. Recently, D-baclofen was reported to antagonize electrophysiological effects of L-baclofen in the trigeminal nucleus (Pharmacology (1983) 27:85) and antinociception following intrathecal administration (Prog. Neuropsychopharmacol.& Biol. Psych. (1984) In Press). In the present study this later effect was examined in more detail. Experiments were conducted in rats implanted with chronic indwelling catheters. Drugs were administered intrathecally (i.t.) and effects on tail flick (TF) latency determined. L-Baclofen (0.01-0.3 µg) and D-baclofen (0.3-75 µg) produced dose-related increases in TF latency when tested at 15 min intervals for 60 mins following injection. The L-isomer was 100 times more potent than the D-isomer, and twice as potent as the racemate. When L-baclofen (0.1 µg) was injected 15 min after D-baclofen 2-20 µg (when the intrinsic effect of D-baclofen was at a plateau level) the antinociceptive effect of L-baclofen was reduced in a dose related manner. When D-baclofen was coadministered with L-baclofen, no significant alteration in the effect of L-baclofen was observed Antagonism by D-baclofen appears specific for baclofen receptors because the antinociceptive effect of morphine and noradrenaline was not affected. An analog of baclofen, β-m-chlorophenyl GABA, was a full agonist in this system while β-hydroxy GABA was a partial agonist. Both were antagonized by pretreatment with D-baclofen. GABA (1-100 µg) did not increase TF latency. Pretreatment with 2, 4-diaminobutyric acid (a GABA uptake inhibitor) and γ-acetylenic GABA (a GABA-transaminase inhibitor) did not unmask significant elevations in TF latency in response to i.t. GABA. Antinociception produced by L-baclofen appears to result from activation of a receptor which is stereoselective for the L-isomer and can be blocked by D-baclofen in doses which have initial agonistic activity. This receptor may not be a GABA receptor subtype because GABA does not mimic the effect of baclofen. Baclofen appears to act at two separate receptors: that described in this abstract (BCF_A receptor?) and the already characterized $GABA_B$ receptor. (Supported by MRC Canada)

32.14 EVIDENCE FOR INTERRELATED YET INDEPENDENT MECHANISMS FOR THIP- AND BACLOFEN-INDUCED ANTINOCICEPTION. J.L. Vaught*, K. Pelley*, L.G. Costa, P.E. Setler, and S.J. Enna, Dept. of Biol. Res., McNeil Pharmaceutical, Spring House, PA and Depts. of Pharmacol. and/or Neurobiology and Anatomy, University of Texas Med. Sch., Houston, Texas.

The antinociceptive activity (AA) of the GABA agonists THIP (4,5,6,7-tetrahydroisoxalo(5,4-c)pyridin-3-ol) and baclofen (BAC) was evaluated in mice using the hot-plate (HP; 48°C and 55°C) and tail immersion (TI; 50°C) procedures. On the 48°C HP, THIP was found to be significantly more potent than BAC (ED50=0.99 vs. 2.45 mg/kg, i.p., respectively). Prior treatment with atropine (10 mg/kg i.p.) significantly blocked THIP- but not BAC-induced AA. Atropine did not reverse THIP-induced motor incoordination as measured by the rotorod assay. This indicates a distinction between behavioral and antinociceptive activity. On the 55°C HP, THIP and BAC were equipotent with ED50 values of 4.62 and 4.88 mg/kg, respectively. At this higher temperature, atropine (10 mg/kg i.p.) and scopolamine (2.5 mg/kg i.p.), but not atropine methylnitrate (10 mg/kg i.p.), mecamylamine (5 mg/kg i.p.), bicuculline (1 mg/kg i.p.) or picrotoxin (1 mg/kg i.p.) significantly blocked the AA of both THIP and BAC; suggesting a central muscarinic cholinergic involvement in the AA of both agonists. Haloperidol pretreatment (0.5 mg/kg i.p.) enhanced the AA response of BAC but had no effect on THIP. In the TI assay, both THIP and BAC were active with BAC being considerably more potent. Tolerance developed to the AA in the TI assay following repeated administration of THIP (7.5 mg/kg i.p., b.i.d., 5 days) or BAC (15 mg/kg i.p., b.i.d., 7 days). While a reciprocal cross-tolerance was found between THIP and BAC, cross-tolerance to morphine was observed only with THIP. These results suggest that while the analgesic response to THIP and BAC is partially mediated by a common pathway, the two agents act by independent mechanisms as well. The elucidation of these mechanisms should facilitate the discovery and classification of GABA analgesics and perhaps lead to a better understanding of the pharmacological basis for clinical management of pain. [S. Enna supported in part by grants from National Science Foundation and Bristol-Meyer Corporation.]

32.15 A CHARACTERIZATION OF THE ANTINOCICEPTIVE ACTIVITY OF KOJIC AMINE, A γ-AMINOBUTYRIC ACID (GABA) ANALOG. K.A. Pelley*, R. Scott* and J.L. Vaught*, (Sponsor: A. Cowan) Dept. of Biol. Res., McNeil Pharmaceutical, Spring House, PA

Certain GABA-related compounds have been shown to produce naloxone insensitive antinociception. Studies were conducted to characterize the antinociceptive activity of kojic amine (2-aminomethyl-5-hydroxy-4H-pyran-4-one). Kojic amine produced dose-related, but short lived, antinociceptive activity in the 48°C [ED50 9.2 (8.2-10.3) mg/kg i.p.] and the 55°C [ED50 13.8 (12.2-15.7) mg/kg i.p.] mouse hot plate assay. The antinociceptive activity of kojic amine at 48°C was found to be bicuculline (1.0 mg/kg i.p.) and picrotoxin (0.5 mg/kg i.p.) insensitive. It was distinctly separate from the impairment of motor function (measured by a rotorod assay) and was not significantly affected by prior treatment with the cholinergic antagonist atropine sulfate (10.0 mg/kg i.p.). However, at 55°C the antinociceptive effect of a high dose (20 mg/kg i.p.) of kojic amine was significantly attenuated by similar pretreatment with atropine sulfate but not by the peripheral cholinergic antagonist atropine methylnitrate (10.0 mg/kg i.p.). Kojic amine exhibited no significant interaction with haloperidol (0.5 mg/kg i.p.) at this temperature. Kojic amine did not produce significant antinociceptive activity in the Haffner tail pinch, the tail flick or tail immersion assays. Previous work in this laboratory indicates that the antinociceptive activity of THIP (GABA-A) and (±)baclofen (GABA-B) can be differentiated. Electrophysiological and neurochemical studies suggest that kojic amine may be classified as a GABA-A agonist. However, our in vivo data suggest that the antinociceptive activity of kojic amine exhibits similarities to both THIP and (±)baclofen.

32.16 MICROINJECTION OF THIP, A GABAERGIC AGONIST, INTO THE VENTROLATERAL PERIAQUEDUCTAL GRAY MATTER OF THE RAT: EFFECTS ON ANALGESIA. K. C. Retz and L. Holaday*. Dept. of Pharmacology, Texas Col. of Osteopathic Med., Ft. Worth, TX 76107.

Several lines of evidence have suggested a physiological role for alteration of GABAergic neurotransmission as a concomitant of the analgesia produced by opiates. Recently the systemically active heterocyclic GABAergic analog, THIP, 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol, was shown to produce analgesia in the rat that was naloxone insensitive, but cross-tolerant with morphine. Since the site where THIP elicits its analgesic effects is unknown, the current study investigated the effects of THIP microinjection into the ventrolateral PAG, an area known to be important in the processing of nociceptive information, and wherein analgesia may be elicited both by administration of opiates and electrical stimulation.

Male HSD:(SD)Br rats weighing 200-225 g. were stereotaxically implanted with chronic indwelling cannulae (Plastic Products Co., Roanoke, VA) under pentobarbital anesthesia and allowed to recover for at least 7 days before receiving drugs. Cannulae placements were made using the coordinate system in the atlas of Pellegrino et al.(1979) at the coordinates: posterior from bregma = 6.0 mm; lateral from bregma = 0.8 mm; ventral from dura = 5.0 mm. In each animal at 60 min. pre-drug, and 15 and 30 min. post-drug, analgesia was assessed by the hot plate test (55°C.) and tail flick to radiant heat test; motor activity was assessed using the EAM system (Stoelting, Chicago, IL).

THIP, 2.0 ug, significantly elevated the hot plate latency at 15 min. (+2.6 sec., N=16, $p<0.05$) and 30 min. (+4.7 sec., N=16, $p<0.05$), had no effect in the tail flick test, and significantly enhanced motor activity at 15 min. post-drug (+43%, N=12, $p<0.05$). Lower doses were without effect in either hot plate or tail flick test, although motor activity was significantly enhanced at 15 min. following a 1.0 ug dose (+28%, N=9, $p<0.05$). Limited studies (N=4) with 5, 10, 20 ug THIP suggested no trends on either the hot plate or tail flick tests, although motor activity seemed to be enhanced at the 10 and 20 ug doses. The increased motor activity seen with ventrolateral PAG microinjections is in contrast to the effects of systemic administration. These preliminary studies suggest that THIP could perhaps be exerting some of its analgesic effects in the ventrolateral PAG. (Texas College of Osteopathic Medicine Faculty Research Grant #34103 awarded to K.C.R.)

32.17 THE PERIAQUEDUCTAL GRAY FAILS TO PARTICIPATE IN THE ANALGESIC ACTION OF KETAMINE. D.J.Smith, J.M.Perrotti*,A.L.Mansell,* & P.J.Monroe. Dept. of Anesthesiology and Pharmacology,WVU Medical Center, Morgantown, WV 26506.

Analgesia associated with the anesthetic agent ketamine is partially related to an action on opiate neuronal processes (Pain 12:57,1982) that may be initiated by an interaction of the drug with opiate receptors (Neuropharmacol 21: 605, 1982). This study was designed to evaluate ketamine analgesia with regards to the participation of the opiate-sensitive, pain-inhibitory neuronal system originating in the periaqueductal gray(PAG)and descending to the spinal cord. Microinjection guide cannulas were stereotaxically implanted over the PAG of male,Sprague-Dawley rats and were used(after 1 wk. had elapsed)for the infusion of drugs(0.5ul/maximum volume delivered over 30 sec). Analgesic responses were measured by the tail-flick test using a high intensity light as the heat source. A 15 sec. maximum exposure of the tail to the light was used to avoid tissue damage. Ketamine was either injected directly into the PAG in an attempt to induce analgesia, or was injected systemically and the ability of naloxone to antagonize the resulting analgesia was tested by injecting the antagonist into the PAG. Morphine was always used as a control in parallel experiments.

Initially it was determined that the microinjection of morphine produced a dose-dependent (1.25-5.0μg) increase in tail-flick latency (TFL)that peaked about 60 min. post-injection. In contrast, no dose of ketamine tested (0.1-100μg) significantly altered TFL when injected into the PAG. This apparent inability of ketamine to activate the descending inhibitory neuronal pathway was verified in studies where the drug was injected systemically (160 mg/kg, ip). In these experiments it was observed that naloxone (3μg/.5μl) administered into the PAG did not antagonize ketamine analgesia, but was capable of reversing the effect of morphine.

Eventhough ketamine acts as agonist of opiate receptors in some tissues, it appears that the drug is unable to initiate a morphine-like opiate effect from the PAG. However, ketamine still appears to have pharmacological effects related to opiate neuronal processes outside of this restricted neuronal circuit. In fact, it was verified in this study that ketamine analgesia was effectively antagonized by naloxone when the antagonist was administered systemically, rather than into the PAG.

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32.18 STUDIES ON THE TRANSMITTER IN THE MEDULLA MEDIATING DESCENDING INHIBITION PRODUCED IN THE MIDBRAIN. L.M. Dilts and G.F. Gebhart., Department of Pharmacology, University of Iowa, Iowa City, Iowa 52242.

The neurotransmitter in the rostral ventral medulla mediating descending inhibition from the midbrain periaqueductal gray (PAG) is unknown. It has been demonstrated that both the n. raphe magnus (NRM) and medullary reticular formation (MRF) lateral serve as bulbar relays between the PAG and spinal cord. The objective of this study is to determine the medullary transmitter functionally important to descending inhibition of the tail flick (TF) reflex produced by electrical stimulation in the PAG.

Rats were anesthetized (45 mg/kg pentobarbital ip) for craniotomy and cannulation of the femoral vein and artery. Blood pressure was monitored continuously and a light level of anesthesia maintained following surgery by iv infusion of pentobarbital (3-6 mg/kg/hr). Guide cannulae were stereotaxically implanted in the PAG, NRM and MRF bilaterally to allow both electrical stimulation and drug injection at the same site. Brain stimulation (100 Hz constant current cathodal pulses, 100 μsec) was started 10 sec before heating the tail. Thresholds for stimulation-produced TF inhibition were determined in the PAG, NRM, and MRFs (62.1 ± 3.1, 30.1 ± .9 and 22.9 ± 1.4 μA, respectively) and the effects of drugs injected into the medulla determined. Artificial CSF (0.5 μl) produced no change in threshold values. Lidocaine (4%, 0.5 μl) produced a non-specific functional block, increasing stimulation thresholds in the NRM and MRFs > 3 times control. Methysergide (5 μg/0.5 μl) increased stimulation thresholds in the NRM and MRFs > 4 times control while naloxone (1 μg/0.5 μl) doubled all thresholds.

So that only the descending pathway through the NRM could be examined, lidocaine was injected into the MRFs. Stimulation thresholds for TF inhibition in the PAG and NRM were unchanged and naloxone was injected into the NRM. While a functional block was present in the MRFs and thresholds were at least doubled in the NRM by naloxone, no significant change in the PAG threshold was seen. That functional opioid receptors in the NRM are capable of modulating the TF reflex was demonstrated; morphine (2.5-10 μg) inhibited the TF reflex when injected into the NRM. Thus, an endogenous opioid is not the functional transmitter in the NRM modulating antinociceptive PAG stimulation. The role of serotonin is being studied. Supported by DA 02879 and NS 19912.

33.1 THE RELATIONSHIP BETWEEN SKIN MECHANICS, PERIPHERAL TACTILE AFFERENT ACTIVITY AND TOUCH SENSATIONS. R. H. Cohen and C. J. Vierck Jr. Dept. of Neuroscience, Univ. of Fla. Col. of Med., Gainesville, FL 32610.

Movements of glabrous skin in primates were produced by a vertically indenting point stimulus and were examined in cross-section and in normal skin, using a video technique. With the same stimuli, the discharges of median nerve afferents supplying the glabrous skin of monkeys were examined to compare properties of skin mechanics with receptor activation throughout the receptive fields. From the unit data, estimates of the total population of afferent activity were calculated and correlated with measures of the intensity of touch sensations in humans.

Estimates of the sensations elicited in human subjects by ramping on, holding and ramping off the skin were obtained, using line-drawing and matching procedures. At a moderate velocity (10 gm/sec, or approximately 2 mm/sec), qualities of the touch sensation during the onset and offset periods were different from the steady state (pressure) sensation, and the intensities could not be compared. Only a pressure sensation was described at a low velocity (1 gm/sec). At a high velocity (100 gm/sec), subjects matched onset, offset and steady state sensation intensities. Subjects produced ratios of 1.6 to 1.3 to 1.0 for onset, offset and steady state magnitudes. The ratio of total afferent spike rate for onset vs. steady state was considerably greater than the psychophysical ratio. Slowly adapting afferent activity was sufficient to account for the magnitudes of the onset and steady state sensations. This suggests that rapidly adapting afferents do not contribute substantially to the intensity of onset sensations. Activation of rapidly adapting afferents adds a distinct sensation of velocity. (Supported by PHS grants NS 07261 and MH 15737).

33.2 AIRPUFF DETECTABILITY ON HAIRY AND GLABROUS SKIN. H.A. Hamalainen, S. Warren and E.P. Gardner. Dept. Physiology and Biophysics, NYU Sch. Medicine, New York, NY 10016.

The high innervation density and large cortical representation of human glabrous skin have suggested that its tactile sensitivity is more acute than that of hairy skin. Experimentally, vibration and touch thresholds have been shown to be lowest on the finger tips. In this study we have used airpuffs, which preferentially excite rapidly adapting mechanoreceptors, to measure relative sensation intensities on both hairy and glabrous skin areas. Controlled airpuff stimuli were delivered at one or three points (15 mm apart, eliciting a funneled sensation) on the hairy dorsum or the glabrous palm of the human hand. Mean reaction times (RTs) and stimulus detectability (d') were determined in six subjects to airpuffs of 800 and 1600 dyn peak force.

Detectability of all airpuff patterns was superior or equal on hairy skin, in spite of the denser innervation and larger representation of glabrous skin. Mean RTs to 3 weak airpuffs, determined in paired sessions, were significantly shorter ($p<0.01$) on hairy skin in 4 subjects, and identical on hairy and glabrous skin in 2 subjects. None showed significantly longer mean RTs on hairy skin. Average RTs measured 297.1±13.7 ms on hairy skin, and 335.6±19.2 ms on glabrous skin. Average d' values were 4.1±0.4 on hairy skin and 3.4±0.9 on glabrous skin. Shortest RTs and largest d's were obtained on both skin areas with three airpuffs at high intensity, whereas the longest RTs and smallest d's were measured to one airpuff at low intensity. The average hit rate (the ratio of detected to total stimuli) decreased from .99 with 3 strong airpuffs to .94 with 1 weak airpuff on hairy skin, and to .88 on glabrous skin. On hairy skin, no difference was found in average RTs or d's obtained with three airpuffs at low intensity (3x800 dyn) and one airpuff at high intensity (1x1600 dyn). However, on glabrous skin, detectability was significantly better when force was concentrated at a single point than when diffused over a wide skin area. These results are in accord with findings of non-linear summation of neuronal activity, and indicate that there is less spatial summation on glabrous skin.

The enhanced sensitivity of hairy skin to airpuffs is attributable to motion of the hairs. After hair removal by chemical depilation, detectability of airpuffs was reduced on hairy skin to a level below that on glabrous skin. Hair follicle units thus provide a sensitive detection mechanism for hairy skin. (Supported by USPHS Grants NS11862 and NS17973).

33.3 EFFECT OF COWHAGE ON CUTANEOUS RECEPTORS WITH MYELINATED AXONS IN CAT. R.P. Tuckett and J.Y. Wei, Dpt. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84108.

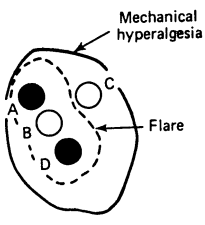
In an earlier report a few of each type of cutaneous receptor conducting in the myelinated range was tested for reactivity to the itch-producing agent cowhage. None responded. To further test for cowhage responsiveness, ongoing activity from cutaneous whole nerve fasciles was recorded before and after cowhage was applied to the whole nerve field.

To determine the limitations of our gross-recording technique, the skin was stimulated electrically and the conduction latency for slowly conduction neurons measured. Impulses were observed with good signal-to-noise ratios and conduction velocities as low as 1.5 m/s. The distributions of interspike interval duration (ISID), before and after cowhage stimulation, were compared (Kolmogorov-Smirnov test). Two of 7 fasicles showed a significantly change in the distribution of ISID ($p<0.001$). To further search for cowhage responsiveness, gross activity from large filaments of nerve (N=9) was recorded (it was estimated that there were about 10-20 mechanoreceptive units/filament) before stimulation, after inactive cowhage and then after active cowhage application. In 5 of 9 samples there was a significantly change in ISID distribution after cowhage ($p<0.001$; four of the 5 showed a decreased mean ISID after cowhage). There was no significant change in ISID after inactive cowhage ($p>0.025$). Because observation of the shapes and sizes of impulses during gross recording did not reveal any obvious recruitment of new units after cowhage, the response of receptor populations reported to exhibit ongoing activity was re-examined. Preliminary observations showed that some type I (2 of 4) and type II (3 of 5) receptors exhibited a significant change in ISID after cowhage application ($p<0.001$).

In conclusion, results obtained from multiunit recordings suggest that some receptors conducting in the myelinated range respond to cowhage. Subsequent single-unit analysis has indicated the involvement of type I and II receptors. It is unknown whether their nerve terminals are reacting to mechanical deformation caused by localized edema and spicule penetration or to the pruritogenic agent present in cowhage.

33.4 CHARACTERISTICS OF PRIMARY AND SECONDARY HYPERALGESIA DIFFER. R. A. Meyer, J. N. Campbell, and S. N. Raja. Applied Physics Lab. and Depts. Neurosurgery and Anesthesiol/CCM, Johns Hopkins Univ., Balto., MD 21205

The characteristics of hyperalgesia that develops within the area of injury (primary hyperalgesia) and surrounding the area of injury (secondary hyperalgesia) following a heat injury to the glabrous skin of the hand were studied using mechanical and heat stimuli. The magnitude of hyperalgesia to mechanical stimuli was comparable in the primary and secondary regions. In contrast, hyperalgesia to heat stimuli occurred only in the primary region. Notably, decreased sensitivity to heat stimuli (hypalgesia) coexisted with hyperalgesia to mechanical stimuli in the uninjured region between two burns. Responses to mechanical and heat stimuli at sites A, B, and C (see figure) were recorded from 10 human subjects before and after a burn (53°C, 30s) at sites A and D. Heat stimuli, which were delivered by means of a non-contact laser thermal stimulator, consisted of 3s stimuli ranging from 41 to 49°C in 1°C increments. Mechanical stimuli were delivered by means of calibrated nylon monofilament probes. Pain intensity was measured with the technique of magnitude estimation. Following injury, the area of mechanical hyperalgesia (20.1 ± 3.6 cm^2, ± S.E.M.) was significantly ($p < 0.01$) larger than the area of the flare (8.3 ± 0.8 cm^2). The pain threshold to mechanical stimuli before injury was similar at each of the three sites (12.0 ± 1.1 bars). After injury, the pain threshold decreased significantly ($p < 0.001$), and again the threshold was similar at each of the sites (Site A: 5.4 ± 0.5 bars, Site B: 5.0 ± 0.7 bars, Site C: 5.1 ± 1.1 bars). Hyperalgesia to heat stimuli occurred at Site A, where ratings increased by 440 ± 120% ($p < 0.01$). At Site B, ratings decreased by 46 ± 10% ($p < 0.005$), whereas at Site C there was no significant change (3 ± 13%). The coexistence at Site B of hyperalgesia to mechanical stimuli and hypalgesia to heat stimuli suggests that the mechanisms of primary and secondary hyperalgesia differ. (Supported by NIH grants NS-14447 and NS-00519).



33.5 EXCITATORY SYMPATHETIC ACTIONS ON A-MECHANICAL-HEAT AFFERENTS: A SOURCE OF CAUSALGIC PAIN? W.J. Roberts and S.M. Elardo. Neurol. Sci. Inst., Good Samaritan Hosp. and Med. Ctr., Portland, OR 97209.

Sympathetic activity was shown in earlier studies to modulate the sensitivity of many classes of mechanoreceptors. Although these peripheral sympathetic actions are suitable for modulating the perception of mechanical stimuli, it is not clear that these actions are involved in the pain syndromes such as causalgia in which sympathetic arousal evokes pain. In this study activity is recorded from single A-mechanical-heat (AMH) afferents from cat's skin. These units respond both as sensitive mechanoreceptors and as thermal nociceptors. Others have shown that they are sensitized to thermal stimuli by repeated heating of the receptive field into the noxious range; that finding is confirmed.

Sympathetic effects on single AMH afferents were tested by comparing responses to thermal or mechanical stimuli during the presence and absence of sympathetic stimulation (SS) at 10 Hz. No AMH units were activated by SS alone prior to thermal sensitization. However, those AMH units with relatively high mechanical thresholds (> 5 gm, von Frey) were activated by SS alone after thermal sensitization (5 of 6 units tested to date). Only a few of the more sensitive AMH units (thresh. < 5 gm) became active during SS alone after sensitization (5 of 17 to date). However some of these units showed reduced thresholds to mechanical stimulation during SS. Units in another functional class, the A-delta mechanical nociceptors, were not sensitized by noxious heat and were not activated by SS, even after thermal trauma to the receptive field (5 tested to date).

Data from the present study show that sympathetic activity does not evoke activity in these afferents in undamaged skin, but it does evoke activity after trauma to the skin. If the AMH afferents have excitatory actions on central "pain" pathways then their activation by sympathetic activity may explain the occurrence of sympathetically evoked pain or hyperalgesia in the sympathetic reflex dystrophies including causalgia.

33.6 COOLING AND ANTIEPILEPTIC DRUGS DEPRESS IMPULSE CONDUCTION AT BRANCH POINTS OF SINGLE MYELINATED AFFERENT FIBERS. S.D. Stoney, Jr. and E. Hershberger*. Dept. of Physiology, Medical College of Georgia, Augusta, GA 30912.

We have studied the effects of phenytoin, phenobarbital and temperature changes on impulse conduction at the branch point of myelinated afferent axons in perfused dorsal root ganglia (DRG) of frogs (Rana pipiens). Intracellular recording from a DRG neuron soma during double-pulse stimulation of peripheral nerve (orthodromic impulses) and/or dorsal root (antidromic impulses) allowed measurement of the least conduction interval (LCI) of its axon branch point within the ganglion. For each neuron, the LCI was determined by gradually reducing the interstimulus interval until the response to the 2nd stimulus failed in an all-or-none manner. Collision tests using oppositely conducting impulses showed that failure of the 2nd response meant that it had not invaded the branch point. Comparison of DRG neuron LCIs for orthodromic impulses with LCIs for afferent axons recorded 1-2 mm before the ganglion showed that the 2nd response was not failing at a node of Ranvier distal to the branch point ($p < .0005$). Average LCIs for the branch points of 4 electrophysiologically-distinct types of DRG neurons were inversely proportional to conduction velocity. At 21-23°C, average LCIs (± SE) were 7.76 ± 0.28 msec for branch points of unmyelinated C fibers (n=6) and 1.79 ± 0.09 msec for the most rapidly conducting A fibers (n=48). The safety factor (SF) for the branch point was derived from the LCI and found to be directly proportional to axon conduction velocity. The SF for antidromic impulses was significantly less than the SF for orthodromic impulses. Phenobarbital and phenytoin were administered via the perfusion stream at 21-23°C and effects were measured after 10-15 minutes. At high concentrations phenytoin (70-100 μM) and phenobarbital (100-500 μM) significantly increased LCIs, i.e. decreased the SF, of branch points of rapidly conducting neurons. Phenytoin was found to increase LCIs in a dose-dependent fashion at 7-70 μM concentrations. LCIs of rapidly conducting neurons were found to be inversely proportional to temperature over a range of 12-33°C. The results show that cooling and antiepileptic drugs depress conduction of closely spaced impulses at branch points of myelinated axons. The sensitivity to phenytoin suggests that depression of branch point safety factor may contribute to phenytoin's antiepileptic action. (Supported in part by BRSG #25-07-RR-05365-23).

33.7 THE ROLE OF IONIC COMPOSITION AND OSMOTIC PRESSURE ON THE EXCITATION OF CORNEAL FREE NERVE ENDINGS. R.W. Beuerman, A.J. Rozsa, and B.M. Dupuy*. LSU Eye Center, New Orleans, LA 70112.

Very little is known of the mechanisms involved in the activation of free nerve endings by sensory stimuli. In the cornea, all axons from the trigeminal ganglion terminate within the epithelium as free nerve endings. In this study solutions of electrolytes and nonelectrolytes at various osmotic pressures were used to investigate the pathway for sensory stimulation. Albino rabbits (1.5-2kg) were anesthetized with urethane (1.5g/kg) and tracheotomized. The long ciliary nerve was isolated in the orbit and prepared for action potential recording by conventional techniques. Care was taken not to damage the epithelial surface. Multi-unit records integrated with a 1.5 sec time constant were used to determine the relative effectiveness of these stimuli on the population of sensory free nerve endings. Electrolytes (NaCl, Na_2SO_4, Na cyclamate and choline chloride) and nonelectrolytes (sucrose and urea), each in a concentration series, covered the range of osmotic pressures from 20 mOsm to 1800 mOsm. Stimulus solutions were maintained at 31°C and applied in 1.5cc aliquots into a chamber, limiting stimulus presentation to the cornea. Following a 10 sec stimulus duration, several wash applications of isotonic NaCl or corneal Ringer reestablished baseline activity. At normal osmotic pressure (305 mOsm), none of these solutions were stimulatory; however, both decreasing and increasing osmotic pressures elicited responses related in magnitude to their departure from isotonic conditions. Hyperosmotic electrolyte solutions were more excitatory than with the nonelectrolytes. However, stimulation in the hypo-osmotic range did not reveal large differences between solutes. The barrier effect of the apical membrane of the corneal epithelium on the movement of Na^+ has been tested. Amphotericin B which creates Na^+ channels in the apical membrane, was applied to the corneal epithelium for 5 min at 10^{-4}M. Comparison of the amplitude and duration of the responses to 0.3M and 0.5M NaCl before and after amphotericin showed these to be unaffected. The results suggest that ions can access the environment of the sensory endings through the paracellular pathway, presumably breaking down the tight junctions of the epithelium. However, excitation by sucrose and hypo-osmotic solutions indicate that the effects of water flow across the apical membrane can also be stimulatory. EY04074.

33.8 RESPONSES OF CAT G1 HAIR RECEPTORS TO RANDOM-SEQUENCE STIMULUS TRAINS. M.D. Goldfinger. Dept of Physiology, Wright State Univ. Sch. of Medicine, Dayton, OH 45435.

Single G1-receptor hairs are waxed to a speaker-driven rod while recording elicited parent axon impulses in the dorsal column (1).For random stimulation, each stimulus pulse (1-ms duration) is triggered from a stack of interevent intervals originally generated by a statistically stable Poisson process (2).Non-Poisson sequences are derived from the Poisson interval stack.Input stimulus pulses and output axon impulses are translated into interevent time intervals and stored for point-process analysis.Impulse trains showing clear fatigue are not studied.

Random stimulus sequences allow convenient assessment of responses via the impulse train conditional probability P as a function of time:

$P(t) = S(t) \cdot R(t)$, where S(t) is the conditional probability of the input stimulus train, and R(t) is a conditional probability function summarizing contributions by the viscoelastic properties of the tissue between stimulus probe and lanceolate terminal, the terminal, the terminal's axonal branch, the electrically-coupled axonal arborization,and the parent afferent axon.R(t) determines the extent to which the net output P(t) differs from the input S(t).Both P(t) and S(t) are represented by the Expectation Density computed from respective event trains.Three types of S(t) trains are used, where S(t) either is a constant (Poisson process), has a slow (4-5 ms) risetime to a constant level, or begins high and declines (in approx. 9 ms) to a constant level.

For each case, P(t) differs from S(t).The deadtime (2-4 ms) exceeds that of S(t).P(t) has a post-deadtime transient exceeding the steady-state envelope level.With slowly-rising S(t) trains, additional later P(t) transients also occur.With each type S(t), the P(t) transients persist at higher or lower stimulus amplitude levels, which may be above, below, or at the amplitude required to elicit an impulse doublet (as determined with 1/sec periodic testing).The onset delay of a P(t) transient may coincide with the doublet impulse interval.

These data illustrate the sensitivity of G1 afferents to trains of randomly-occurring constant-amplitude short-duration pulses. Persistence of P(t) transients with varied stimulus amplitude suggests they are not due to a monotonic recovery cycle (2),and implies that under these experimental conditions impulse initiation by individual components of the G1 afferent unit is mediated by a damped oscillatory process.

(1) Goldfinger,M.D.&V.E.Amassian. J.Neurophysiol.44:979-1001, 1980.
(2) Goldfinger, M.D.Soc.Neurosci.Abst. 8:858, 1982.

33.9 THE RESPONSE OF DIGITAL NERVE FIBERS TO GRATINGS MOVED SINUSOIDALLY ACROSS THE FINGERPADS OF ANESTHETIZED MONKEYS A.W. Goodwin*, J.W. Morley* and I. Darian-Smith. Dept. of Anatomy, University of Melbourne, Parkville, Victoria, 3052. Australia.

A human typically assesses a textured surface by rubbing his fingerpads back and forth across the surface sinusoidally. We have previously shown that humans can discriminate two gratings (simple textures) that differ in spatial period by about 5% (Morley, J.W., Goodwin, A.W. and Darian-Smith, I., Expl.Brain.Res., 49: 291-299, 1983). We recorded from afferent fibers with mechanoreceptive fields (RFs) on the fingerpads of anesthetized monkeys; these fibers responded to gratings moving back and forth sinusoidally across the pads. Stimulus parameters controlled were the amplitude and frequency of the sinusoid, the contact force and the spatial period of the grating (ratio of ridge width to groove width held constant at 1:7).

Generally, slowly adapting mechanoreceptive fibers responded to coarse surfaces (spatial period > 1.75mm), quickly adapting fibers to coarse and medium surfaces (periods > 1.25mm) and Pacinians to all surfaces (periods between 0.75 and 3.0mm). With other parameters held constant, a change in the spatial period of the grating produced a change in the fiber's response. For some fibers the response profile (discharge frequency as a function of time) was approximately sinusoidal in synchrony with the movement of the grating. During the portion of the cycle around peak velocity, the response was entrained to the temporal frequency of the ridges. However changes in either the temporal frequency of the ridges or in the spatial period of the ridges often led to non-sinusoidal response profiles. Some fibers never exhibited simple sinusoidal discharge profiles.

A common finding was direction selectivity, that is asymmetry in the fiber's response to the grating moving in opposite directions. For most fibers with RFs at the center of the fingerpad, this selectivity could be eliminated by changing the angle of the grating with respect to the tangent to the finger at the RF. However, for many fibers with RFs not at the center of the pad, this asymmetry could not be eliminated. Such asymmetrical responses will occur in a proportion of the peripheral afferents when a human scans his fingers over a textured surface.

33.10 INFORMATION CONVEYED BY PACINIAN AFFERENTS IN MAN ABOUT POLYHARMONIC VIBRATIONS. K. W. Horch. Dept. Physiology, Univ. Utah Sch. Medicine, Salt Lake City, UT 84108.

The ability of subjects to distinguish pure sinusoidal from square- or triangular-wave vibrations of the same fundamental frequency applied to glabrous skin raises the question: can the vibrotactile system, like the auditory system, distinguish polyharmonic from pure tones, irrespective of changes in fundamental frequency or amplitude? This possibility was tested using low amplitude (< 10 μ peak to peak displacement), high frequency (> 100 Hz) vibrations applied to the finger tips of human subjects. The stimuli were chosen so as to selectively activate only the Pacinian corpuscle mechanoreceptor population.

For a given trial the subject was presented with alternating pairs of tones, one of which was a pure sinusoid and the other of which had a second or third harmonic component of fixed relative amplitude and phase. The fundamental frequency and amplitude of the two tones were varied independently. The task of the subjects was to identify which of the two tones was diharmonic, ignoring changes in apparent pitch or intensity. Only subjects who could successfully perform this discrimination using auditorily presented tones were selected for the study.

As of this writing, despite intensive training on the task, no subjects have been able to reliably identify the diharmonic stimulus in all the vibratory pairs used, although they could do so for the same pairs presented acoustically. These results suggest that, unlike the auditory system, the Pacinian component of the vibrotactile system cannot resolve the component frequencies of polyharmonic stimuli. Distinguishing sinusoidal from non-sinusoidal tactile stimuli must rely on apparent changes in some other dimension of the stimulus, such as its intensity.

33.11 UNMYELINATED JOINT AFFERENTS IN RAT, CAT AND HUMAN. L.A.Langford. Div. of Neuropathology. Univ. of Texas Medical Branch,Galveston,TX 77550.

The traditional concept of knee joint innervation has implied that the majority of axons supplying a joint are myelinated with the majority of the less numerous unmyelinated axons being postganglionic autonomic, innervating the joint vasculature. Langford and Schmidt (Anat.Rec.206:71-78, 1983) demonstrated the medial and posterior articular nerves supplying the cat knee joint are predominantly unmyelinated (80%,78%) with 50% of these axons sensory and the remaining 50%, autonomic.

In this study the unmyelinated sensory axons and their terminals from rat and cat medial articular nerves are being examined after removal of the autonomic axons with a bilateral lumbosacral sympathectomy (L_3-S_3). The nerve with the entire medial aspect of the joint is removed and prepared for electron microscopy. Single gold serial sections are viewed in an electron microscope and individual unmyelinated axons are traced to their terminals. Preliminary results show the majority of the sensory axon terminals occur near blood vessels; nevertheless, they also occur in dense regular and irregular connective tissue, areas dense with fat cells and occasionally accompanying Ruffini's corpuscles. All of these endings are associated with Schwann cell cytoplasm but most are only partially surrounded by the Schwann cell with separation from connective tissue by a lamina externa.

In the human studies the ultrastructure of normal medial articular nerves and terminals in the medial joint capsule are being examined. Perhaps reassessment of human joint innervation, in conjunction with extensive animal experimentation, will aid in further clarifying joint kinesthetics and pain.

33.12

WITHDRAWN

33.13

WITHDRAWN

33.14 SENSORY TRIGEMINAL INNERVATION OF THE TONGUE OF THE GALAH (CACATUA ROSEICAPILLA). R.A.Carr* and J.M.Wild, Dept. of Behavioural Biology, R.S.B.S., Australian National University, Canberra, A.C.T. 2601. Australia.

In mammals the sensory innervation of the tongue is supplied by cranial nerves V, VII and IX, but in birds a lingual contribution by the trigeminus has usually been denied, and a lingual role for the facial nerve is in doubt because of the small number and position of taste buds in most species. The glossopharyngeal nerve is therefore said to be the sole source of sensory lingual innervation. Recent studies have shown, however, that some birds which husk seeds, e.g. parrots and finches, have a substantial lingual sensory innervation by the hypoglossal nerve (Wild, J.M., J. Comp. Neurol., 203: 351, 1981; Bottjer, S.W. and Arnold, A.P., J. Comp. Neurol., 210: 190, 1982). In this study we investigate the possibility of a sensory innervation of the parrot tongue by the trigeminal nerve.

Dissection of the ramus mandibularis in the jaw of the galah revealed a large branch coursing through the upper part of the proximal mandible to enter the lateral margin of the tongue directly behind the keratinised, bulbous tip. In different experiments horseradish peroxidase (HRP) was either applied to the proximal severed stump of this branch or injected into the tongue subsequent to cutting the hypoglossal and glossopharyngeal nerves proximally. In both types of experiment the results were the same, as determined by standard processing of 40µ sections with TMB and H_2O_2. HRP-positive somata were located in the maxillary-mandibular portion of the Gasserian ganglion, and extra-perikaryal reaction product suggestive of afferent terminals was localized within two specific regions of the principal trigeminal sensory nucleus (PrV); one dorsomedial at rostral levels of the nucleus, and the other ventromedial at more caudal levels. Labeled fibers were also traced into the descending trigeminal tract as far as the upper cervical spinal cord with presumed terminals in the dorsomedial dorsal horn. A sparse projection was also observed to lateral portions of the rostral solitary nucleus.

These data suggest that, like the glossopharyngeal and hypoglossal lingual nerve sensory components, that of the trigeminus in the galah is importantly concerned with transmitting mechanoreceptive information from that part of the tongue in direct contact with the seed during the husking process. This information is passed to the trigeminal column, and via PrV, probably to the telencephalon.

33.15 STUDIES ON PRIMARY AND PERMANENT TEETH: COMMON INNERVATION FEATURES. T.E. Jones*, K.V. Anderson and N.F. Capra. Department of Anatomy, University of Mississippi Medical Center, Jackson, Mississippi 39216.

The pulps of both deciduous and permanent teeth are innervated by neurons whose cell bodies reside in the trigeminal ganglia (TG). While much is known about the general anatomical and physiological features of TG cells, little is known about target innervation mechanisms that direct TG neurons during the development of specific target tissue. Our present experiments, with cats, were designed to determine whether some TG neurons that supply the first teeth to be formed, the deciduous teeth, also come to provide a portion of the innervation of the final teeth to be developed, the permanent teeth.

Multiple labeling methods were used to accomplish this goal. Cell bodies which innervate deciduous teeth were labeled by exposing their terminal branches in the pulp cavity of maxillary canines to the fluorescent label Fast Blue (FB). After natural replacement of the deciduous dentition, the pulp chamber of permanent maxillary canines were exposed to a second tracer compound, Lectin-Horseradish Peroxidase (L-HRP). Following these procedures, TG tissue was examined to permit the identification of single or double labeled neurons.

The results of our experiments showed that three populations of labeled cells could be identified within the TG. The first population of cells contained only the FB fluorescent label, suggesting their involvement in the innervation of deciduous maxillary canine teeth. A second population within the TG contained only the L-HRP label, indicating that they innervated the permanent maxillary canine teeth. The third population of cells were double labeled and contained both FB and L-HRP, indicating that these neurons first projected to deciduous teeth and, later, came to supply the neural pulp of permanent canine teeth. Taken together, these results suggest that permanent teeth may derive their innervation from at least two categories of TG cells. In one instance, permanent teeth appear to receive a unique innervation from TG neurons; these TG neurons project their afferents only to permanent teeth and do not appear to participate in the innervation of primary teeth. In a second instance, certain TG neurons seem to serve a kind of "dual" purpose, first supplying primary teeth and, later, supplying permanent teeth.

34.1 **PENTOBARBITAL-INDUCED CHANGES IN THE MOUSE AUDITORY BRAINSTEM RESPONSE AS A FUNCTION OF STIMULUS REPETITION RATE AND TIME POST-DRUG.** M.W. Church, T.M. Welch* and D.W. Shucard. Brain Sciences Labs., National Jewish Hospital, Denver, CO 80206.

Anesthetics are commonly used in animal auditory brainstem response (ABR) studies as a form of chemical restraint. While the prevailing view maintains that anesthetics do not alter the ABR, few studies have systematically studied the effects of anesthetics on the ABR. The present study examined the time-dependent effects of pentobarbital anesthesia as well as the combined effects of pentobarbital and stimulus repetition rate on the mouse ABR. Both fast stimulus repetition rates and pentobarbital can be regarded as 'synaptic stressors.' It was predicted, therefore, that any pentobarbital-induced effect on the ABR would be increasingly more pronounced at progressively faster stimulus repetition rates.

The subjects were 10 young adult female BDF_1 mice. A newly developed restraining device allowed for recordings to be obtained with and without anesthesia. ABRs were recorded from each animal during a baseline period as well as after saline and pentobarbital (80 mg/kg, i.p.) treatments. Rectal temperatures were continuously monitored and maintained, on the average, within $0.1^{o}C$ of baseline values.

Pentobarbital produced prolongations in the latencies of ABR components P2, P3 and P4 that were significant relative to baseline and saline control values. These latency shifts were greater for each successive component. Latency shifts were maximal 15 min after drug administration and dissipated over time in a pattern similar to a drug elimination curve. Additionally, pentobarbital produced significant increases in the amplitudes of P1, P2 and P4. Only the pentobarbital-induced changes in P4 amplitude and latency were significantly dependent on stimulus repetition rate. The pentobarbital-induced changes in the mouse ABR proved to be independent of core temperature, circadian variation, stress, etc. Moreover, significant ABR changes were still present as animals 'awakened' from anesthesia.

The fact that pentobarbital can significantly influence the mouse ABR has major implications for auditory neurophysiological studies in which subjects are under the influence of a sedative/hypnotic. For example, such drugs may affect the ABR sufficiently to influence the interpretation of clinical and experimental studies. That is, such drugs may mask, exaggerate, or even be mistaken for the clinical or experimental effects being studied. The fact the ABR can be influenced by pentobarbital also indicates that the ABR may be a useful measure for studying pharmacological and neurotoxic agents.

34.2 USE OF ABR TO STUDY ERYTHROMYCIN OTOTOXICITY. L.B. Wright*, L.P. Rybak and C. Whitworth*, Dept. of Surgery, Southern IL Univ. Sch. of Med., Springfield, IL 62708.

Erythromycin has been shown to cause reversible hearing loss in humans as well as in an animal model. Erythromycin has been used primarily for the treatment of mild to moderate infections in humans. The present series of experiments was designed to determine which frequencies of hearing are altered by erythromycin in the chinchilla. Auditory Brainstem Response (ABR) was performed in a sound proof room using a Nicolet 1170 averager. Each chinchilla had a control ABR followed by the administration of erythromycin. The data indicates that alteration of ABR amplitude appears within the first four days of high-dose erythromycin therapy. The reduction of wave V amplitude induced by erythromycin was in the higher frequencies. The discontinuation or reduction of the dosage resulted in rapid recovery. Advanced studies using the present model will help to further define the mechanism of erythromycin-induced hearing loss. (This research was supported by NIH (NINCDS) Teacher Investigator Development Award #KON-NS 00705).

34.3 OBSERVATIONS ON THE DEVELOPMENT OF THE BRAINSTEM ELECTRIC RESPONSE TO TONE PIPS IN RAT. B. Blatchley*, W.A. Cooper*, and J. Coleman (SPON: J. Freeman). Depts. of Psychology and Communicative Disorders, University of South Carolina, Columbia, SC, 29208

Rat pups, anesthetized with ether, were presented with acoustic signals at 9, 10, 12, 14, 16, 20, and 24 days after birth. The signals were 3, 8, and 40 K Hz tone pips of 0.5 msec rise/fall and 1.0 msec at maximum amplitude. These signals were presented to the left ear at 90 dB PeSPL via sound field at a 90 degree azimuth, with the right ear occluded with bonewax. Brainstem electric responses were obtained via subcutaneous needle electrodes located at vertex and chin with the indifferent at the base of the tail. A separate group of normal adults (70 days old) were used as controls.

The first response was observed on day 12 at 1.22 msec at all three frequencies. By day 14, the first wave had migrated to 1.8, 1.54 and 1.32 msec for 3, 8, and 40 K Hz respectively. Wave I latency increased further at day 16, and on subsequent days decreased to the adult value of 1.6, 1.39 and 1.37 at 3, 8, and 40 K Hz respectively.

8 K Hz produced a second wave at 2.20 msec on day 12. All three signals produced a second wave on day 14. The systematic inverse relationship between latency and frequency observed for wave I was not apparent for wave II.

Wave III appeared on day 14 for signals at 3 and 8 K Hz, but not until day 16 at 40 K Hz. Wave IV did not appear until day 20 at any frequency.

Wave V appears at all frequencies on day 16. The I-V interpeak latency ranged from 3 to 4.5 msec at 3 and 40 K Hz respectively on day 16. By day 24, this latency difference was 3.6 and 3.4 at the same frequencies, which approximate adult values.

It appears that the cochlear-Nerve VIII part of the auditory system matures most rapidly and the more central structures at a later time. Further responses to higher frequencies appear at a later date than do those elicited by lower frequencies.

34.4 WIDE BAND NOISE MASKS BRAINSTEM RESPONSES EVOKED BY CLICKS MORE EFFECTIVELY THAN RESPONSES EVOKED BY ANGULAR ACCELERATION IN RATS. L. Hoffman* and J. Horowitz. Dept. of Animal Physiology, Univ. of California, Davis, CA 95616.

The question posed in this study is whether wide band auditory noise can block auditory brainstem responses (ABRs) to a greater extent than it can block responses evoked by a different stimulus modality, angular acceleration. The experiments were thus designed to determine if there were interactions between vestibular and auditory evoked responses.

ABRs and angular acceleration evoked responses (AAERs) were recorded in rats chronically implanted with skull screws, a thermistor, wire leads, and an electrical connector (J. Neurosci. Meth. 7:261, 1983). Under pentobarbital anesthesia (usually immediately following surgical preparation) ABRs were recorded in response to bone-conducted auditory clicks (approximately 40 dbSL). Following a recovery period of at least one week, rats were anesthetized and AAERs were recorded in the same animals for comparison with ABRs previously obtained. To record AAERs, the animal was placed on a platform so that a reference point equidistant between the external auditory meatus was over the platform's center of rotation. AAERs were recorded in response to displacing the platform through an angle of 1.5 degrees in less than 7 milliseconds. ABRs and AAERs were recorded before, during, and after trials during which a consistent level of wide band noise was presented to each animal. Brain temperature was monitored and maintained at 37 +/- 0.5 ^{o}C throughout each recording session.

The wide band noise either severely attenuated or completely masked the ABRs in response to 40 dbSL clicks, whereas the noise had at most only a small attenuating effect on AAERs. Thus, in rats white noise more completely blocks brainstem potentials evoked by clicks than by angular acceleration. The masking effect of noise on ABRs in rats using bone-conducted stimuli has been shown previously (J. Neurosci. Meth. 7:261, 1983). The persistence of brainstem responses following angular acceleration in the presence of noise appears to reflect a response traveling centrally over vestibular pathways. This observation tends to support the proposal by Elidan et. al. (Electroenceph. Clin. Neurophys. 53:501, 1982) that vestibular brainstem potentials can be recorded in rats, although our responses are somewhat different in form.

(Supported by NASA Grant 2234.)

34.5 ADAPTATION AND SIMULTANEOUS MASKING BEHAVIOR IN THE AUDITORY NERVE NEUROPHONIC (ANN) AND FREQUENCY FOLLOWING RESPONSE (FFR). R.L. Snyder and C. Schreiner *. Epstein Lab., Dept. of Otolaryngology, Univ. of Calif., San Francisco, CA 94143

When the auditory system is stimulated with sustained stimuli, sustained neural ensemble responses can be recorded from several intracranial areas ('neurophonics') as well as from the scalp (frequency following response). In this paper we are reporting on the time course of adaptation and recovery from adaptation in the ANN (auditory nerve neurophonic) and FFR as well as the simultaneous masking behavior of these responses.

The averaged ANN response was differentially recorded using platinumiridium ball electrodes placed on either side of the auditory nerve as it exits the internal meatus. The averaged FFR was differentially recorded using silver wire electrodes placed in the skin at the vertex and under the pinna of the stimulated ear.

The amplitude (RMS) of both responses display adaptation in their AC and DC components. Adaptation in the AC components of the ANN can be described by two time constants, a short time constant between 7-30ms and a longer 250ms time constant. The value of the short time constant is intensity dependant, the higher the stimulus level the shorter the time constant. In contrast, the long time constant is relatively independant of stimulus intensity. Adaptation of the AC component in the FFR has a similar time course to that seen in the ANN.

Like adaptation, the recovery from adaptation in both responses is similar. Recovery from adaptation was examined using a forward masking paradigm. The probe was an 800Hz, 50dB tone that was 60 ms in duration. The masker was a tone of variable frequency 60 ms in duration at various levels. The rate of recovery from adaptation was dependant upon stimulus level of masker and probe and consisted of two time constants. For example, a tonal masker between 500 and 1400Hz at 70dB produced recovery time constants of 28 ms and 150ms.

As suggested by the adaptation data, both the ANN and FFR are maskable. We have previously reported upon the forward masking behavior of these neurophonics. These data are best summarized by forward masking tuning curves (30% depression contours) which are relatively sharp(Q10 dB=3-8) and which have tips that are displaced from the probe in both frequency and amplitude. The tips of these curves can be up to an octave above the probe in frequency and 30dB below the probe in level. In a simultaneous masking paradigm where 60ms maskers and probes are strobed on and off together, the tips of the tuning curves are similarly displaced. However, as might be expected, the curves are broader (Q10 dB=1-3). Although these tuning curves are unusual, they are not without precidence when low frequency stimuli are employed to examine unit responses (Rose et al., J. Neurophysiol. 1974) and ensemble responses (Harris, Hearing Res. 1979) in the auditory nerve. Such masking data should effect the representation of complex waveforms when the stimuli consist of low frequency components.

Supported by the Colman and Mainprice Funds UCSF.

34.6 THE FREQUENCY FOLLOWING RESPONSE TO CONTINUOUS AND AMPLITUDE MODULATED TONES IN HUMANS. R. Batra, S. Kuwada and V.L. Maher*. Department of Anatomy, University of Connecticut Health Center, Farmington, CT 06032.

The frequency following response (FFR) is a scalp-recorded electrical potential which faithfully mimics the periodicity of low frequency tones. The FFR is usually recorded in response to tone bursts. Continuous tones have several advantages: simplicity of generation, reduction in data collection time and elimination of transient effects which may complicate the response. With a view to refining the use of the FFR as an audiological tool and extending it to high frequencies we measured the FFR from human subjects in response to continuous pure tones and continuous tones that were sinusoidally amplitude modulated (AM).

Tones were presented to the left ear via a μ-metal shielded headphone. Responses were differentially amplified between vertex and right mastoid, and were then averaged. We assessed the response amplitude by computing the Fourier transform at the stimulating frequency.

The response to continuous pure tones as a function of frequency was measured at 95-100 dB SPL. The response increased from 125 Hz to a peak of 150-400 nV at about 200 Hz and then declined. In most subjects no response could be discerned above 500 Hz. Responses were graded with intensity. We found that at frequencies below about 250 Hz the response increased from roughly 75 to 100 dB SPL. At higher frequencies the response was still detectable at 75 dB, but did not increase above about 85 dB SPL.

Responses to AM tones were measured at an intensity of 85-90 dB HL. Frequencies of modulation between 30 and 250 Hz produced a FFR to the envelope of the tone for carrier frequencies up to at least 6 kHz. In general, the response is largest at low modulation frequencies (about 40 Hz) but the size of this response is variable from record to record and may change by as much as a factor of 10. This variability may be related to the degree of arousal of the subject.

Our data show that FFR's can be obtained in response to continuous pure tones and AM tones. At a given frequency the amplitude of the response to continuous tones is similar to that reported for tone bursts, but usually we could not detect FFR's above 500 Hz, although responses to tone bursts at frequencies up to 2000 Hz have been reported. Employing AM tones may make it possible to use the FFR to assess high frequency hearing in a frequency specific manner.

This work was supported by a NIH grant (NS18027) and the University of Connecticut Research Foundation.

34.7 DERMATOMAL SOMATOSENSORY EVOKED POTENTIALS AND SEGMENTAL SPINAL CORD ANALYSIS IN HUMANS. J.C.Slimp and W.C.Stolov*. Department of Rehabilitation Medicine, University of Washington School of Medicine, Seattle, WA 98195.

Identification of focal abnormalities of the contents of the vertebral canal (e.g., spinal cord and roots) may be attempted electrophysiologically by stimulating a large peripheral nerve (e.g., posterior tibial) and recording the conducted activity at different vertebral levels as it travels proximally through the cauda equina and spinal cord into the brain. The extremely small amplitude of the signal when recorded from the skin along the spine, particularly at thoraco-cervical levels, makes this technique relatively impractical as a clinical diagnostic tool.

On the other hand, direct skin stimulation of dermatomal segments with scalp recording does produce responses of sufficient size and shape to permit accurate amplitude and latency measurements after averaging a much smaller number of repetitions. Even with the possibility of dermatomal overlap, latency and amplitude comparisons of the cerebral responses from adjacent dermatomes, both ipsilateral and contralateral, permits the localization of abnormalities.

Studies of neurologically normal subjects verified that good cerebral evoked potentials could be recorded to dermatomal stimulation of arms, trunk, and legs, extending from C5 to S1. Several case studies demonstrate the ability of this method to identify localized segmental abnormalities of the spinal cord and/or single nerve roots, especially where peripheral nerve stimulation was not appropriate or inconclusive. The diagnoses so clarified included nerve root disease, radiation myelopathy, and spinal cord contusion.

34.8 THE SOMATOSENSORY EVOKED POTENTIAL TO MUSCLE STRETCH IN THE MONKEY: RELATION TO STRETCH AMPLITUDE AND EVIDENCE FOR DIURNAL RHYTHM. R. Dowman, J.A. O'Keefe* and J.R. Wolpaw. Ctr. for Labs & Research, NYS Dept. of Hlth. & Depts. of Neurol. & Anat., Albany Med. Coll., Albany, N.Y. 12201.

We have recently shown that the amplitude of the primate spinal stretch reflex (SSR) can be brought under operant control without changing background muscle activity or initial muscle length (Wolpaw et al., J. Neurophysiol. 50: 1296-1319, 1983). We now seek to use the stretch-evoked somatosensory evoked potential (SEP) as a measure of activity in afferent pathways to help determine the origin of SSR amplitude change. This preliminary study investigated SEP amplitude as a function of muscle stretch amplitude and observed the stability of the SEP over extended periods.

Five monkeys (Macaca nemestrina) were chronically implanted with fine-wire EMG electrodes in biceps and triceps muscles and screw electrodes over contralateral somatosensory cortex and frontal sinus. Each trained by computer to keep elbow angle at 90° (±1.5°) against steady extension (or flexion) torque. If the animal held this angle for a randomly-selected 1.2-1.8 sec period, and if the average absolute value of biceps (or triceps) EMG for the final 100 msec was within a preset range, a brief torque pulse extended (or flexed) the elbow, eliciting the SSR and the SEP. Liquid reward occurred 200 msec after pulse onset. Each animal completed 3000-6000 trials/day.

The first components of the stretch-evoked SEP were a 15 ms positive peak (P15) and a 25 ms negative peak (N25). Baseline-to-peak amplitude of each averaged about 10 μV. Each was stable in amplitude (±10%) and latency (±1 ms) over periods as long as 100 days. For each, amplitude was a nonlinear function of stretch amplitude: the slope of peak amplitude vs. stretch amplitude decreased slightly as stretch amplitude increased.

All animals showed evidence of a diurnal rhythm in SEP amplitude similar to that previously described in SSR amplitude (Wolpaw & Seegal, Br. Res. 244: 365-369, 1982). The rhythm was most apparent in 3 monkeys who worked a significant number of trials at night as well as during the day. As with SSR amplitude, SEP amplitude was about 15% larger from midnight to 3 a.m. than from noon to 3 p.m. In 2 monkeys, we also monitored the SEP to electrical stimulation over 24 hrs. These data showed no evidence of a diurnal rhythm, suggesting that the rhythm in the stretch-evoked SEP originates peripherally, probably in the muscle spindle.

34.9 Spatial and Temporal Properties of, and Neuronal Contributions to the Somatosensory Evoked Potential P.B. Hoeltzell* and R.W. Dykes, Dept. of Physiology, McGill University, Montreal, Quebec, Canada

The cortical somatosensory evoked potential produced by a brief puncture stimulus to the forearm of Nembutal-anesthatized cats was examined in three dimensions and in time. Fixed amplitude, 0.1s duration mechanical stimuli delivered every 3.5s generated a reproducible potential that was sampled at 200 um steps horizontally and every 100um through the cortical depth. The resulting three dimensional potential matrix was subjected to current source density analysis (CSD). The potentials recorded were classified into three types: (1) one that was initially positive on the cortical surface but inverted deeper to become an initially negative waveform (2) an initially positive waveform that did not invert deeper in the cortex and (3) an initially surface negative waveform that did not invert within the cortex. Potential types 2 and 3 were distinguishable in separate locations away from the region of maximum activity but summed at the focus to produce the Type 1 wave. CSD analysis suggested that several different classes of neurons contribute to the observed potentials. The distribution of sources and sinks in space and time were consistant with the hypothesis that excitation was followed by inhibition in at least two neuronal populations in two different cortical layers. Our data support the idea that afferent fibers excite inhibitory aspinous and sparsely spinous stellate cells which feedback onto several pools of pyramidal cells and that both the stellate and pyramidal cell populations contribute significant currents to the observed surface primary response.

34.10 EARLY LATENCY SOMATOSENSORY EVOKED POTENTIALS (SEL's) RECORDED FROM DEEP BRAIN SITES IN HUMANS. P. Newlon*, R. Greenberg* and D. Becker. Div. of Neurosurg., Med. Col. of Va., Richmond, VA 23298.

SEL's (0-25 ms) in response to median nerve stimulation were recorded from 8 patients with depth electrodes (D) placed for relief of pain. Each electrode had 4 recording points arranged vertically, separated by 5 mm (deepest: D1, most shallow: D4). Five patients had D1 in the periventricular gray (PVG). One of these 5 also had an internal capsule (IC) placement. Two patients had D1 in the periaqueductal gray (PAG) and 1, in the VPM nucleus of the thalamus. Surface SEL's were recorded in a standard manner from the brachial plexus and neck (C7) referenced to Cz. Depth recordings were unipolar (D1-4 - A1,2) and/or bipolar (D1-4 - C7, Cz, D1-4). In 5 cases, SEL's both ipsi- and contralateral to the D electrodes were obtained.

The SEL's from D were generally characterized by a triphasic Pos.-Neg.-Pos. potential which matched the latency of the N-P-N complex commonly recorded from the neck (N13, P17,N22). The triphasic potential had small oscillatory potentials superimposed on it that peaked approximately every 0.2 ms. In contralateral bipolar D recordings of close proximity (D1-D2), the P-N-P complex disappeared, but the oscillations remained, suggesting that they represent a local "closed field." The latencies of the P-N-P complex shifted slightly at D, usually occurring earlier than at the surface by tenths of a ms. The amplitudes of the "P17" and "N22" (N,P at D) were 2-8 times greater than at surface and sometimes showed a steep gradient across different D sites. The greatest amplitude effect was seen at caudal sites (PAG) and was 2 or more times greater with contra- than ipsilateral stimulation. There was a small amplitude enhancement (X2) of the "N13," although no dissociation between the different sides was observed for this component.

These findings support a subcortical origin of several peaks of the SEL recorded from the neck surface, which may also contribute to the scalp recorded SEL up to 25 ms. While it seems likely that the P-N-P complex reflects the lemniscal volley, the small oscillatory potentials, not seen in surface recordings, may be synaptic in origin, possibly arising from thalamus, but also possibly from a separate, parallel pathway. It is not likely that the thalamus is a primary generator of the surface-recorded SEL. This work was supported in part by NIH grants NS 12587 (PGN) and NIDR 263-77-C-0623.

34.11 INTERHEMISPHERIC COMMUNICATION TIME ESTIMATED BY VISUAL EVOKED MAGNETIC FIELDS G.W. Lewis, M.R. Blackburn and M. Metcalfe*. Navy Personnel Research and Development Center, San Diego, CA 92152.

Neuromagnetic recording can provide better definition of source location than neuroelectric techniques. A neuromagnetic probe situated over one visual projection area should be sensitive primarily to activity in the underlying cortex and relatively insensitive to activity in the contralateral cortex. Stimulation within a hemiretinal field should activate first the hemisphere which receives the thalamic projection and then, after a delay associated with transmission across the corpus callosum, the contralateral visual cortex. The objective of the present research was to apply the visual evoked field (VEF) to study this delay. VEF recordings were obtained in an unshielded environment from three-adult human subjects at sites O1 and O2 (10/20 system) using a DC SQUID, second derivative gradiometer (S.H.E. Corp. model 600B). Visual stimulus was a black and white checkerboard pattern (5.6° visual angle, 0.36°/8mm check, back luminated by a 2msec flash, 69cd/m^2 luminance). Subjects fixated either on the checkerboard center or on dim red LEDs located 2.8cm to its right and left. Under the three fixation conditions and two recording sites, series of 40 stimuli were presented aperiodically with mean inter-flash-intervals of 750±150msec. Responses within a 512msec. post-stimulus epoch were averaged for each series. Amplitude and latency measures of the four major VEF deflections, averaged over subjects and sites within conditions are presented below. The hemiretinal conditions had lower amplitudes of all components than the central. Latencies of contralateral responses were similar to the central condition but the ipsilateral condition resulted in earlier deflections after the first component. The delay of activity in the contralateral hemisphere after the first VEF component may reflect interhemispheric communication. The first component may originate in the thalamus. Phase and amplitude results do not support an hypothesis of field detection across the midline. (Abstract does not necessarily reflect views of the Department of the Navy or Defense Nuclear Agency.)

Component	1	2	3	4	
Condition	x cv				
Central	104(36)	145(51)	115(28)	100(30)	Amplitude
Ipsilateral	57(52)	74(28)	98(25)	88(14)	(femto-
Contralateral	75(36)	81(65)	88(34)	67(36)	Tesla)
Central	115(10)	164(6)	283(10)	430(10)	
Ipsilateral	110(20)	144(9)	213(8)	363(15)	Latency
Contralateral	108(23)	171(16)	266(3)	426(13)	(msec)

34.12 INVESTIGATION INTO POSSIBLE CENTRIFUGAL MODULATION OF RETINAL ACTIVITY DURING SELECTIVE ATTENTION. G.R. Mangun*, J.C. Hansen and S.A. Hillyard*. Department of Neurosciences, University of California, San Diego, La Jolla, CA 92093.

Centrifugal modulation of retinal activity has been demonstrated in fish, reptiles, birds, and with less certainty, in some mammalian species. Evidence for a centrifugal neural pathway to the human retina, however, has not been unequivocally established. Nonetheless, recent reports by Eason et al. (Soc. Neurosci. Abstr., 7:659, 1981; Physiol. Psych., 11:18, 1983) described changes in the B-wave of the human electroretinogram (ERG) during selective attention which were interpreted in terms of centrifugal modulation of retinal input. This remarkable finding raises the possibility that central control of retinal activity might occur in association with visual selective attention. We investigated this effect using procedures similar to those employed by Eason and associates.

Subjects attended selectively to a sequence of flashes in one visual field while ignoring a concurrent sequence in the opposite field. A target detection task was used to accomplish directed attention. Stroboscopic checkerboard displays were flashed at 30 degrees to the left or right of a central fixation point in random order. Stimuli were presented at interstimulus intervals of 300-700 msec at an intensity of approximately 3 log units above a background luminance of 0.3 millilamberts. The ERG was recorded using a gold foil electrode contacting the corneal-scleral surface of the right eye. Simultaneously, visual event-related potentials (ERPs) were recorded from electrodes placed at the internal and external canthi of the right eye, and from frontal, central and occipital scalp sites. The subject's left eye was occluded.

Selective visual attention was evident in an enhancement of the visual evoked potential to flashes in the attended field. A main effect of attention was demonstrated at frontal, central and occipital scalp sites on the N170 component for both attend left and attend right conditions. However, no effect of attention was observed upon the B-wave (latency of 65 msec) of the ERG recorded from the foil electrode. Possible attention related modulation of longer latency (150-700 msec) activity in the corneal-scleral recording is under investigation.

34.13 EVOKED POTENTIALS ELICITED BY VERNIER OFFSET TARGETS: ESTIMATING VERNIER THRESHOLDS, AND THE PROPERTIES OF THE NEURAL SUBSTRATE. R. Zak* & M.A. Berkley. (SPON: J. Tunkl). Florida State University, Tallahassee, FL 32306.

A three-part evoked potential (EP) study was undertaken in an attempt to better understand the neural mechanisms underlying vernier acuity. In Experiment I, VEPs were recorded in response to a single vernier offset presented for 100 msec every .67 sec., parameters similar to those used in psychophysical studies. The latency to first peak of the VEP was about 220 msec and its amplitude varied linearly with the log of the target offset magnitude. Extrapolation of the EP-amplitude offset-magnitude function to the zero EP amplitude value yielded threshold estimates in good agreement with psychophysical thresholds (replicating the initial report by Levi et. al., Invest. Ophthal. & Vis. Sci., 1983, 24, 92, despite differences in stimulus configuration and duration).

In Experiment II, behavior of the offset-evoked response was examined in the presence of contiguous contours (lines), a target configuration shown to decrease psychophysically measured sensitivity (Westheimer & Hauske, Vis. Res., 1975, 15, 1137-1141). When the extraneous contours were placed within 5 min. or less of the offset, the amplitude of the EP was attenuated. Control measures showed the contribution of retinal glare to the EP attenuation effect to be minimal.

Experiment III examined interaction between vernier offset-evoked EPs and luminance-evoked EPs. Pilot data showed the absence of occlusion of the vernier EP by the luminance EP response and approximately linear summation of flash and vernier EP amplitudes in response to a composite stimulus. This finding suggests independence of the two generating mechanisms.

These results indicate: a) systematic variation of offset-evoked EP amplitude with vernier offset magnitude is a reliable phenomenon and can be used to estimate vernier thresholds; b) offset-evoked EPs appear to be direct measures of a vernier processing mechanism because of the similarity of the effects of spatial interference lines on both VEP amplitude and psychophysical sensitivity; and c) the source of the vernier EP may be outside striate cortex because of its relatively long latency and the apparent independence of luminance and vernier EP generators. (Supported by NEI grant EY00953-12).

34.14 PATTERN EVOKED POTENTIAL CHANGES AT HIGH BUT NOT LOW CONTRAST PRODUCED BY CHLORDIMEFORM INSECTICIDE. W.K. Boyes and R.S. Dyer. Northrop Services, Inc., and Neurotoxicology Division, U.S.E.P.A., Research Triangle Park, NC 27711

Acute dosage with chlordimeform (CDM) insecticide increases the amplitude of pattern reversal evoked potentials (PREP)s elicited with a high-contrast square wave grating, while leaving flash evoked potential amplitude unchanged (Dyer and Boyes, The Toxicologist, 3:13, 1983). The PREP amplitude increase occurs when using large (0.1-0.4 cpd) but not small-sized (0.8 cpd) bars (Boyes and Dyer, Soc. Neurosci. Abstr., 9:369, 1983). The apparent spatial frequency dependence of CDM action may be due to relative contrast sensitivity differences to the high and low spatial frequency gratings. To address this possibility, stimuli of high and low spatial frequency were presented at different contrasts. The stimuli were vertical gratings with a sinusoidal spatial luminance profile at two spatial frequencies. At 0.2 cpd, contrast values were 1.5, 3, 5, 10, 30, and 65%, and at 0.8 cpd 40, 50 and 65%. One hour before testing Long-Evans hooded rats received either saline (n=14) or 40 mg/kg CDM HCl ip (n=15). Each rat was tested at every spatial frequency and contrast combination. PREP amplitudes increased with increasing contrast, regardless of spatial frequency, but were greater at 0.2 than 0.8 cpd. Action of CDM was both contrast and spatial frequency dependent. At 0.8 cpd, PREP amplitudes were unchanged by CDM. At 0.2 cpd, amplitudes were increased by CDM between contrast values of 10 and 65%, but were unchanged between 1.5 and 10%. Over the high contrast range, CDM appeared to increase the slope of the amplitude-contrast function. Murray and Kulikowski (Vision Res., 23: 1741-43, 1983) suggest that the two limbs of the PREP amplitude vs stimulus contrast function represent the operation of two different visual mechanisms, specifically that the high contrast limb corresponds to motion detectors and the low contrast limb to pattern detectors. Elsewhere it has been suggested that the high contrast limb may represent the function of Y cells and the low contrast limb of X cells (Kulikowski, Vision Res. 18: 183-189, 1978). If these hypotheses are correct, then CDM exposure may selectively enhance the responsiveness of motion detectors, or Y-like cells, in the rat visual system.

This is an abstract of a proposed presentation and does not reflect EPA policy.

34.15 BINOCULAR INTERACTION IN NORMAL AND DEPRIVED CATS; EVOKED POTENTIALS G. Sclar*, I. Ohzawa*, R. D. Freeman, (SPON: W. Makous) Ctr. for Vis. Sci., Univ. of Rochester, Rochester, NY, 14627 and Sch. Optom., Univ. of Calif., Berkeley, CA, 94720

We have examined binocular interactions in normal cats and animals reared with experimental strabismus or monocular deprivation (MD). Our purpose was to determine the degree of summation in visual evoked potentials (VEPs) elicited by binocularly presented sinusoidal gratings. Cats were anesthetized and paralyzed during recordings. Stimuli were of high contrast and were presented under computer control at a phase reversal rate of 1 Hz. Spatial frequency was varied and presentations were randomly interleaved.

In normal animals we find typical response patterns: potential amplitudes are reduced at high and low spatial frequencies and values for left and right eyes are typically well matched. Based on the average of all spatial frequencies tested we find that the ratio of binocular to monocular responses ranges from 1.27 to 2.12 (mean = 1.58). Since stimulus disparity might affect these findings control tests were run during which the relative phase of dichoptically presented gratings was varied. In general, responses were constant and not influenced by such phase changes, although some small effects were apparent at very low frequencies.

If the summation we have found were the product of a binocular process, it ought to be reduced in strabismic and MD'd cats in which binocular vision is disrupted. While summation was reduced in 5 esotropic animals (mean = 1.18) it was approximately normal in 2 exotropic animals (mean = 1.36) despite the fact that single-unit recordings in all these cats showed that similar breakdowns in cortical binocularity had occurred. This suggests that the "binocular summation" seen in the VEP cannot be simply interpreted. Recordings in 3 monocularly deprived cats, however, showed that response amplitudes obtained following binocular stimulation were consistently smaller than those obtained from the normal eye alone (mean = .80). Although some form of binocular interaction may underlie this effect, results from single unit recordings in striate cortex of MD'd cats have so far failed to provide any indication of similar interactions. These findings emphasize the difficulty of comparing results from single unit and evoked potential experiments. (NIH grant: EY01175)

34.16 VISUAL EVOKED RESPONSES IN RABBITS WITH EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS. N.E. Brennan*, C.F. Brosnan*, M.B. Bornstein* and J.C. Arezzo. Depts. of Neuroscience and Neuropathology, Albert Einstein Coll. of Med., Bronx, N.Y. 10461.

Non-invasive electrophysiological procedures were used to assess the integrity of specific portions of the visual pathways in twelve rabbits with experimental allergic encephalomyelitis (EAE). In addition, half the animals received subsequent lymphokine injections, inducing inflammatory demyelination, in one eye, and saline in the other. Progression of pathology was monitored by flash evoked electroretinogram (ERG), optic nerve potential (ONP), and cortical visual evoked potential (VEP), recorded epidurally. Data were sampled at several time points and correlated with structural changes in the corresponding tissue.

The initial electrophysiological change was noted 5.5 hours after lymphokine injection and consisted of a delay in both onset and peak of the primary cortical component contralateral to the injected eye. Slowing continued until final stabilization which occurred between 24 and 48 hours post-injection. At this time there was an approximate 50% reduction in amplitude and a greater than 20% increase in the latency of all cortical components. A concomitant slowing of the ONP without change in the ERG suggested that the principal focus of demyelination was in the optic nerve. In contrast, the electrophysiological change in animals not treated with lymphokines showed a delayed cortical response with no change in either ERG or ONP, suggesting post-chiasmal pathology. Histological evaluation reveal inflammation and demyelination in the superficial layers of the retinal strip and extensive perivascular cuffing and demyelination in the proximal portion of the optic nerve in lymphokine injected eyes, while the retinal strip and the optic nerve remained unaffected in the saline injected eye and all animals not treated with lymphokines.

Our results demonstrate that non-invasive electrophysiological measures are a sensitive index of early onset of demyelination and are capable of distinguishing the affected region. Thus, these techniques can be used as means of longitudinal assessment of the factors involved in primary demyelinating disease.

This work was supported by grants HD01799, MH06723 and NS11920 from the USPHS.

34.17 PAIRED FLASH RECOVERY IN RATS FOLLOWING PICROTOXIN AND VALPROIC ACID. R.S. Dyer, W.K. Boyes and B.E. Hetzler, Neurophysiology Branch, NTD, HERL, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Picrotoxin is a convulsant with GABA antagonist properties. Valproic acid is believed to elevate GABA levels, and functions as an anticonvulsant for certain types of seizures. To the extent that GABA is involved in the elaboration of the flash evoked potential, administration of these compounds would be expected to produce distinguishable effects. Paired stimulus recovery functions may be useful for detecting and characterizing neurotoxicants (Dyer and Boyes, Fed. Proc. 42:3201, 1983). Alterations in the recovery function are presumed to reflect alterations in the excitability of the system, with slowed recovery reflecting depression and speeded recovery reflecting excitation. While this paradigm has been explored with several suspected neurotoxicants, there are no studies showing clearly that either convulsant or anticonvulsant drugs affect the paired flash recovery function in rats. In the present study 36 Long-Evans hooded rats were implanted surgically with chronic visual cortex electrodes one week before testing. 30 min prior to testing, the rats received topical atropine to dilate the pupils, and either the convulsant picrotoxin (n=14, 2 mg/kg i.p. 15 min before testing), the anticonvulsant valproic acid (n=12, 100 mg/kg i.p. 30 min before testing), or saline (n=10, 1 ml/kg 15 min before testing). Flash-evoked potentials were recorded at interstimulus intervals of 400 msec, 300 msec, 200 msec and 100 msec. Analyses were performed upon latency of peak N1, and amplitude of peak P1N1. While both picrotoxin and valproic acid depressed the amplitude of P1N1 by about 20%, neither drug altered the N1 latency, or the extent to which either the amplitude or the latency recovered from the first of the paired flashes. The picrotoxin-induced and valproic acid-induced depression of P1N1 are consistent with earlier reports. The similar effects of picrotoxin and valproic acid on P1N1 amplitude, combined with the lack of a differentiating effect on the recovery function, suggest that GABA may not be involved in the synaptic activity specific to the recoverability of the visual system to paired flashes. Alternatively, sufficient dosages of the test compounds may not have been given to detect changes. However, since the CD50 for picrotoxin is about 4 mg/kg, the paired flash paradigm may not be useful for detecting subtle alterations in CNS excitability.

34.18 TEMPERATURE-DEPENDENT CHANGES IN VISUAL EVOKED POTENTIALS OF RATS WITH PREOPTIC/ANTERIOR HYPOTHALAMIC LESIONS. B.E. Hetzler[1], W.K. Boyes, J. Creason* and R.S. Dyer. Health Effects Research Laboratory, US EPA, and Northrop Services, Inc., Research Triangle Park, NC 27711.

Many studies have examined the effects of body temperature (Tb) on evoked potentials recorded from anesthetized animals. However, potential interactions between Tb and drug actions confound these data (Doull, Essays Toxicol. 3:37-63, 1972). To circumvent this problem, the effects of Tb on flash and pattern reversal evoked potentials (FEPs and PREPs) were examined in hooded rats whose thermoregulatory capacity was compromised with lesions of the preoptic/anterior hypothalamic area. Tb was manipulated via exposure to different ambient temperatures (Ta). In the first experiment, lesioned animals (n=24) and controls at 85% body weight (n=15) were tested following 2 hrs at Ta's of 18°C and 30°C. Tb ranged from 33.3-42.0°C for lesioned animals and from 37.6-40.0°C for controls. For each animal, changes per °Tb (i.e., slopes) were calculated for each of the following variables: FEP: P1N1, N1P2 amplitudes, P1, N1, P2 latencies; PREP: N1P1, P1N3 amplitudes, P1, N3 latencies. No significant differences in mean values of slopes were detected between the lesioned and control animals, indicating equal responses to changes in Tb. For the lesioned animals, the mean values of the latency slopes (FEP: -1.11, -1.62 and -1.95 msec/°C for P1, N1 and P2; PREP: -2.38 and -4.28 for P1 and N3, respectively) were significantly different from 0, indicating reliable relationships with Tb. Slopes for FEP and PREP amplitudes did not differ from 0. The second experiment involved 11 lesioned animals which were tested following 0.5 hr exposure to 4°C and 23°C while restrained in a harness. Tb ranged from 27.2-40.0°C. Mean values of the latency slopes (FEP: -1.68, -2.13 and -3.71 msec/°C for P1, N1 and P2; PREP: -4.90 and -6.53 for P1 and N3) were again significantly different from 0. The slope for PREP P1N3 amplitude (-2.90 μv/°C) also differed from 0. These data demonstrate the temperature-dependence of FEP and PREP latencies, as well as PREP amplitude, independent of anesthetic or other drugs. Previously reported increments in FEP amplitude in hypothermic rats may reflect drug x temperature interactions.

[1]On leave from Lawrence University, Appleton, WI 54912.

This is an abstract of a proposed presentation and does not reflect EPA policy.

METABOLIC STUDIES

35.1 THE TRANSPORT OF [3-^{14}C]-3-HYDROXYBUTYRATE BY DISSOCIATED BRAIN TISSUE FROM RATS OF DIFFERENT AGES. J. T. Tildon* and L. M. Roeder* (SPON: M. L. Rennels). Dept. of Pediatrics, Univ. of Maryland Sch. of Med., and Walter P. Carter Ctr. for Mental Retardation, Baltimore, MD 21201.

It is now well established that under certain conditions the brain can use alternative substrates, in addition to glucose, for energy and the synthesis of structural components. Recent studies suggest that the utilization of oxidizable substrates by the brain may be regulated, in part, by transport across the plasma membranes. In order to investigate this possibility, dissociated tissue obtained by mechanical disruption of rat brain was used to measure the uptake of [3-^{14}C]-3-hydroxybutyrate (3HOB). The uptake increased with time with a rapid initial phase followed by a more linear second phase suggesting two mechanisms. The rate of 3HOB uptake was also proportional to substrate concentration and the kinetics of these reactions revealed two components; a non saturable component that reflected simple diffusion with a rate of 0.9 nmoles/min/mg protein/mM 3HOB and a saturable component which followed Michaelis-Menten kinetics with a Km of 1.47 mM and a Vmax = 5.0 nmoles/min/mg protein. The rates of uptake were temperature dependent and they were significantly higher at pH 6.2 as compared to the rates at pH 7.2 or 8.2. Increasing the intracellular substrate concentration by pre-incubating the tissue in a reaction mixture of 12.5 or 25 mM 3HOB increased the rate of uptake 143% and 216%, respectively, suggesting a counter-transport effect. The net uptake was inhibited 50% by phenylpyruvate, α-keto isocaproate, KCN and $NaAsO_3$. However, little or no effect was observed with lactate, methylmalonate and α-cyanohydroxy cinnamate. The rates of uptake by preparations from 14-16 day old animals was about 2-fold the rates obtained using preparations from 2 and 28 day old animals or adults. Cells dissociated by trypsinization rather than by mechanical disruption showed a reduced uptake of 3HOB suggesting the possible loss of receptors or transport protein. Collectively, these data are consistent with the proposal that uptake at the cellular level contributes to the regulation of substrate utilization by the brain. The data also suggest that 3HOB enters the brain by both a diffusional component and a carrier mediated transport system. However, additional studies are required to determine if this latter component is a generalized monocarboxylic carrier or if it is specific for 3HOB. (Supported in part by NICHD Grant # 16596)

35.2 REGULATION OF pH IN LAMPREY CENTRAL NEURONS. M. Chesler and C. Nicholson. Department of Physiology and Biophysics, New York University Medical Center, New York, NY 10016.

In the nervous system, studies of pH_i regulation have been limited to giant invertebrate cells (Roos and Boron, Physiol. Rev., 61: 296, 1981). The mechanisms which regulate H^+ in the vertebrate CNS are not known. Double barreled H^+ selective microelectrodes were used to study pH_i regulation of Muller and Mauthner cells in the brainstem of the larval sea lamprey, Petromyzon marinus. Our data suggest two mechanisms for pH_i regulation. One is sensitive to the diuretic amiloride and can operate in the nominal absence of HCO_3^-. The second mechanism is insensitive to amiloride but is HCO_3^- dependent.

The lamprey brain was dissected under cold Ringer, and pinned in a recording chamber. H^+ microelectrodes contained a neutral carrier liquid membrane (Amman et al, Anal. Chem., 53: 2267, 1981) and had tip sizes of 0.5-2 μm. Cells were penetrated following equilibration in either zero-HCO_3^-, HEPES buffered Ringer (5 mM, pH 7.4-7.6), or 23 mM HCO_3^-/5% CO_2 Ringer (pH 7.4) at 23°C.

In HCO_3^- Ringer, pH_i was 7.44 ± 0.03 with a membrane potential of 54 ± 4 mV (N = 6). pH_i regulation in HCO_3^- free solutions was tested by acid loading cells with a 5 min. exposure to 10 mM NH_4Cl in Ringer (Boron and DeWeer, J. Gen. Physiol., 67: 91, 1976). pH_i recovered exponentially (rate constant = 0.069 ± 0.006 $min.^{-1}$, N = 5) against the electrochemical gradient for H^+ and was reversibly blocked by 1 mM amiloride. Na^+ substitution with bis(2-hydroxyethyl)dimethylammonium caused a slow acidification. Return of Na^+ produced an immediate, amiloride sensitive pH_i recovery suggesting that Na^+-H^+ exchange mediates acid extrusion.

Transition to HCO_3^- Ringer increased the rate of pH_i recovery. Regulation in HCO_3^- Ringer was insensitive to amiloride but was blocked by the anion transport inhibitor 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (0.1 mM). Na^+ substitution in the presence of amiloride reversibly blocked HCO_3^- dependent recovery. These data suggest that lamprey central neurons utilize an Na^+/HCO_3^- dependent, acid extrusion mechanism in addition to an Na^+-H^+ exchanger. Use of two systems would allow pH_i control despite changing HCO_3^- levels. Supported by NINCDS grant NS-13742 and NIGMS grant 5 T32 GM 07308.

35.3 UTILIZATION OF BLOOD-DELIVERED RETINOL BY THE FROG RETINA. Andrew T.C. Tsin and Coriene Hannapel*. Division of Life Sciences, The University of Texas at San Antonio, San Antonio, Texas 78285.

Leopard frogs (Rana pipiens, 20 gm) were injected intravenously with [^{3}H] all-trans retinol (30 Ci/mmol; each frog received 1.9 nmol). After a period of 2 hrs, 10 hrs, 24 hrs, 15 days and 46 days (12L/12D, room temperature), they were placed in the dark room for 2 hrs before they were sacrificed. The specific activities (cpm per nmol) of rhodopsin in the retina and of retinyl esters in the retinal pigment epithelium (RPE) and in the liver were determined. In the liver, the specific activity of retinyl esters increased from 35,000 cpm per nmol at 2 hrs postinjection to 46,400 cpm per nmol and 43,400 cpm per nmol at 10 and 24 hrs postinjection. Specific activities of rhodopsin (in the retina) and retinyl esters (in the RPE) increased throughout the experiment from about 3,200 cpm per nmol (2 hrs) to 9,300 cpm per nmol and 7,200 cpm per nmol (24 hrs), respectively. This relationship persisted the duration of the experiment (i.e. at the 46th day, the specific activity of liver esters exceeded that of rhodopsin which exceeded that of retinyl esters in the RPE). These observations suggest that in the eye, the blood-delivered retinol served as precursors for the syntheses of both rhodopsin in the retina and retinyl esters in the RPE cells. Supported by the MBRS grant RR-08194 from the Division of Research Resources, NIH.

35.4 Mn^{54} UPTAKE BY HOMOGENATES OF RAT STRIATUM. EFFECT OF Mn^{+2}, Fe^{+3}, Ca^{+2}, AND Mg^{+2}. H. Suárez* and E. Bonilla. INBIOMED-FUNDACITE and Instituto de Investigaciones Clínicas. Apartado 376. Maracaibo-Venezuela.

It is known that chronic manganese poisoning in humans as well as in some other animals invariably and severely affects the corpus striatum causing various disorders. As a preliminary to obtaining some insight about the underlying chemical and/or biochemical changes occurring in the striatum we have studied the uptake of Mn^{54} by rat striatum homogenates.

Sprague-Dawley male rats, weighing 300-400 g,were killed by decapitation. The brains were quickly removed and the neostriatum dissected out at 4°C and homogenized in 0.32M sucrose. The homogenate was incubated with $Mn^{54}Cl_2$ (0.02 uCi) in buffer tris/HCl 0.05 M, pH 7.4. After one minute incubation the reaction was stopped by the addition of cold absolute ethanol. The incubation tubes were centrifuged at 1600 x g x 15' and the pellet washed and centrifuged twice with tris buffer. The radioactivity in the final pellet was measured in a gamma counter.

We studied the effects of Mn^{+2}, Fe^{+3}, Ca^{+2}, and Mg^{+2} on Mn^{54} uptake over a wide concentration range. By using atomic absorption spectroscopy we found the following concentrations of these elements in the neostriatum: 6.87±0.76 n moles Mn/g wet weight; 0.256±0.016 umoles Fe/g w.w.; 6.458±1.368 umoles Mg/g w.w.; 0.84±0.20 umoles Ca/g w.w.

Ca^{2+} and Mg^{2+} produced a maximum stimulation of Mn^{54} uptake at concentrations similar to those found in normal striatum. The saturability of this effect supports a biochemical mechanism of this action for both metals. Ca^{2+} possibly accelerates the transport of Mn into mitochondria.

Mn^{+2} and Fe^{+3} produced a maximum uptake when their concentrations were three to four times higher than normal.Fe^{+3} by a redox mechanism could oxidize Mn^{+2} to Mn^{+3} the latter being the preferred way of manganese uptake by tissues. We do not know the reason why Mn^{+2} at this concentration favors the uptake of Mn^{54} by the homogenates, since in rats chronically intoxicated by oral intake of $MnCl_2$ (0.1 and 1.0 mg Mn/ml of water) for 2 months we found a decrease in Mn^{54} uptake by striatum homogenates in spite of having increased the endogenous manganese content. The latter decrease in Mn^{54} uptake could possibly be due to the binding of Mn^{55} to the tissue anionic sites.

35.5 IN VIVO VOLTAMMETRIC MONITORING OF URIC ACID IN RAT BRAIN, K. Mueller, Dept. of Psychology, Texas Christian Univ., Fort Worth, TX 76129

Semidifferential linear sweep recording (with carbon paste electrodes) from the anterior caudate of rats reveals 3 distinct peaks. The identity of the substances contributing to peak 1 is controversial; peak 1 may be a composite peak reflecting the oxidation of dopamine, DOPAC, and ascorbic acid. Peak 2 is generally thought to reflect the oxidation of 5HT or 5HIAA; microinfusions of either onto the electrode in situ increase peak 2. However, in anterior caudate peak 2 appears to represent solely the oxidation of uric acid (UA).

In vitro the oxidation of UA does not produce a well defined peak. But microinfusions of UA in situ cause a dramatic increase in peak 2. Microinfusion of uricase, which enhances conversion of UA to allantoin (a nonelectroactive substance) eliminates peak 2 in anterior caudate. Microinfusion of xanthine oxidase, which enhances conversion of xanthine to UA, increases peak 2. Finally, allopurinol, which inhibits xanthine oxidase and thereby reduces UA, reduces peak 2 when given either IP or directly into anterior caudate. These data strongly suggest that the oxidation of UA is the sole source of peak 2 in anterior caudate.

UA has been thought to be absent in mammalian brain (Al-Khalidi & Chaglassian, Biochem. J., 97, 318(1965)) but recent assays of dialysate from rat caudate confirm the presence of UA (Zetterstrom, Sharp, Marsden & Ungerstedt, J. Neurochem., 41, 1769(1983)). Apparently the UA is formed locally since implantation of allopurinol directly into caudate can reduce or eliminate peak 2.

Increased UA levels may be indicative of increased cell death or of increased neuronal activity since either case is likely to result in greatly increased purine metabolism. In support of the former hypothesis peak 2 is increased on the day following microinfusion then gradually decreases over days. In support of the latter hypothesis, peak 2 is increased by 4 mg/kg amphetamine; the increase does not occur in the presence of uricase.

If UA is present throughout brain, which seems likely (Zetterstrom et al), one must exercise caution when recording from 5HT-rich areas of brain. In such areas peak 2 may be a composite of UA, 5HT, and 5HIAA.

35.6 THE EFFECTS OF NEOSYNEPHRINE-INDUCED HYPERTENSION ON BRAIN METABOLIC ACTIVITY FOLLOWING CRYOGENIC INJURY IN THE RAT. M.E.Friedlander, D.Dow-Edwards and T.H.Milhorat. Laboratory of Cerebral Metabolism, Department of Neurosurgery, SUNY Downstate Medical Center, Brooklyn,N.Y. 11203

The cold freeze lesion, as originally described by Klatzo (Klatzo et al., Brain Edema, p.554 967), is a well established model for vasogenic brain edema. Klatzo showed the qualitative relationship between hypertension and the extent of vasogenic cerebral edema. Using this freeze lesion model Pappius has shown the quantitative alterations in local cerebral glucose utilization (Pappius, Ann. Neurol. 9:484-49 1981).

To test the hypothesis that hypertension alters the brain metabolic response to the cold freeze lesion, we employed the deoxyglucose method in four groups of animals: freeze-lesion/normotensive, freeze-lesion/hypertensive, sham-op/normotensive, and sham-op/hypertensive. Male Sprague-Dawley rats (340-440 grams) were anesthetized with Halothane and subjected to a small craniectomy at the level of coronal suture four m.m. to the left of midline. A solution of 2% Evan's Blue (1cc/kg) was then injected intravenously, as a marker for blood brain barrier breakdown. In those animals designated to be lesioned, a two m.m. metal probe, cooled to -89°C, was placed directly on the intact dura for 45 seconds. Thirty minutes after surgery a continious intravenous infusion of either 0.1% Neosynephrine or normal saline was started. The Neosynephrine was titrated to maintain a mean blood pressure between 150-160 torr(normotensive <130 torr). Local cerebral glucose utilization was determined at 4 hours using the quantitative [^{14}C] deoxyglucose method of Sokoloff (Sokoloff, et al., J. Neurochem 29:13-26 1977).

The Evan's Blue dye reconfirms the Klatzo work showing that hypertension increases the extravasation of dye throughout the lesioned cortex. The metabolic studies show that in the lesion/normotensive group there was a significant decrease in glucose utilization in the cortical structures in the lesioned hemisphere compared to the contralateral hemisphere. These differences were not as marked in the lesion/hypertensive group. These data suggest that while hypertension appears to increase vasogenic edema, the brain metabolic activity appears to be normalized.

35.7 STORAGE AND DEPLETION OF NEURONAL GLYCOGEN IN THE CNS OF POSTNATAL RATS. R.C. Borke and M.E. Nau*, Dept. of Anatomy, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Progressive changes in the postnatal incidence, distribution and duration of glycogen in neurons of the pons, medulla and spinal cord were studied by light and electron microscopy using cytochemical methods. Albino rats of eleven ages ranging from newborn to adult were used. Methacrylate sections stained with periodic acid-Schiff-dimedone (PAS) were surveyed to identify cell groups containing neuronal glycogen. PAS-positive neurons were detected in the hypoglossal nucleus, mesencephalic nucleus of V, abducens nucleus, nucleus ambiguus, facial motor nucleus and anterior horn cells of the spinal cord. Of these cell groups, the intensity and duration of the PAS reaction appeared greatest in hypoglossal neurons. The PAS reaction, although of decreasing intensity and frequency, was apparent in these neurons until 21 days postnatal (dpn). The neurons of the mesencephalic nucleus demonstrated a PAS reaction of moderate intensity and duration. All of the other cell groups revealed a weak, diffuse reaction that disappeared by 7 dpn. Ultracytochemical staining for glycogen with periodic acid-thiosemicarbizide-silver proteinate resulted in a heavy metallic staining of the granules that correlated with PAS-positive sites in the neurons of the specified cell groups. At birth, the most striking accumulations of glycogen were massive deposits up to several microns in diameter filling portions of some neuronal perikarya. Most neurons however contained small groups of particles in the juxtanuclear region and multiple, variable-sized aggregates of glycogen bordering the perikaryal margin. Changes in the concentration and pattern of glycogen deposits took place chiefly during the first postnatal week; juxtanuclear and marginal glycogen deposits were replaced by a small number of particles randomly dispersed in perikarya. In most of the specified sites neuronal perikarya contained little if any glycogen by 5-7 dpn. The majority of hypoglossal neurons however were not glycogen-free until 24 dpn. The normal incidence and subsequent depletion of neuronal glycogen from selective cell groups during development of the mammalian CNS may be of relevance to the mechanism involved in infantile glycogenosis Type II disorders in which pathological accumulations of neuronal glycogen occur in many of the same CNS sites. (Supported by USUHS Grant C07019.)

35.8 PHOSPHOCREATINE IN NIE 115 NEUROBLASTOMA AND DISASSOCIATED RAT BRAIN CELLS AS STUDIED BY 31P NMR. P. Glynn*, S. Ogawa*, T. M. Lee*, (Spon.: D. Ready) AT&T Bell Laboratories; R. Chappell, Hunter College, NY; Z. Ahmed, Univ. of Iowa.

There have been several reports of phosphocreatine (PCr) levels measured by 31P NMR in rat brains measured under normal ischemic and hypoischemic conditions. What portion of the PCr NMR spectra arises from the glia and/or neuronal cells has been in dispute. NMR measurements on intact brains cannot distinguish between spectra arising from glia and neuronal cells, the former of which constitute the largest cell volume in the brain. We have studied NIE 115 neuroblastoma cells in the differentiated and undifferentiated states, and also disassociated fetal rat brain cells (70% neuron type). The cells were maintained on microcarriers in a closed perfusing system buffered with bicarbonate at 37°C.

We found no detectable levels of PCr in cells grown on DMEM supplemented with FBS or in fetal rat brain cells cultured for 20 days on defined medium. When the medium was supplemented with .1mM creatine (Cr) for 10 days, PCr was observed at a level of 8% of the ATP. The PCr level rose, in a graded fashion, to 280% of ATP with a supplement of 15 mM Cr., a PCr level comparable to that reported in the literature in intact animals as measured with surface coils. When both cell systems were subjected to an ischemic condition, the PCr levels diminished before ATP levels, and returned to preischemic levels after ATP levels became high upon resumption of normal perfusion.

The present results show that PCr could be present in neuronal cells and could serve as an energy reservoir. However, the PCr level in both cell systems reached the level observed in vivo only after the external Cr concentration was raised to 15 mM or higher, far beyond the physiological concentration of 0.1 mM. Therefore, it is possible that the dominant portion of PCr in vivo observed by NMR comes from glia cells.

35.9 ONTOGENY AND LESION INDUCED CHANGES IN CYTOCHROME OXIDASE (CO) LEVELS IN THE OLFACTORY SYSTEM. R. Costanzo, Medical College of Virginia, Richmond, VA 23298, M.T. Shipley and S. Van Ooteghem, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0521.

The distribution of CO corresponds to the location of major synaptic fields in the olfactory bulb and piriform cortex (Shipley et.al., this meeting). If CO levels reflect synaptic activity, the ontogenetic development of CO should parallel the development of synaptic systems in the bulb and cortex. Removal of specific synaptic inputs might decrease CO activity. In the visual system CO is reduced in functional anatomical loci by sensory stimulation, deprivation and eye removal (Wong-Riley, '79; Horton and Hubel, '81) but there have been no previous studies of the development of CO activity.

Pre- and postnatal rats were perfused and stained for CO. Adult hamsters were stained for CO 4-10 days after olfactory nerve (ON) transection or bulb removal. At E-15 there are no 1° synapses in glomeruli and there is no discernible, organized CO activity in the bulb. At E-18, some 1° synapses have formed (Farbman, et.al., '80) and CO was distinctly visible in tiny glomeruli. At birth (P-1) glomeruli were larger, contained more CO and CO was present in the external plexiform layer. From P-1 there is a progressive increase in CO; the pattern of maturation is being studied as is the development of CO in retrobulbar areas.

ON transection sharply reduced CO activity in all bulb layers. Glomeruli became smaller and the amount of CO/unit area was less than on the normal side indicating that the decline in CO is not due to the reduced size of the glomeruli alone. There was a reduction in layer I of AON and piriform cortex. Thus, the present method reveals transynaptic changes in CO. Partial or complete destruction of the bulb results in nearly complete loss of CO staining in layer IA of the AON and piriform cortex.

CO histochemistry shows changes in metabolic activity associated with normal synaptic development and deafferentation. It may be possible to detect bulbar and transynaptic responses to different odor conditions.

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35.10 OLFACTORY BULB CYTOCHROME OXIDASE (CO) STAINING PATTERNS SUGGEST THAT GLOMERULI ARE FUNCTIONAL UNITS. M.T. Shipley, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0521 and R. Costanzo, Medical College of Virginia, Richmond, VA 23298.

CO is an intramitochondrial enzyme essential to aerobic cellular respiration. CO is high in auditory and visual structures and the hippocampus in loci where both spontaneous neural activity and 2-Deoxyglucose uptake are high (Kageyama and Wong-Riley, Neuroscience, '82). We report the distribution of CO in the olfactory bulb and piriform cortex of normal adult rats and hamsters.

Cryostat (4-16μm) or freezing microtome (40-50μm) sections were reacted for 15-120 min. in a modification of Wong-Riley's (Brain Res. '79) histochemical medium.

High levels of CO were present in glomeruli, the external and internal plexiform layers and in the neuropil of the granule cell layer. CO was low in the olfactory nerve layer and low to moderate in most cell bodies. Different glomeruli had different amounts of CO. The differences did not appear to relate to the size or location of the glomeruli. To evaluate this impression, sections were studied with an image analysis computer system. The results showed that different glomeruli have different amounts of CO/unit area than others. By contrast, there is no discernible variation in CO within individual glomeruli. The physiological bases for inter-glomerular differences in CO activity remain to be determined but the present results may imply that glomeruli are functionally homogeneous and that from moment to moment some glomeruli are more active than others. CO is also very high in the molecular layer of the anterior olfactory nucleus and piriform cortex corresponding to the location of the terminals of olfactory bulb efferents.

CO levels demonstrated by enzyme histochemistry corresponds in location and intensity to regions known to contain high concentrations of synapses in olfactory structures. CO staining and markers for other metabolic enzymes may be useful in unraveling the development and functional organization of the olfactory system.

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35.11 **INCREASED CYTOCHROME OXIDASE STAINING IN RAT SPINAL CORD SUBSEQUENT TO PERIPHERAL NOXIOUS STIMULATION.** **T. Vaughn*, M.M. Behbehani, F.P. Zemlan and M. Shipley (Sponsored by G. Khodadad), Dept. of Physiology and Biophysics & Dept. of Anatomy and Cell Biology, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267-0576.**

Cytochrome oxidase (CO) is a mitochondrial enzyme central to cellular metabolism. CO in the brain is high in regions of pre- and post-synaptic specializations. We find that CO levels are markedly different for individual glomeruli in the olfactory bulb (Shipley et al., this meeting). To date most studies of CO staining have reported patterns that could reflect chronic variations in metabolic activity. Thus, CO is a useful indicator of long-term differences in neural activity between different functional loci. In this study we examined the possibility that short-term changes in localized activity can also be detected by CO staining.

Single units in the dorsal horn of the lumbar region of the spinal cord were recorded in Sprague-Dawley rats anesthetized with choral hydrate (400 mg/kg). Peripheral receptive fields (RF) on the hindlimbs were mapped using noxious stimuli (pinch). Formalin (0.1 cc-5%) was injected subcutaneously in the region of the RF. Elevations in ongoing firing were recorded and supplemental injections of formalin were given to maintain the increased firing rate in the dorsal horn for 45 minutes after which the animals were perfused and processed for CO (Wong-Riley, Brain Res., 1979), with or without cobalt chloride intessification. The site of the recording was determined by reference to a lesion made after the recording electrode was advanced to the ventral horn.

CO staining was visibly greater in the dorsal horn of the stimulated side. In some cases, computer image analysis showed that the staining density was 1.5-2x higher on the stimulated than the non-stimulated side. The differences between the two sides were restricted to the dorsal horn.

Despite the fact that the genome for CO is located in the mitochondria, the period of peripheral stimulation used in this study would seem too brief a time for the synthesis of sufficient new CO to produce the observed changes in staining. It is possible that stimulation (demand) alters the configuration of the mitochondrial inner membrane bringing more CO into an active, stainable state. EM observations of neuropil in stimulated areas may shed light on this and other possible alternatives.

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35.12 Astrocyte Swelling and Respiration during Exposure to Octanoate: A Model of Brain Edema in Reye's Syndrome. J E Olson, R Sankar, and D Holtzman. Dept. of Psych. and Neurol., Tulane Univ. Sch. of Med., New Orleans, LA 70112.

Reye's Syndrome (RS) is characterized by fatty infiltration of the viscera and by brain edema. Increased serum fatty acids may play a role in the pathophysiology, as octanoate (OCT) infusion produces similar changes in rabbits (Trauner, Ped Res, 16:950, 1982). Astrocytes may contribute to the production and resolution of brain edema. These cells behave as perfect osmometers in hypertonic media. In hypotonic media, they display a capacity for cell swelling which is greater than that predicted by osmotic theory (Olson and Holtzman, Brain Res 246:273, 1982). This increased capacity to take up water is associated with an ATP-independent, sodium-dependent component of respiration. To study the relationship of respiration and brain edema in RS, we have investigated the effect of OCT on oxygen consumption and osmotically-driven cell swelling in cerebral astrocytes.

Astrocytes grown in primary culture were suspended in normal saline (360 mOsm) or in saline containing 2x or 1/2 x the normal concentration of NaCl. In respiration experiments, cells were placed in a sealed chamber with a Clark electrode to record O_2 concentration. OCT was added after a basal respiratory rate was determined or after the addition of dinitrophenol (DNP, to stimulate respiration) or oligomycin (to inhibit oxidative phosphorylation). For cell volume measurements, OCT was added to the cell suspension and volumes determined immediately and after 15 and 30 min.

In all osmolarities, basal, oligomycin-insensitive, and DNP-stimulated respiratory rates were inhibited by 4 mM and higher concentrations of OCT. In 1 mM OCT, oligomycin-insensitive respiration was increased by 40% while basal and DNP-stimulated respiratory rates were inhibited.

In hypotonic saline, astrocytes swelled immediately to 141% of their normal volume. After 15 min, the volume had decreased to 131%. This volume was stable for up to 1 hr. OCT (4 mM) reduced the cell volume at 0 and 15 min compared with controls. A lower concentration (1 mM) caused a reduction in swelling only after 15 min. OCT did not affect cell volumes in hypertonic saline.

The data indicate that, in cerebral astrocytes, OCT inhibits or uncouples respiration and reduces the degree of cell swelling in hypotonic saline. The pathophysiology of brain edema in RS may include an inhibition of astrocyte swelling due to an alteration in respiratory control. (Supported by the Robert Katz Medical Research Foundation).

35.13 THE EQUILIBRIUM CONSTANT FOR THE GLYCINE SYNTHASE REACTION. J.M. Liegel* and R.W. Guynn, Dept. of Psychiatry, Univ. of Tex. Med. Sch., Houston, TX 77025.

The activity of glycine synthase (GS) is defective and the concentration of brain glycine (Gly) is increased in infants with non-ketotic hyperglycinemia. Mortality results from intractable seizures. The equilibrium constant (K_{obs}) under physiological conditions (38° C, 0.25 M I, pH 7.0) for the GS reaction has been determined as part of a larger project whose goal is directed toward understanding the mechanisms and consequences of seizure to brain metabolism. The K_{obs} for the GS reaction is the product of the K_{obs} for partial reactions catalyzed by the GS enzyme complex. These reactions are the lipoate (Lip) dehydrogenase reaction (Reaction 1), H_4folate-formaldehyde ($CH_2(OH)_2$) condensation reaction (Reaction 2) and the Gly-Lip decarboxylase reaction (Reaction 3) forming reduced lipoate ($Lip(SH)_2$), NH_4^+, CO_2 and $CH_2(OH)_2$.

$Lip(SH)_2 + NAD^+ \rightarrow Lip + NADH + H^+$ (1)

$H_4folate + CH_2(OH)_2 \rightarrow 5,10\text{-}H_4folate$ (2)

$H^+ + Gly + Lip \rightarrow Lip(SH)_2 + NH_4^+ + CO_2 + CH_2(OH)_2$ (3)

The K_{obs} for Reactions 1 and 2 have been reported by this laboratory (respectively 2.08 x 10^{-8} M and 3.0 x 10^4) at 38° C, 0.25 M ionic strength. The determination of the K_{obs} for Reaction 3 and the calculation of the overall K_{obs} for GS

$$K_{obs} = \frac{[5,10\text{-}CH_2\text{-}H_4folate][NADH][CO_2][NH_4^+]}{[H_4folate][NAD^+][Gly]}$$

are reported in this work.

The K_{obs} for Reaction 3 is the product of Reactions 4-7

$LipSHSCH_2OH + H_2O \rightarrow Lip(SH)_2 + CH_2(OH)_2$ (4)

$H_2O + LipSHSCH_2NH_3^+ \rightarrow LipSHSCH_2OH + NH_4^+$ (5)

$LipSHSCH_2NH_2 + H^+ \rightarrow LipSHSCH_2NH_3^+$ (6)

$Gly + Lip \rightarrow LipSHSCH_2NH_2 + CO_2$ (7)

Reactions 4-6 are non-enzymatic reactions who constants were determined spectrophotometrically. Reaction 7 was catalyzed by the partially purified P-protein of GS with equilibrium approached from both directions. The value for K_{obs} for this reaction is 8.15 x 10^{-3}. The overall K_{obs} for the GS reaction has been determined to be 1.18 x 10^{-3} by combination of values for Reactions 1-3. (Sponsored in part by NIH Grant GM 26488)

35.14 SPECIFICITY OF HEXOSAMINIDASE AND GLYCOSYLTRANSFERASES IN THE MOUSE BRAIN AND IN NEURAL CELL HYBRIDS. C.Ruppert*, D.Barthels*, and W. Wille, Inst. f. Genetics, University of Köln, D-5000 Köln 41, Fed. Rep. Germany

During the cerebellar development significant glycoconjugate alterations have been demonstrated (Edelman & Chuong, PNAS 79:7036, 1982; Schaal & Wille, submitted), which ,in part, correspond to changes of glycosidase activities. Thus, the activities of the membrane-bound neuraminidase (Wille & Trenkner, J Neurochem 37:443, 1981) and the ß-hexosaminidase (ß-hex) (Wille et al.,J Neurochem 40:235, 1983) are age- genotype-, and region-dependently expressed in the murine brain.

Measuring several physico-chemical parameters the identity of cerebral and cerebellar ß-hex has been established. Because of the divergent behavior of mouse brain ß-hex in native starch gel electrophoresis compared to the human liver enzyme, we investigated the murine ß-hex by immunochemical techniques. The enzyme has been purified and antisera against the purified ß-hex have been raised. The most immuno-active antiserum IV-16-3-3 was used for the identification of ß-hex on immunoblots. As the most striking difference between the the murine and the human ß-hex, it turned out, that the former enzyme is posttranslationally processed to a much lesser degree than the latter.

The other class of glycoconjugate metabolizing enzymes we investigated, are the glycosyltransferases sialyl- and galactosyltransferase. Using derivatized glycoproteins and glycolipids as acceptor molecules for radioactively labeled sugars, we defined the differences between the protein- & the lipid-specific brain enzymes with sialyl- & galactosyltransferase activities, respectively. By aid of somatic cell hybrids of neural and non-neural cells we demonstrated a linear relationship between the content of ganglioside-bound neuraminic acid and the sialyltransferase activity. Data concerning the genetics of ganglioside patterns in hybrid cell lines will be presented.

35.15 PYRUVATE PARTICIPATION IN THE LOW-MOLECULAR WEIGHT TROPHIC ACTIVITY FOR CNS NEURONS IN GLIA-CONDITIONED MEDIA. S.D. Skaper, I. Selak, L. Facci* and S. Varon. Dept. of Biology, School of Med., Univ. Calif. San Diego, La Jolla, CA 92093.

Conditioned media from glial cell cultures contain low molecular weight agents which can support survival of CNS neurons in the absence of recognized protein neuronotrophic factors. A similar support is provided to CNS neurons by selected basal media, and pyruvate is the critical medium constituent responsible for their trophic competence. Eagle's basal medium, which contains no pyruvate, acquires pyruvate when conditioned over astroglial cell cultures. Enzymatic degradation of the pyruvate in the astroglia-conditioned medium leads to corresponding losses in its low molecular weight trophic activity for CNS neurons. Quantitative correlations between pyruvate content and CNS trophic activity demonstrate that i) pyruvate is the main trophic ingredient of the glia-conditioned medium, and ii) other low molecular weight substances, acquired during conditioning, reduce the pyruvate concentration required for its trophic effect. The "pyruvate-sparing" substances, as yet unidentified are not the serine and Fe^{3+} which have pyruvate-sparing competence for peripheral, ciliary ganglionic neurons. These findings, together with previous observations, propose that prenatal neurons fail to generate or retain endogenous pyruvate at the levels needed for their survival-sustaining activities.

35.16 IN VITRO INHIBITION OF PHOSPHATIDYLETHANOLAMINE METHYLATION BY ZINC. C.E. Leprohon. Dept. of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada. M5S 1A8.

We have previously shown that rat brain synaptosomes synthesize phosphatidylcholine (PC) by the stepwise methylation of phosphatidylethanolamine (PE), utilizing S-adenosylmethionine (SAM) as a methyl donor, catalyzed by the exzyme complex phosphatidylethanolamine-N-methyltransferase (PeMT). Current interest in central nervous tissue PeMT is largely based on the observation that PeMT activity in a variety of tissues including brain is stimulated by receptor agonists, such as the catecholamines norepinephrine and dopamine. However, since this newly formed PC is rapidly hydrolyzed liberating free choline, this pathway may also be providing the brain with a source of choline for acetylcholine biosynthesis. Since PeMT requires the divalent cation Mg for maximal activity, we decided to look at the effect of other divalent cations. We now report that hepatic and brain PeMT activity, measured in vitro, is inhibited by zinc with a K_i of approximately 100 µM. Rat hepatic microsomes or the synaptosomal-enriched P2 pellet from whole brain were resuspended in buffer (pH 7.5) containing 2.5 µCi (^{3}H-methyl)-SAM (15 Ci/mmol) and 0 - 2000 µM $ZnSO_4$. When phosphatidyl-N-monomethylethanolamine (PME) and phosphatidyl-N,N-dimethylethanolamine (PDE) were used as PeMT substrate, they were dispersed (by sonication) in buffer before being added to the assay mixture. Zn inhibited the incorporation of ^{3}H-methyl groups into the PE-methylated products PME, PDE and PC in a dose-dependent fashion with a K_i of approximately 100 µM. Sensitivity of PeMT to Zn was similar in both hepatic and brain tissue. This inhibition of PeMT was independent of pH (pH 7.5 and 10.0) and anion (SO_4 and Cl). Since 100 µM zinc is within the reported range of total hippocampal zinc concentration, we examined PeMT activity in P2 pellets isolated from hippocampus. A similar K_i of 100 µM Zn for PeMT was observed in this preparation. The results of this study indicate that Zn is an inhibitor of PE methylation, when measured in vitro, and that this inhibition may be occurring at physiologically relevant concentrations. (Supported by the Ontario Mental Health Foundation).

35.17 ALTERATIONS IN BRAIN MITOCHONDRIAL FUNCTION DURING THE RECOVERY PERIOD IN HYPERGLYCEMIC CATS EXPOSED TO ANOXIA. K.R. Wagner and R.E. Myers. Research Service, VA Medical Center, Cincinnati, OH 45220 and Department of Neurology, Univ. Cinci. College of Medicine, Cincinnati, OH 45267.

Ultrastructural and biochemical studies of brain mitochondria have demonstrated disorganization and dysfunction of these organelles as a result of exposure to ischemia. It is unclear if these alterations are irreversible and if they relate to the development of brain injury. To gain insight into these questions we examined brain mitochondrial function during the recovery period following anoxia in cats infused either with glucose or saline prior to exposure. Glucose infused cats develop diffuse brain injury in the hours following resuscitation and ultimately die while saline infused cats remain brain intact and survive following identical exposures. Glucose (serum glucose concentration 50 mM) or saline infused (serum glucose concentration 5 mM) cats were exposed to 6 to 8 minutes of anoxia produced by nitrogen breathing. All cats reduced their blood pressures to tissue pressure values before the end of exposure. The cats were resuscitated and their brain mitochondria isolated during the recovery period using the procedure described by Clark and Nicklaus. Mitochondria isolated at the end of exposure from both groups before resuscitation showed decreases in state 3 (ADP stimulated) and state 4 (substrate alone) oxygen consumption rates and respiratory control ratios (state 3 rate/state 4 rate) with the NAD-linked substrates glutamate plus malate. By 5 hours of recovery when fasciculations were present in the tongue musculature of glucose infused cats, state 3 and state 4 respiration were decreased as compared to controls for both NAD- and FAD-linked (succinate) substrates. In contrast, state 3 and state 4 respiration by mitochondria from saline infused cats had recovered and did not differ from control values. Brain mitochondria from glucose infused cats showed unaltered ADP/O ratios as compared to controls. However, their maximal phosphorylation rate (state 3 rate x ADP/O ratio) was decreased. Thus, although the capacity of these mitochondria to phosphorylate ADP was unaltered, the rate at which this process occurs was significantly reduced. These results show reduced mitochondrial function during the recovery period in brain tissue from glucose as compared to saline infused cats exposed to anoxia. (Supported by Veterans Administration Medical Research Service.)

35.18 REDUCED NICOTINAMIDE ADENINE DINULEOTIDE LEVEL AND TRANSRETINAL POTENTIAL AND MASS RECEPTOR POTENTIAL IN TOAD'S IN VITRO PREPARATIONS. Carlos Rodríguez-Estrada. Cátedra de Fisiología.I.M.E.,Facultad de Medicina, U.C.V. Ap.Postal 50587 Sabana Grande, CARACAS, Venezuela.

In an earlier repor I found (Neuroscience Abs. 2:613) that anoxia increases the NADH level and decreases the transretinal potential. This work was intended to correlate respiratory chainactivity and mass receptor potential and transretinal potential in order to find out if the decrease of the transretinal potential dued to anoxic action in the synaptic events or if dued to anoxic action in the receptors. Fluorometric determinations of reduced nicotine adenine dinucleotide level (NADH) was made during a period of anoxia. Then a same period of anoxia measuring the transretinal potential without measuring the NADH level. And this was followed by measuring the mass receptor potential using the same period of anoxia which produced a decrease of 50% or more of the transretinal potential amplitude. Each retina of the toad's eye adapted to light and pigmented free was placed in a transparent chamber which was circulated with moistened oxygen or nitrogen. Mass receptor potential was recorded after a drop of 10 mM solution of Glutamic acid on the retina. Under dim light illumination a flash light of 5 second was applied every 30 or 60 seconds. It was found that anoxia obtainedafter 5 minutes of circulating nitrogen produced the largest NADH changes level. In a repeated period of anoxia the amplitude of the transretinal potential decreased 50% or more. In the same period of anoxia the mass receptor potential did not change at all. All changes observed were reversed in oxygen. These results indicate that respiratory chain supplies the energy required for synaptic events of the transretinal potential. And suggest that there is a dependence of the energy released from the respiratory chain activity. On the contrary the energy store of the receptor provides the supply of it for the receptor response is maintained long after 50% or more of the synaptic events are not longer seen. The decrease of transretinal potential is dued to anoxic action on the synaptic events. Partially supported by a Grant of Fundación Vargas.

35.PO IMPORTANCE OF ANAEROBIC GLYCOLYSIS FOR SYNTHESIS OF ATP IN SYMPATHETIC NEURONS CULTURED IN SERUM-FREE MEDIUM, AND RELATIONSHIP BETWEEN ATP CONTENT AND THE UPTAKE OF NOREPINEPHRINE IN THESE NEURONS. Taruna D. Wakade* and Arun R. Wakade. (SPON: H.L. Cohen) Dept. of Pharmacology, SUNY, Downstate Medical Center, Brooklyn, NY 11203.

It is recognized that nerve cells utilize their energy in the form of ATP for different types of activities such as maintenance of ionic gradient, and synthesis, storage and transport of neurotransmitters and possibly of other molecules. However, very little is known about the source of ATP synthesis in sympathetic neurons (SN) and its requirement for a specific nerve function such as uptake and storage of norepinephrine (NE). The present experiments were carried out on SN derived from paravertebral sympathetic ganglia of 11- to 13-day-old chick embryos and maintained in serum-free culture medium supplemented with 1 µg/ml each of insulin and transferrin and 20 ng/ml nerve growth factor. About 30,000 cells were plated on polyornithine-coated dishes. After 2 days, cultures were treated with different types of metabolic inhibitors commonly used to block anaerobic and oxidative metabolism of cells. Treatment with inhibitors of oxidative metabolism such as 2-4-dinitrophenol (0.25 mM), dicumarol (1 mM), cyanide (1 mM), azide (3 mM), and arsenate (1 mM) for 2 to 8 hr did not significantly change ATP content of SN in comparison to those of sister cultures (83±2.3 ng/dish). On the other hand, procedures which interfere with glycolysis (i.e., iodoacetate (IAA), 0.25 mM; meta-arsenite (2 mM), or glucose deprivation up to 4 hr) caused a significant drop in ATP levels. Thus, 50% reduction in ATP content occurred within 1-hr exposure to IAA, and essentially complete depletion was seen in 4 to 5 hr. Dependency on ATP for uptake and storage of ^{3}H-NE was examined at various levels of ATP in IAA-treated SN. A marked reduction in tissue ATP stores (over 80%) was not accompanied by any reduction in uptake and storage of ^{3}H-NE. Only when ATP levels were reduced below 90% was uptake significantly reduced. 50% uptake occurred at only 5% of total tissue ATP stores. Specificity of neuronal uptake in untreated controls and IAA-treated SN was ascertained by using 0.3 µM desipramine, which caused over 90% blockade of ^{3}H-NE uptake in both groups. It is concluded that SN generate their ATP primarily by metabolizing glucose via an anaerobic glycolytic pathway, and only a fraction of the total ATP pool is essential for transport of NE across neuronal and vesicular membranes. (Supported by NIH Grant #HL18601 and NSF Grant #BNS7923019.)

LEARNING AND MEMORY: PHYSIOLOGY I

36.1 CHANGES IN I_A AND I_C BUT NOT I_{Na} ACCOMPANY RETENTION OF CONDITIONED BEHAVIOR IN HERMISSENDA. R. Forman, D.L. Alkon, M.Sakakibara*, J.Harrigan*, I.Lederhendler*, and J.Farley. Section on Neural Systems, Lab. of Biophysics, NINCDS at MBL, Woods Hole, MA 02543.

Several studies have indicated that changes in membrane currents of the type B photoreceptors play a causal role in the acquisition and retention of conditioned behavior in the nudibranch mollusc Hermissenda crassicornis. In the present study, measurements of an early outward K^+ current (I_A), an outward Ca^{++}-dependent K^+ current (I_C) and a light induced inward Na^+ current (I_{Na}) were made in the somata of axotomized medial type B photoreceptors of animals belonging to an experimental group or to one of two control groups. The experimental group (P) was exposed to paired light and rotation, the controls were exposed to random light and rotation (R) or to no treatment (N). Phototactic responses of animals were assessed prior to and ∿ 18 hrs following training or control treatments. In the post-treatment test, paired animals were significantly slower to respond to light than were control animals ($p<0.01$). Membrane currents were measured in ASW in nervous systems isolated 20-48 hrs after treatment. Outward currents were measured during command depolarizations to -10 mV and 0 mV from a holding potential of -60 mV. I_A was taken as peak outward current 20-40 msec following, and I_C was taken as outward current 300 msec following, onset of the command step. Mean peak amplitudes of I_A and I_C at -10 mV were significantly different among the groups (I_A: $F_{2,42}=9.37$, $p<0.005$; I_C: $F_{2,43}=7.33$, $p<0.005$). I_A (-10 mV) was significantly smaller for P ($\bar{X}=25.1$ nA) vs. R ($\bar{X}=34.0$ nA) groups ($p<0.001$) and for P vs. N ($\bar{X}=33.1$ nA) groups ($p<0.001$). I_C (-10 mV) was significantly reduced for P ($\bar{X}=6.8$ nA) vs. R ($\bar{X}=11.6$ nA) groups ($p<0.002$) and for P vs. N ($\bar{X}=10.6$ nA) groups ($p<0.001$). Mean peak amplitude of I_A but not of I_C at 0 mV was significantly different among the groups (I_A: $F_{2,21}=9.57$, $p<0.005$; I_C: $F_{2,22}=2.80$, $p<0.1$). I_A (0 mV) was significantly smaller for P ($\bar{X}=44.6$ nA) vs. R ($\bar{X}=58.0$ nA) groups ($p<0.001$) and for P vs. N ($\bar{X}=60.4$ nA) groups ($p<0.001$). Random and naive animals did not differ significantly from each other in any case. I_{Na} was measured as the peak inward current elicited by a 2 sec light following 11-12 min of dark adaptation. Mean peak I_{Na} showed no significant differences between groups. I_C (-10 mV) values for those paired animals which took > 25 min to respond to light in the post-treatment test were responsible for the significance of the paired vs. control group difference for this current.

36.2 ASSOCIATIVE CHANGES IN THE SPATIAL AMPLITUDE PATTERNS OF RABBIT OLFACTORY EEG ARE NOREPINEPHRINE DEPENDENT. C. M. Gray*, W. J. Freeman and J. E. Skinner. Neurophysiology Section and Neuroscience Program, Baylor Coll. of Med., Houston, TX 77030.

The spatial distribution of the 40-80 Hz electric activity that exists on the surface of the olfactory bulb shows a fixed focus of high-amplitude activity in the attentive rabbit. The focus changes shape within a contiguous area after acquisition of conditioning to a new odor (Freeman and Schneider, Psychophysiol. 19:44-56, 1982). The electric events evoked by an odor during acquisition, which may underlie the spatial change of the focus, include a phasic change in the neural activity. This response is thought to represent ongoing modification of synaptic efficacies among mitral-tufted cells during temporally combined afferent and centrifugal inputs to the bulb. In the visual cortex and hippocampus, use-dependent changes in synaptic efficacies have been demonstrated to require beta-receptor activation by norepinephrine (NE). A noradrenergic projection to the bulb is known to exist. We tested the hypothesis that the acquisition of the phasic spatial pattern change depends on beta-receptor activation.

Each of 6 rabbits was implanted with a 64-electrode array (3.5 X 3.5 mm) covering the lateral bulbar surface of the left bulb and an infusion cannula into the ventricle of each bulb. The rabbits were conditioned to an odor paired with cutaneous shock while receiving a continuous intrabulbar infusion of a vehicle solution (0.9% NaCl, 0.1% Na-Ascorbate) or the vehicle containing dl-propranolol (100 uM, 1 ul/hr, 1 wk). Statistical computations revealed that the phasic pattern response to the reinforced odor was acquired only in those animals infused with vehicle. No phasic pattern change was observed for unreinforced odors in either condition.

In a second experiment, the animals were given intrabulbar injection of vehicle or vehicle and NE (100 uM 10 ul, 2 min) followed by 10 presentations of a novel odor. A significant pattern change was observed during a single session with NE injection; this effect was not observed when the odor was paired with vehicle injection. It is concluded that the acquisition of the phasic spatial pattern change, which is thought to depend on the long term alteration of bulbar synapses, depends on the beta-receptor effects of intrabulbar NE. Supported by MH06686 from NIMH.

36.3 ACQUISITION OF THE PAVLOVIAN CONDITIONED EYEBLINK RESPONSE IS RELATED TO THE PERIOD DURING THE CARDIAC CYCLE IN WHICH THE CS IS INITIATED. D. A. Powell, Linda L. Hernandez and Shirley L. Buchanan. WJB Dorn VA Hospital, and University of S.C., Columbia, SC 29201

It is known that during classical (Pavlovian) conditioning of skeletal reflexes other autonomic responses also become conditioned. The latter are acquired prior to the skeletal response, and a major question concerns whether acquisition of these so-called non-specific responses hastens or retards acquisition of skeletal behaviors. One model of cardiac-somatic relationships suggests that the heart rate (HR) decelerations and blood pressure depressor responses elicited by classical conditioning contingencies may be causally related to acquisition of accompanying skeletal behaviors. This suggestion, advanced by the Laceys (Psychophysiology, 1980, 17, 209-221), is that the cardiovascular system functions as an inhibitory feedback system to the CNS affecting the sensory processing of salient stimulation.

According to this hypothesis the presentation of stimuli during diastole, when blood pressure is at its lowest, should be more easily processed than stimuli presented at systole when the blood pressure wave is at its peak. Accordingly, in the present study different groups of New Zealand albino rabbits received a tonal conditioning stimulus (CS) either coincident with the R-wave of the ECG or either 100 msec or 200 msec after the occurrence of an R-wave. A periorbital electric shock train was the unconditioned stimulus (UCS). Both eyeblink (EB) and heart rate CRs were recorded. Two delay conditioning experiments were performed in which either a 500 or a 1000 msec stimulus served as the CS. A third experiment involved a trace conditioning procedure in which the ISI was 1 sec in duration, but a 500 msec CS was employed. In the latter two experiments (i.e., 1 sec delay and 0.5 sec trace) animals which received the 200 msec CS delay showed faster acquisition of the EB CR than animals which received either the 100 msec delay or the 0 msec delay. The HR CR in all three experiments consisted of HR slowing which reached its greatest magnitude in the interbeat interval immediately following CS onset.

These results support the notion of the Laceys that inhibition in the cardiovascular system facilitates CNS processing of sensory information, and thus indirectly affects skeletal conditioning.

36.4 INVOLVEMENT OF THE INFERIOR OLIVE IN CLASSICAL CONDITIONING OF THE RABBIT EYELID. Joseph E. Steinmetz*, David A. McCormick, Carl A. Baier*, & Richard F. Thompson. Department of Psychology, Stanford University, Stanford, CA, 94305

Recent studies have suggested that critical neural alterations underlying classical conditioning of the rabbit eyelid response occur in the dentate-interpositus region of the cerebellum (McCormick & Thompson, Science, 223, 1984). A major source of input to the cerebellar nuclei is projected along climbing fibers that originate in the inferior olive (IO). The present study evaluated the role of the IO in classical eyelid conditioning. Subjects were New Zealand white rabbits. Bilateral electrodes were first chronically implanted into the rostromedial portion of the right and left dorsal accessory olive (DAO). After a 1 wk recovery period, the left eyelid was trained with daily sessions consisting of 117 paired presentations of a tone CS and an airpuff UCS. Animals were trained to criterion, overtrained, then given bilateral DAO lesions through the previously implanted electrodes. Beginning 24 hr after the lesion, nine additional training sessions were given (four with the left eyelid, four with the right eyelid, and a final session with the left eyelid).

Animals that received rostromedial DAO lesions (n=6) demonstrated conditioned responses during the first 20-50 postlesion paired trials but with continued paired training the conditioned responses rapidly disappeared as if UCS presentations were discontinued and extinction procedures begun. Subsequent postlesion training of the right eye failed to produce conditioned responses. Lesions placed in other portions of the IO as well as in various reticular formation sites failed to produce this effect (n=10). A number of animals failed to develop conditioned responses prior to DAO lesion even though as many as 10 days of training were given (n=5). Histological examination revealed rostromedial damage that most likely occurred during electrode placement. These data suggest that the rostromedial DAO may provide essential input to the cerebellum concerning the UCS/UCR; a finding consistent with previous speculation that climbing fiber input from the IO supplies "teaching" or reinforcing input to the cerebellum (Albus, Math. Biosci., 10, 1971; Marr, J. Physiol., 202, 1969). These data also suggest that the memory trace essential for classical eyelid conditioning is not in the rostromedial DAO.

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36.5 CLASSICAL CONDITIONING USING STIMULATION OF THE INFERIOR OLIVE AS THE UNCONDITIONED STIMULUS. M.D.Mauk & R.F.Thompson. Psychology Dept., Stanford Univ. Stanford, CA 94305.

Recent studies have shown that lesions of the inferior olive produce effects on conditioned responding that resemble removal of the unconditioned stimulus (US). In previously trained animals the lesions produce a decline in conditioned responses (CR) similar to extinction (McCormick & Thompson, Neurosci Abstr., 1983; Steinmetz et al Neurosci. Abstr., 1984). These data suggest that the inferior olive forms a portion of the pathway between the sensory neurons activated by the US and the brain region(s) where the plasticity essential for the CR occurs. If so, stimulation of the inferior olive should be an adequate substitute for an external US. We report here preliminary data suggesting that stimulation of the inferior olive is an effective US for classical conditioning in the rabbit.

Male albino rabbits were prepared with stainless steel electrodes directed toward the region of the inferior olive. Subsequent stimulation using brief stimulus trains (0.1 msec pulses, 400 Hz, 100-msec duration) elicited a variety of movements at stimulus intensities ranging from 75-450 uA. Most of the responses involved lateral or upward head movements. However, in one animal the response was a fairly discrete contralateral eyeblink with a latency of approximately 30 msec. By contrast, single-pulse stimulation (0.1 msec, up to 500 uA constant current) failed to elicit unconditioned responses (UR) and was not effective as a US.

Conditioned responses developed when inferior olive stimulation was paired with an auditory conditioned stimulus (CS). In each case the CR was virtually identical to the UR elicited by the electrical stimulation. The CRs appeared within 100-200 trials, similar to conditioning with external USs such as a corneal airpuff. Unpaired presentation of the CS and US produced no CRs in naive animals and fairly rapid extinction in trained animals. The inter-stimulus interval (ISI) function using inferior olive stimulation is similar to that observed for external stimuli: no conditioning at 50-msec ISI, reliable conditioning at 150 msec, and the most robust conditioning at 250-msec ISI.

Thus, with the possible exception of more rapid extinction, conditioning using stimulation within the region of the inferior olive as the US appears identical to conditioning using external USs.

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36.6 SINGLE UNIT ANALYSIS OF CEREBELLUM DURING CLASSICALLY CONDITIONED EYELID RESPONSE. Michael R. Foy, Joseph E. Steinmetz* and Richard F. Thompson. Dept. of Psychology, Stanford University, Stanford, California 94305

In the present study, we have analyzed the electrophysiological activity of individual neurons in the cerebellar deep nuclei through extracellular single unit recording during classical conditioning of the nictitating membrane (NM)/eyelid response in the awake rabbit. In previous multiple unit recordings in the dentate-interpositus (D-I) region of the cerebellum, increases in the firing pattern that well correlate with the amplitude and time course of conditioned eyelid behavior have been observed (McCormick and Thompson, Science, 223, 1984).

One week prior to training, a microdrive adaptor was implanted over a hole in the skull drilled above the cerebellum. Behavioral training consisted of paired presentations of a CS (1 kHz tone) and UCS (corneal airpuff) with the behavioral NM response monitored by a potentiometer. Animals displayed consistently conditioned NM responses within 1-2 days, so that the majority of neurons sampled in this study were recorded when animals were well trained. During recording sessions, single unit electrodes were lowered with a microdrive into the D-I region of the cerebellum and single units were isolated and monitored for 1 to 6 blocks of paired training trials (9 trials/block).

Subsequent analysis of single unit activity revealed several classes of nuclear cells. Among these classes, we found cells which showed relatively short latency and short duration increases in firing patterns in response to the presentation of both CS and UCS stimuli. Several of these cells responded only to tone (CS) onset; others responded only to airpuff (UCS) onset while a third class of cells responded to both CS and UCS onsets within the same trial. Another class of cells displayed an increase in rate of firing that preceded and modelled the learned behavioral eyelid response. As of yet, no cells have been found to respond to both stimulus presentations and also model the conditioned behavior, suggesting that different cell populations may account for these two distinct patterns of responses.

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36.7 CEREBELLAR DEEP NUCLEI LESIONS ABOLISH OR IMPAIR AN INSTRUMENTAL AVOIDANCE RESPONSE IN RABBIT. B. E. Polenchar and M. M. Patterson. Department of Psychology, Ohio University, Athens, Ohio 45701.

Recent electrophysiological and pharmacological studies (e.g. McCormick, et al., Bulletin of the Psychonomic Society, 1981, 18 :103) indicate that certain deep nuclei of the cerebellum, including the dentate and interpositus, are obligatory components of the classically conditioned nictitating membrane (NM) and leg-flexion responses in rabbit. We report here that similar lesions of the cerebellar deep nuclei selectively abolish or severely impair an instrumental avoidance response (AR), while leaving the animal's unconditioned response (UR) to an aversive stimulus intact. In our paradigm, rabbits were trained to avoid the presentation of a corneal airpuff unconditioned stimulus (US) by extending the NM shortly after a tone stimulus which regularly preceeded the airpuff US by an interval of 500 msec. Unlike the classically conditioned NM response, animals trained using an instrumental paradigm continue to improve their performance even in the nearly complete absence of US presentations. After 5 days of avoidance training, animals were lesioned ipsilateral to the trained eye, then retrained using the same eye (for 3 days), contralateral eye (2 days) and same eye (2 days) in that order. Animals who sustained damage to the vermis of the anterior lobe in the most rostral extent and to the dentate/interpositus areas in the medial to caudal extent, showed little or no evidence of reacquisition of the AR when trained using the eye ipsilateral to the lesion. However, rapid acquisition of the avoidance response was acomplished when training was shifted to the contralateral eye. Animals that sustained damage to the regions just ventral and medial to the deep nuclei did not show impaired reacquisition of the AR. These results suggest that lesions of the cerebellar deep nuclei have the same effect on an instrumental avoidance response as has been reported for the classically conditioned NMR. Current theory suggests that the cerebellar deep nuclei may be part of the essential neuronal circuitry coding associative learning which involves a voluntary skeletal response (the "CR pathway"). We are currently completing similar work with the cat NM and hindlimb flexion responses which suggests that the importance of these nuclei in associative learning may be a general phenomena. This research was supported by the Ohio University College of Osteopathic Medicine.

36.8 ENHANCEMENT OF SEPTO-HIPPOCAMPAL ACTIVITY AND NICTITATING MEMBRANE CONDITIONING BY WATER DEPRIVATION. S.D. Berry, A.T. Salvatierra and R.A. Swain (SPON: R. Sherman). Dept.of Psychology, Miami Univ., Oxford, OH 45056.

The distribution of hippocampal EEG frequencies has been shown to predict neural responsiveness and behavioral acquisition rate in the rabbit nictitating membrane (NM) paradigm (Berry & Thompson, Science, 1978, 200, 1298). Their predictive measure was recorded prior to the initial training trial, and thus, presumably reflected nonassociative factors. In this study, we manipulated one such variable, motivational state, by imposing water deprivation prior to NM conditioning.

Twenty New Zealand White rabbits were anesthetized with Ketamine (50 mg/kg) and implanted with chronic bilateral hippocampal, or septal plus hippocampal, recording electrodes. One week after surgery, 10 animals were placed on a 23 hr water deprivation schedule, while 10 had water available ad libitum. In the 2 days following an adaptation session, 26 blocks of training trials were given (13 each day) to 6 deprived and 6 ad lib animals, while the remainder of each group received explicitly unpaired stimulation. Each block consisted of 8 paired (tone-air puff) trials and 1 tone-alone test trial. Learning criterion was 8 conditioned responses in any 9 consecutive trials. EEG was sampled for 2 min. prior to each session, and unit activity was recorded during each trial. Quantification of EEG patterns was done using a zero-crossing frequency analysis; 8-trial averaged histograms were computed for unit responses.

Water deprivation produced a significant shift in EEG frequencies such that deprived rabbits had a higher proportion of 2-8 Hz activity than controls ($t=2.08$, $df=10$, $p < .05$). In addition, they took fewer trials ($\bar{X}=66$) to reach behavioral criterion than controls ($\bar{X}=117.2$; $t=12.5$, $df=10$, $p < .001$). A correlation coefficient based on each animal's pretraining EEG pattern and subsequent learning rate was highly significant ($r=.84$, $df=10$, $p < .001$). Preliminary unit analyses indicated that deprivation enhanced neural responsiveness to the conditioning stimuli.

These results confirm the prediction of hippocampal responsiveness and behavioral conditioning rate using the frequency distribution of the hippocampal EEG. In addition, they suggest that the predictive measure is based on, or modulated by, the motivational state of the animal--even in situations where the motivation is not essential for the establishment of the conditioned response.

36.9 MEDIAL SEPTAL CONDITIONED RESPONSES PRECEDE HIPPOCAMPAL RESPONSES DURING APPETITIVE CLASSICAL CONDITIONING. C.G. Oliver* and S.D. Berry. Dept. of Psychology, Miami Univ, Oxford, OH 45056.

A number of experiments on the nature of limbic system involvement in learning have used classical conditioning of the rabbit's nictitating membrane (NM) response. Historically, the limbic system has been implicated in motivational processes as well as learning so that exclusive use of an aversive paradigm may restrict the interpretation of limbic conditioned responses. Therefore, we observed unit responses in the hippocampus and medial septum during appetitive jaw movement conditioning (CJM) in which the motivational aspects of the task were manipulated by changing the nature of the unconditioned stimulus (UCS).

Chronic recordings of multiple unit activity (MUA) were made from the dorsal hippocampus and the medial septum (MS) during CJM training of 8 New Zealand White rabbits. The parameters of the conditioning were identical to those used for NM training except that rabbits were deprived of water for 22 hr prior to training and the UCS was 1 cc of 0.02% saccharin. Control subjects were given explicitly unpaired tone and saccharin presentations. Analysis consisted of peristimulus block histograms of unit activity averaged across 8 trials, standard scores for each block of unit activity, and jaw movement latency.

As in NM training, the conditioned hippocampal response developed early in training and the pattern of MUA corresponded to the topography of the behavior. In contrast to the NM results, in which evoked MS unit responses to paired and unpaired stimuli occurred, MS responses in CJM were clearly associative, occurring only to the CS in the paired group.

The associative nature of the MS response in jaw movement conditioning suggests that the MS may discriminate between appetitive and aversive stimuli such that the MS provides learned input to the hippocampus in the appetitive CJM situation.

36.10 HABITUATION OF THE MONOSYNAPTIC PERFORANT PATH TERMINATING IN THE CA1 REGION OF THE IN VITRO HIPPOCAMPAL SLICE. P.C.Rinaldi. Div. of Neurosurgery, Dept. of Surgery, UCI School of Medicine, Irvine, CA. 92717.

In the hippocampal formation, fibers originating in the entorhinal area give rise to projections to pyramidal cells in the CA1-CA2 transition of the ipsilateral regio superior (Steward, J.Comp.Neur. 167,285-314,1976). This projection has been shown electrophysiologically to be a direct monosynaptic connection between perforant path (PP) and CA1 pyramidal cells (Doller & Weight, Br.Res.237,1-13,1982). Study of habituation in this pathway is of interest since it terminates on the same cells as do the Schaffer/Commissural Projections (SCP). This latter pathway does not exhibit habituation.

Hippocampal slices from 14 male Sprague-Dawley rats were prepared and maintained by standard techniques. A bipolar stimulating electrode was positioned in the entorhinal area in fibers comprising the projections to CA1. Two glass recording pipettes were positioned for simultaneous recordings of extracellular synaptic field potentials in the CA1-CA2 transition area, one in the distal dendrites of the s. lacunosum-moleculare which receive the PP input and the second in dendrites of the s. radiatum which receive the SCP input. Current sink/source relationships were observed for optimizing stimulation to insure that SCP excitation did not contaminate PP responses. The stimulus intensity was below that required to elicit a population spike. In some slices SCP fibers were interrupted by knife cut or lesion to insure that this input did not contribute to the PP - CA1 dendritic field potential. An habituation series typically consisted of delivery of 3 stimuli at 1 minute intervals for baseline, 10 to 20 stimuli at 2 to 8 second intervals for habituation, and 3 stimuli at 1 minute intervals for recovery.

It appears that habituation can be demonstrated in the monosynaptic PP - CA1 pathway synaptic field potential in terms of six characteristics tested thus far that are considered fundamental to habituation (Thompson & Spencer, Psychol.Rev.73,16-43,1966). The response to repeated stimulation: 1) follows a negative exponential function, 2) exhibits spontaneous recovery, 3) decays more rapidly upon repeated habituation, 4) exhibits an inverse relationship to stimulus intensity, 5) exhibits below zero habituation, and 6) within qualifying limits, is directly related to stimulus frequency.

36.11 A MODEL FOR THE EFFECTS OF HIPPOCAMPAL LESIONS ON PAVLOVIAN CONDITIONING. N.A. Schmajuk. Department of Psychology, University of Massachusetts, Amherst, MA 01003.

Some of the effects of hippocampal lesions are described in terms of changes in Pearce and Hall's(Psych.Rev., 87: 532, 1980) algorithm for Pavlovian learning.

According to Pearce and Hall's model, the associative strength gained by a stimulus CS in each trial is given by $\Delta V_A = S_A \cdot \alpha_A \cdot \lambda$; where S is the CS saliency, α is the CS associability, and λ is the US intensity. The associability of the CS is determined by how well the US is predicted by the aggregate associative strength of all the stimuli present on a given trial n: $\alpha^n = |\lambda^{n-1} - \Sigma V_i^{n-1}|$.

It is proposed that the effects of hippocampal lesions on classical conditioning can be interpreted as a change in the computation of the associability α , which would be determined by how well the US is predicted by the individual associative strength of each particular stimulus:

$$\alpha^n = |\lambda^{n-1} - V_A^{n-1}|.$$

Computer simulations of this model show deficits in extinction, latent inhibition, blocking, overshadowing, and discrimination reversal; but not in acquisition. The simulations are consistent with experimental data available in the literature (see Isaacson, The Limbic System, 1982).

The results suggest that the model adequately describes the behavior of hippocampally lesioned animals in classical conditioning paradigms.

36.12 HIPPOCAMPAL CORRELATES OF INSTRUMENTAL BEHAVIOR. L. Holt* and R.F. Thompson (SPON: K. Pribram). Dept. of Psychology, Stanford Univ., Stanford, CA 94305.

It has been shown that both hippocampal EEG (Berry & Thompson, 1978) and hippocampal unit activity (Thompson, Berger, Berry, Hoehler, Kettner, & Weisz, 1980) reflect the development of simple associative learning. In the present study, the involvement of hippocampal EEG and unit activity in the acquisition of free-operant behavior was investigated. Over a 10-day period, 4 male New Zealand white rabbits were shaped to lever-pressing on a DRL 10-sec reinforcement schedule. Later, recording electrodes were implanted unilaterally in the dorsal hippocampus. Following surgical recovery, both EEG and unit activity were monitored continuously during a further 6 days of DRL acquisition. Hippocampal EEG was sampled for both the 5-sec periods immediately preceding, as well as immediately following lever-pressing; unit activity was sampled from 300 msec immediately preceding, to 450 msec immediately following the lever press. Generally, the instrumental response was accompanied by a rhythmic slow-wave EEG pattern ("theta") having a peak frequency of 6-7 Hz. A frequency-distribution comparison showed a relative rise in 4-6-Hz activity associated with the adequate (rewarded) response, while inappropriate (nonrewarded) behavior was marked by a selective increase in 7-9-Hz activity. The acquisition of the appropriate response was also marked by the development over sessions of a distinct temporal pattern of hippocampal unit activity which predicted this behavior. There was no corresponding unit model for nonrewarded responses nor any obvious change in hippocampal activity which could characterize or otherwise predict such inappropriate behavior. Inter-animal comparison indicated that the development of the hippocampal unit model was roughly related to the overall strength of learning (r= +.71). There was no correspondence between unit activity and motor performance (r=+.03). The unit model disappeared during extinction. These overall findings support earlier results obtained using a classical conditioning technique. More important, the present study reinforces (1) the hypothesis that generally during learning, hippocampal activity forms a temporal model of the appropriate response being acquired, and (2) the idea that this model provides an unambiguous dissociation between learning and motoric aspects of behavior.

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36.13 SEQUENTIAL DEPENDENCIES REGULATE SENSORY EVOKED RESPONSES AND PERFORANT PATH FIELD POTENTIALS IN THE DENTATE GYRUS. T.C. Foster*, R.E.Hampson*, and S.A. Deadwyler, (SPON: J. McCormick). Dept. of Physiology & Pharmacology, Bowman Gray Sch. of Med., Winston-Salem, NC 27103

Past investigations of auditory evoked responses recorded from the outer molecular layer of the dentate gyrus (OM AEPs) have suggested that afferent pathways synapsing on the outer two-thirds of the granule cell dendrites are responsible for its two distinct negative components. Lesion experiments have verified that the short latency N1 component is dependent upon the integrity of the perforant path-to-granule cell synaptic connection (Deadwyler et al., Science, 211:1181, 1981). Depth profile analysis suggests that the N1 component reflects synaptic activity of the perforant path.

In the present study 5 animals were prepared for recording both perforant path elicited field potentials (PP FP) and OM AEPs during the performance of a two-tone auditory discrimination task similar to that employed in previous studies (Deadwyler et al., Brain Res., 169:29, 1979). Amplitude fluctuations in the PP FP elicited by electrical stimulation of the angular bundle and entorhinal cortex 200 msec prior to tone onset were examined on the basis of trial sequences and correlated with changes in amplitude of the N1 component.

Results showed that PP FP amplitude exhibited an endogenous fluctuation dependent on the preceding trial sequences as previously demonstrated for the N1 component of the OM AEP (West et al., Neurosci. Lett., 28:319, 1982). Amplitude changes in the PP FP (100-700 uV) were 2.5 times greater than the range of fluctuation in the OM AEP (100-300 uV). Two types of trial sequences proved to be effective in changing the amplitude of the PP FP: 1) single alternation sequences; and 2) double alternation sequences, the latter being the most influential. In two animals, sequences of 3 to 5 like trials continued to increase N1 amplitude but not the PP FP, suggesting that other factors in addition to PP activity may influence N1 amplitude during longer runs of positive or negative trials. The similarity between the sequential dependencies of the PP FP and the N1 component of the OM AEP provides further evidence that changes in the efficacy of the PP synapses are responsible for the now well characterized trial-to-trial fluctuations in sensory evoked potentials recorded from this region.

36.14 SPATIAL FIRING OF HIPPOCAMPAL CELLS: PUTTING BARRIERS IN THE ENVIRONMENT. R.U. Muller* and J.L. Kubie (SPON: B. Altura) Dept. of Physiol., Downstate Med. Ctr., Brooklyn, NY 11203.

We have shown that the place fields of hippocampal cells in freely moving rats are extremely sensitive to the geometry of the rat's environment. Imagine we know the cell's place field in a cylindrical enclosure. Doubling cylinder's size leaves the RELATIVE position of the field unchanged and results in similar expansion of the field's size for many cells. In contrast, if the cylinder is replaced with a rectangle, it becomes impossible to predict where the place field (if any) will be. We now focus on the effects that internal walls (barriers) have on place fields.

Our recording chamber is a gray cylinder (76 cm diam) with a white polarizing stimulus that covers 100 deg of internal arc. Between trials, the rat is put in its home cage, the gray paper floor is replaced and required changes of scenery are made. We record the cell's firing and the rat's position in a 64x64 grid; spatial firing patterns are displayed as color-coded, time-averaged firing rate maps for each 16 minute trial. An initial no-barrier trial is run. In the next trial, the fiber-board barrier (23x23x0.5 cm) is set to bisect the now-known place field. Another no-barrier trial is then run to see if changes produced by the barrier persist. Finally, trials with the barrier in other parts of the cylinder are run, interspersed with no-barrier trials.

We find that if the barrier bisects the place field, the spatial firing of the cell is drastically disrupted; the firing in the erstwhile field decreases to the background rate in the rest of the chamber. This effect is transient; the field returns when the barrier is removed. When the barrier is far from the field, location-specific firing is unaffected. Intermediate cases are also of interest. When the barrier cuts about one-third of the field, the field size is reduced and the firing rate in the remnant is much lower. If the barrier grazes the field, the field size is slightly reduced without much effect on the firing rate.

Thus, the effect of a barrier within an enclosure is purely local. Cells which happen to have fields in the vicinity of the barrier undergo strong shifts in their location-specific firing, whereas those with distant fields are unaffected. We conclude that the hippocampal, map-like representation of an environment is stable when the structure of the environment is altered with a barrier; only the "image" of the region of the barrier is re-mapped.

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36.15 SINGLE UNIT ACTIVITY IN THE HIPPOCAMPUS: BEHAVIORAL CORRELATES IN NONSPATIAL AND SPATIAL REFERENCE MEMORY TASKS. C. G. Wible*, E. J. Lang*, and D. S. Olton (SPON: E. Blass). Psychology Department, The Johns Hopkins University, Baltimore, MD 21218.

During performance of a working memory nonspatial discrimination, the activity of single units in the hippocampus was correlated differentially with the discriminative stimuli that the rat was required to remember to solve the task. The present experiment was designed to determine the extent to which this behavioral correlate depends on the type of memory involved in the discrimination.

Unit activity in the hippocampus was examined during a nonspatial cued discrimination and a spatial cued discrimination, each of which required reference memory but not working memory. The same apparatus was used in both discriminations; it consisted of two goal boxes, side by side, on the end of a maze stem. In both discriminations the position of the goal boxes was switched between trials in a random counterbalanced fashion so that each color was on the right for half of the trials and on the left for half of the trials. In the cue discrimination, the rat was rewarded for choosing the same colored goal box on each trial regardless of its spatial position. In the spatial discrimination, rats were rewarded for choosing the goal box on the same side for each trial regardless of the color of the goal box. A ten pin electrode was chronically implanted over the CA1 layer of the hippocampus and lowered into the CA1 layer until a unit was isolated. Activity was recorded while the rats performed the cued discrimination and the spatial discrimination. The data from this experiment in conjunction with that from similar single unit recordings during tasks that require working memory (Findling, Shapiro and Olton, Soc. Neurosci. Abstr., 9:646, 1983) further delineates the role of the hippocampus in memory processing. This research is supported by NIMH P316123.

36.16 HIPPOCAMPAL STIMULATION DIRUPTS SPATIAL WORKING MEMORY EVEN AFTER EIGHT HOURS FOR CONSOLIDATION. B. Knowlton*, M. McGowan*, and D.S. Olton. Department of Psychology, Johns Hopkins University, Baltimore, MD, 21218, E. Gamzu (SPON: J. Sepinwall). Department of Pharmacology, Hoffman-La Roche, Inc., Nutley, NJ, 07110.

The present experiment used hippocampal stimulation to demonstrate the following: 1) that storage of information in spatial working memory requires normal hippocampal function, and 2) that consolidation of this information does not occur even after 8 hours, suggesting a difference between reference and working memory. Rats were trained to perform a spatial working memory task on a 12 arm radial maze. Each rat was forced to the ends of 6 arms, randomly chosen by the experimenter, to obtain a food reward. After 8 hours, the rat was allowed to choose among all the arms to find the ones not previously chosen. During some sessions the hippocampus was stimulated through an electrode placed in the CA1 layer, producing an electrophysiological seizure. The current level used for seizure stimulation ranged from 10 to 150 uamps. Stimulation occured 0, 2, 4, 6 or 8 hours after the first 6 choices. During other sessions, the hippocampus was not stimulated, or was stimulated at a sub-threshold current level which was 50-75% of the current level used for seizure stimulation for a particular rat. After seizure stimulation, the number of retroactive errors (returning to arms visited prior to stimulation) increased uniformly at all intervals. Proactive errors (returning to arms visited during the free choice session) increased only after 8 hour seizure stimulation. Sub-threshold stimulation did not increase errors. These results indicate that normal hippocampal function is required for the storage of spatial information in working memory. These results also suggest that the time course of consolidation of working memory is greater than that seen in other types of memory, if consolidation of working memory takes place at all. An important difference between reference and working memory may be their respective rates of consolidation.

36.17 BRAIN TRANSPLANTS CAN RESTORE SINGLE UNIT ACTIVITY IN THE HIPPOCAMPUS. M.L. Shapiro, D. Simon*, & D.S. Olton, Dept. of Psychology, Johns Hopkins University, Baltimore, MD, 21218, F.H. Gage, A. Bjorklund*, & U. Stenevi*, Dept. of Histology, Universtiy of Lund, Lund, Sweden.

Transplants of embryonic septal tissue can restore maze performance in rats given fimbria-fornix (Ffx) lesions. However, transplants can occasionally lead to greater impairments in the performance of maze tasks than lesions alone (Gage, Bjorklund, and Stenevi, in press). The pattern of innervation produced by these two types of septal transplants can not be distinguished histologically. The present study investigated the effects of transplants of embryonic septal tissue upon single unit activity in the hippocampus of four groups of rats: normal rats, those given Ffx lesions, those given Ffx lesions and transplants that improved performance on spatial memory tests (imp/smart), and those given Ffx lesions and transplants that impaired maze performance (imp/impaired).

Theta unit activity was recorded from the CA-1 layer of the dorsal hippocampus during two behaviors: (1) an appetitive behavior in which rats walked on an elevated track during recording, (2) a consummatory behavior in which rats drank chocolate milk from a drinking tube.

In normal rats, high frequency theta unit activity was organized into 7 hz spike trains during appetitive behavior, while low frequency activity was not organized into rhythmic spike trains during consummatory behavior. In rats given Ffx lesions, the activity of theta units was not organized into rhythmic spike trains, while overall firing frequency still increased during appetitive behaviors relative to consummatory behavior. In imp/smart rats, the activity of theta units were somewhat organized into rhythmic spike trains, and overall firing frequency increased during appetitive behavior relative to consummatory behavior. In imp/impaired rats, the activity of theta units was not organized during appetitive behavior, and high frequency firing appeared during both behaviors.

These results suggest that (1) transplants alter the activity of hippocampal neurons, (2) the type of alteration may be critical to recovery of function brought about by transplants, and (3) restoration of behavior may occur to the extent that patterns of modulation resemble those found in normal rats. (Supported by NIMH P316123.)

36.18 HIPPOCAMPAL CELLS WHICH HAVE PLACE FIELD ACTIVITY ALSO SHOW CHANGES IN ACTIVITY DURING CLASSICAL CONDITIONING P. J. Best and L. T. Thompson. Department of Psychology, University of Virginia, Charlottesville, Virginia 22901

A large number of studies have found that hippocampal neurons show conditioned responses during Pavlovian classical conditioning. Another set of studies has found that hippocampal neurons fire faster when the animal is in a specific location or "place" in an environment. Simultaneous recording from more than one cell reveals that different cells can have different fields. Such place field activity appears to show little modification in an environment. Further, cells which have well defined place fields in one environment can be totally silent in another environment. So the possibility exists that the cells which show place field activity are not the same cells which show conditioned responses.

The present study determines if cells which show place field activity also show conditioned responses in a classical conditioning paradigm.

Rats were implanted with a movable bundle of ten 32u microwire electrodes. The electrode bundle was advanced until well isolated hippocampal unit activity with 4:1 signal-to-noise ratio was found on one or more electrodes. The animals were then placed on a six-arm radial arm maze until they made at least eight visits to each arm. Place fields were determined by automatic registration of the rats' location along with on line computer analysis of unit activity. Rats were then placed in a conditioning chamber where they received pairings of tones (3 KHz, 30 sec) and footshock (60 Hz, 1 sec, 1 ma) in a Conditioned Emotional Response (CER) paradigm. The rats were than placed back in the radial arm maze and the place field activity was redetermined.

Every cell studied showed reliable place field activity on the radial arm maze and showed reliable conditioned responses in the CER paradigm. Further, when the rats were replaced on the maze following conditioning, the cells showed the same place field as before conditioning. In no case did conditioning in one environment modify place field activity in the other environment.

Therefore, we must conclude that the same cells subserve these two functions; or, more likely, that conditionability is a necessary component of place field processing.

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36.19 DIMINUTION OF LONG-TERM SYNAPTIC ENHANCEMENT DURING SLEEP. B.Jones Leonard*, B.L.McNaughton, and C.A.Barnes, Behavioral Neurosci. Program, Dept Psych., Univer. of Colorado, Boulder, Colorado 80309.

Neuronal transmission in the hippocampus depends upon behavioral state. In the fascia dentata, extracellularly recorded synaptic responses are smaller, and population spikes are larger during slow-wave sleep than during quiet wakefulness (Winson and Abzug '78). The present experiment addressed the influence of behavioral state on long-term enhancement (LTE) of perforant path synapses following brief high-frequency stimulation (HFS). Evoked responses and 5 sec epochs of EEG were recorded once every 15 sec from the fascia dentata of chronically prepared animals during the latter half of their normal sleep cycle. Recording was carried out with the animal in its home cage, inside a recording chamber. Each animal received two HFS sessions; once during behavioral and EEG signs of sleep and again after awakening the animal. Care was taken that the animal was in the same behavioral state for at least 10 min before and after the HFS. LTE was calculated as the change in EPSP amplitude 10 min after HFS relative to the preHFS baseline. There was a large and significant reduction in the amount of LTE induced during sleep compared to the awake state. Indeed, the average "LTE" during sleep was not significantly different from baseline. Seven of 7 cases for which the animal remained asleep during the first HFS session showed more LTE in the subsequent session while awake. For 5 other cases, in which the animal woke up during the "sleep" session, LTE was greater than for the subsequent session in which the animal was intentionally awakened. This is consistent with the fact that, given equivalent conditions, most LTE occurs during the first HFS session. It is also of interest that the maximum LTE in this experiment was somewhat lower than has generally been obtained with the same apparatus. The procedural differences were that normally animals are removed from their home cages, and HFS is generally carried out within several minutes of connection to the recording apparatus. This suggests that arousal level, perhaps increased by the less familiar situation or by recent handling, may also modulate LTE under waking conditions.

The results presented here are consistent with the observation that stimulation of the reticular formation increases LTE (Laroche and Bloch '81). They also provide a useful control procedure to dissociate nonspecific effects of HFS in studies seeking the biological basis or functional consequences of LTE. (Supported by PHS Grant NS20331.)

36.20 RETROGRADE SPATIAL AMNESIA GRADIENT FOLLOWING HIPPOCAMPAL LTE. G. Rao, B.L.McNaughton, and C.A. Barnes. Behavioral Neuroscience Program, Dept. Psych., Univer. of Colorado, Boulder, Colorado 80309.

Increasing evidence suggests that long-term enhancement (LTE) of hippocampal synaptic transmission following high-frequency activity may be involved in the storage of spatial information. According to this hypothesis, information about the animal's environment is contained in a specific distribution of synaptic weights in the hippocampus. The hypothesis predicts that disruption of this specific distribution by experimental saturation of LTE should produce both retrograde and anterograde disruption of spatial memory and learning. We previously reported that such saturation of LTE bilaterally in rats produced no deficit on a well learned spatial reference problem involving escape from a brightly lit surface into a concealed goal tunnel (Soc. for Neurosci. Abs. 191.16, 1983). These same animals, however, were severely impaired on the acquisition of a new problem involving a change in the location of the tunnel relative to the extramaze environment. The present experiments were to determine whether retrograde effects could be induced by induction of LTE a short time following training on the spatial reversal problem. Animals with bilaterally implanted electrodes for stimulation of perforant path fibers and recording of population synaptic responses in fascia dentata were trained to asymptotic performance on the circular platform problem as described previously (16 days, one trial per day). Within 5 min of each training session, the evoked responses of both himispheres were recorded using low-frequency (non-LTE inducing) stimulation. On the day following the last training session, the goal tunnel was rotated to a new location 135° from the original one. The animals were given one training session on this new problem. Within 5 min of this training, half of them received high-frequency stimulation which induced LTE bilaterally. The remaining animals received low-frequency stimulation only. The following day, the LTE animals were significantly impaired on their retention of the new location relative to the low-frequency group.

These results demonstrate that spatial memory can be disrupted by the experimental saturation of LTE at perforant path synapses if this treatment is delivered sufficiently soon after training. This is consistent with the hypothesis (among others) that the hippocampus serves as a primary but temporary memory buffer whose contents can be relocated to other parts of the brain within a limited time after initial storage. (Supported by PHS Grant AG003376.)

LEARNING AND MEMORY: PHYSIOLOGY II

37.1 BEHAVIORAL SEQUELAE FROM 5-MINUTE BILATERAL CAROTID OCCLUSION IN THE MONGOLIAN GERBIL. A.H. Tang,* L. Hudson* and A. Salvatierra* (Spon: M.A. Travis). CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

Bilateral carotid occlusion for 5 min. under halothane anesthesia in Mongolian gerbils produced selective ischemic cell loss in the CA1 subfield of the dorsal hippocampus (Kirino, Brain Research 239:57-69, 1982). The behavioral characteristics of gerbils subjected to this procedure of cerebral ischemia were investigated.

Male gerbils weighing 45 to 60 g were anesthetized with 2% halothane in a mixture of 95% 0_2 and CO_2. Both common carotid arteries were occluded with aneurysm clips for a duration of 5 min. The global ischemia resulted in no death, convulsive seizure, or postural abnormality. However, all gerbils exhibited greatly increased locomotor activities, developed within hours after the carotid occlusion. The hypermotility gradually returned to normal in about 2 weeks. During the height of the hyperactivity, spontaneous alternation in Y-maze exploration was replaced by perseveration. The post-ischemic gerbils were also tested for acquisition of active and passive avoidance responses one week after carotid occlusion. In the two-way shuttle avoidance paradigm, the post-ischemic gerbils avoided better than the sham-operated controls and moved more between trials. In another study where movement in the shuttle box was punished by shock, the post-ischemic gerbils learned to suppress their movement at a slower rate. Retention of the inhibitory response, once learned, appeared to be unimparied. The behavior of the post-ischemic gerbils resembled what was observed in rats after extensive lesion to the dorsal hippocampus (Jarrard, Physiol. Psychol. 8:198-206, 1980).

Histological examination of brains of post-ischemic gerbils found swelling of perikaryon in the pyramidal cells of the CA1 subfield 24 hours after carotid occlusion. Little change was seen in the other parts of the hippocampus. Extensive cell loss in the same area was found when the gerbils were sacrificed one week after carotid occlusion.

Pretreatment with pentobarbital sodium (50 mg/kg) before the carotid occlusion protected the gerbils from cerebral ischemia as measured by the development of hyperactivity and pyramidal cell loss in the hippocampus. Administration of the drugs immediately after the carotid occlusion was without effect. A weak protective effect was also demonstrated with pretreatment with naloxone (3 mg/kg) or morphine (10 mg/kg).

37.2 NEURAL AND ENDOCRINE EFFECTS ON LEARNING: A CONTRAST OF HIPPOCAMPAL AND ACTH INFLUENCES. R.L. Port, A.G. Romano, A.A. Mikhail*, and M.M. Patterson, Ohio University, Athens, Ohio 45701.

The interrelationship between the limbic system and the pituitary-adrenal axis has led to speculation that the parallel effects of independent manipulation of neural or endocrine variables may indicate a common substrate. Lovely (J. Comp. Physiol. Psychol. 89: 224, 1975) has postulated that the learning deficits found in hippocampal lesioned animals may be attributable to elevated levels of ACTH consequential to the primary lesion.

Our interpretation of hippocampal participation in learning involves two distinct processes (Port, Mikhail and Patterson, Beh. Neurosci., in press). Initially the hippocampus provides a "stimulus map", a neural representation of the temporal configuration of stimuli. Later, a "neural model" of the learned motor response develops. We suggest that the behavioral effects of ACTH are mediated by the stimulation of hippocampal neurons by the hormone. Further, this excitation may impede the development of the "neural model", a dynamic process, while permitting the "stimulus map" to proceed intact. Two learning tasks were investigated: classical delay conditioning, a paradigm in which the stimulus map and neural model are involved; and sensory preconditioning, a task presumed to rely on the stimulus map.

Hippocampal lesioned (n=6), ACTH elevated (n=6) and normal (n=6) rabbits were classically conditioned using a tone CS, shock US and 150 msec ISI. Both manipulations were found to facilitate acquisition and shorten response onset latency. No effects were found during extinction. An earlier study (Port and Patterson, Beh. Neurosci. 98: 1984) had shown an abolition of SPC by fimbrial damage. The influence of ACTH elevation on SPC was evaluated in a second study. Preconditioned ACTH-injected animals performed no differently than preconditioned normals. Thus, the effects of ACTH on learned behavior may be restricted to tasks which involve the hippocampal "neural model".

37.3 OPPOSITE EFFECTS OF CINGULATE CORTICAL AND SUBICULAR LESIONS ON AVOIDANCE BEHAVIOR IN RABBITS. M. Gabriel, S. P. Sparenborg, N. Stolar*, J. Dreyzehner*, P. Colletier*. (SPON: J. Malpeli). Dept. Psychol., Univ. Illinois, Champaign, IL 61820.

Active avoidance behavior in rodents and carnivores with hippocampal lesions is typically facilitated but behavioral impairment has frequently followed damage to the cingulate cortex. We have studied the effects of these lesions on the avoidance behavior of rabbits, to relate the behavioral consequences to neuronal correlates observed in the same paradigm.

The rabbits were trained to locomote in an activity wheel to avoid a footshock US whenever they heard a tone CS+. They also learned to ignore a second tone (CS-) that differed in auditory frequency from the CS+, and that was not predictive of the US. Sixty trials daily with each stimulus were presented in a random order until a criterion of discriminative performance was attained. After criterion, overtraining, an 8-day retention interval, extinction, reacquisition, and reversal training to criterion, were carried out.

There were no significant effects of the lesions on behavior during acquisition. Rabbits with subicular lesions (N=8) made a significantly greater mean frequency of avoidance responses than controls (N=13) during extinction ($P< .01$) and during reversal training ($P< .05$). A significantly reduced frequency of avoidance was manifested by the rabbits (N=7) with area 29 damage during reacquisition ($P< .05$) and reversal training ($P< .05$). These results considered along with the extant neuronal data suggest the following interpretation. Inputs from the subiculum and area 29 suppress the firing of the anteroventral thalamic nucleus (AVN), and retard the development of discriminative firing in that structure (Gabriel et al.; Ragsdale et al., Neurosci. Abstr., 1984). Removal of the subicular projection to AVN facilitates behavior during retention testing by enhancing the discriminative discharge in AVN. However, removal of the projection from area 29 to the AVN yields deficient performance during retention. The deficiency is virtually identical to that produced by AVN lesions (Gabriel et al., Behavioral Neuroscience, 97: 675, 1983). This is so because the output of AVN must traverse area 29 if it is to affect behavior. (Supported by NIMH 31351 to M.G.)

37.4 LESIONS IN THE CINGULATE CORTEX AND NEURONAL ACTIVITY IN THE ANTERIOR THALAMUS DURING CONDITIONING IN RABBITS. D. Ragsdale*, S. Sparenborg*, N. Stolar*, S. Mills*, and M. Gabriel. (SPON: P. Teitelbaum). Dept. Psychol., Univ. Illinois, Champaign, IL 61820.

Studies of the neuronal correlates of conditioning in rabbits have demonstrated changes in neuronal activity in a triad of limbic structures (the cingulate cortex [area 29], the anterior ventral nucleus [AVN] of thalamus, and the hippocampal formation). Discriminative neuronal discharges (i.e., greater discharges to the positive CS [CS+] than to the negative CS [CS-]) develop in the deep cortical layers (5 & 6) in the first training session. The AVN and the upper cortical layers (1-4) develop discriminative firing later, as asymptotic discriminative behavior is first performed (e.g., Gabriel, et al., Science, 208: 1050, 1980).

A recent study has verified the hypothesis that direct driving of upper cortical neurons by AVN neurons accounts for the late discriminative activity in the upper layers [Gabriel et al., Behavioral Neuroscience, 97: 675, 1983]. Here, we test the hypothesis that the discriminative volleys of the deep cortical neurons, projected along the corticothalamic pathway in the early training stages, are causally relevant to the discriminative discharges that develop in the AVN in the later training stages. Bilateral aspirative and electrolytic lesions of area 29, and implantation of chronic microelectrodes in the AVN were performed in 8 rabbits. Unlesioned controls (N=13) underwent surgery for implantation of recording electrodes. Following recovery, all rabbits received discriminative avoidance training to criterion, and overtraining. The AVN records of the controls replicated past results in manifesting development of discriminative neuronal discharges in the late training stages (the criterial stage in which asymptotic discriminative performance was first attained, and the postcriterial overtraining stage). The AVN records in rabbits with lesions manifested a significantly greater neuronal discharge throughout training, and an accelerated acquisition of discriminative firing, relative to controls. These results, similar to the effects of subicular complex lesions (Gabriel et al., Neurosci Abstr., 1984), suggest that the area 29 projection to the AVN suppresses the CS-elicited neuronal discharge, and delays the advent of discriminative activity in AVN until the late training stages. (Supported by NIMH 31351 to M.G.)

37.5 POSTTRAINING ELECTRICAL STIMULATION OF THE MEDIAL DORSAL NUCLEUS OF THE THALAMUS IMPAIRS RETENTION OF CONDITIONED AVOIDANCE TASKS. D. Tam*, C. Bennett*, and J.L. McGaugh (SPON: P. Gold). Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717

Postmortem examination of Korsakoff patients reveals that a common sign of the disease is damage to the medial dorsal nucleus of the thalamus (MDT). Lesions of this region are highly correlated with the amnestic syndrome associated with this disease (Ann. Rev. Neurosci. 5). Further, experimental lesions of the MDT in rats and cats impair retention of avoidance tasks (Behav. Brn. Res. 4, 263). Anatomically, the MDT has reciprocal connections with the amygdala (Brain 85, 505), a sensitive locus for memory modulatory treatments including electrical brain stimulation (Physiol. Behav. 9, 513). We investigated the effects of posttraining electrical stimulation of the MDT on retention of two conditioned avoidance tasks.

Male Sprague-Dawley rats (60 days old) were stereotaxically implanted with insulated stainless steel electrodes aimed bilaterally at the medial dorsal nucleus of the thalamus (AP -1.0 mm; lateral +/- 1.0; and 5 mm below the dura, Peligreno and Cushman, 1965). Two weeks following the surgery, the rats were trained on a one-trial, step-through inhibitory avoidance task (footshock, 1 mA, 2 s). Immediately or 6 hours following training, the rats received stimulation (MDTS) (125 uA/electrode, 30 s) or sham stimulation (SHS). Retention was tested 24 hours later as the latency to step through (maximum of 600 seconds). Two weeks after the inhibitory avoidance task, the rats were given three training trials on a one-way active avoidance task (FS 640 uA, 30 s). Immediately or 6 hours after training, they received MDTS or SHS according to a schedule of treatment counterbalanced for their treatment after inhibitory avoidance training. Twenty-four hours after training, the rats were tested using eight trials as on day 1.

Either immediate or 6 hour posttraining MDTS impaired retention on both inhibitory and active avoidance tasks. These data suggest that the coherent activity of the medial dorsal nucleus of the thalamus is essential to memory formation or retention processes for up to 6 hours after training.

The present study is supported by USPHS Research Grants MH12526 and AG00538 (to JLMcG).

37.6 SUPPRESSION OF CUE-ELICITED UNIT RESPONSES IN THE RAT FRONTAL CORTEX BY MICROINJECTION OF PROCAINE OR GABA INTO THE NUCLEUS BASALIS MAGNOCELLULARIS. G.C. Rigdon* and J.H. Pirch (SPON: J.B. Lombardini). Dept. of Pharmacology, Texas Tech Univ. Hlth. Sci. Ctr. Lubbock, TX 79430.

Male rats were implanted to allow the recording of single units in the frontal cortex during a cue-event paradigm. Rats were sedated and restrained during the experiments. Animals were first trained to associate a 2 sec. tone cue with rewarding medial forebrain bundle stimulation. After training, units responded to the cue by an increase or decrease in discharge rate. Cumulative histograms of the unit response to the cue were obtained and then either procaine hydrochloride or GABA was microinjected into the nucleus basalis magnocellularis (nBM). Immediately after drug administration another histogram was obtained to ascertain the drug effect. Procaine microinjections into the nBM suppressed the frontal cortex unit responses in 9 of 10 units that had previously responded with an increase in firing rate and 10 of 12 units that had decreased their firing rate before drug. GABA microinjections antagonized the response in 15 of 19 excited units and 2 of 2 inhibited units. Recovery from the drug effect was obtained in all units which could be held for a sufficiently long recording period (23 units). The nBM supplies the frontal cortex with up to 70% of its cholinergic innervation. These results indicate that neurons in the nucleus basalis magnocellularis region are involved in the cue-elicited changes in the rate of discharge of units in the rat frontal cortex. (Supported by Tarbox Parkinson's Disease Institute and USPHS MH29653.)

37.7 ROLE OF THE NUCLEUS BASALIS IN GENERATION OF CONDITIONED CORTICAL SLOW POTENTIALS IN THE RAT. J.H. Pirch, M.J. Corbus*, G.C. Rigdon* and W.H. Lyness. Dept. of Pharmacology, Texas Tech Univ. Hlth. Sci. Ctr., Lubbock, TX 79430.

Although event-related slow potentials have been recorded from the cortex of various animals and from the scalp of humans for many years, the transmitter systems involved in the generation of these potentials have not been determined. These experiments were conducted to gather information regarding the role of cholinergic innervation in the generation of conditioned cortical slow potentials.

Since we had previously shown that conditioned slow potential (SP) responses recorded from the rat frontal cortex are bilaterally equal, the effects of unilateral drug treatments or lesions on ipsilateral and contralateral cortical SP responses were examined. The SP responses were recorded with Ag-AgCl electrodes and were generated by a 2-sec light cue which preceded medial forebrain bundle stimulation (Pirch et al., Brain Res. Bull. 7:399, 1981). The following approaches were used: 1) Microinjection of procaine, GABA or saline into the nucleus basalis; 2) Microinjection of atropine or saline subdurally in the SP recording area; 3) Electrolytic lesion of the nucleus basalis; 4) Kainic acid lesion of the nucleus basalis. The following bilateral measurements were obtained in the lesion studies: choline acetyltransferase (ChAT) in cortex (CTX) and hippocampus (HIPP); serotonin in CTX, HIPP, striatum and nucleus accumbens (NAC); norepinephrine in CTX and HIPP; dopamine in striatum and NAC.

The cortical SP responses were reduced ipsilateral to the injections of procaine and GABA into the nucleus basalis, and on the side of the atropine injection. With either lesion, the SP responses on the lesioned side were reduced as compared to the control side. Reductions in CTX ChAT and other measures occurred ipsilateral to the electrolytic lesion, but only CTX ChAT was reduced in the kainic acid animals. Thus, pharmacological depression of nucleus basalis neurons, blockade of cholinergic receptors in the cortex and lesions that reduce cortical ChAT depress conditioned SP responses in the frontal cortex. These results provide evidence for a role of cholinergic innervation in the generation of event-related slow cortical potentials. (Supported by USPHS MH29653 and by the Tarbox Parkinson's Disease Institute at Texas Tech Univ. Hlth. Sci. Ctr.)

37.8 RESPONSES OF NEURONS IN THE BASAL FOREBRAIN OF THE BEHAVING MONKEY. F.A.W.Wilson*, E.T.Rolls, S.Yaxley*, S.J.Thorpe*, G.V.Williams* and S.J.Simpson* (SPON: D.W.Lincoln). Dept. Exptl.Psychol., Oxford University, Oxford, England.

Neuronal activity was recorded in the basal forebrain in monkeys performing memory tasks. Neurons in this region are known to degenerate in Alzheimer's disease. In a visual discrimination task the monkeys could initiate a lick movement to obtain fruit juice when one stimulus (S+) was shown; responses to the other stimulus (S-) produced aversive saline. In a serial visual recognition memory task each stimulus was shown twice per day, once as novel and once as familiar. Lick responses to stimuli when novel produced saline, and when familiar produced fruit juice reward. One population of neurons (104/2004) responded differentially to the presentation of the S+ and S-. Of these neurons, some (41/61 tested) responded differentially to novel and familiar stimuli. Neurons responding to the S+ also responded to familiar stimuli; neurons responding to the S- also responded to novel stimuli. The responses of these neurons thus reflected the association of visual stimuli with reinforcement in both memory tasks. The differential response to novel and familiar stimuli was maintained when many trials separated the novel and familiar presentations of a stimulus. Some of this population also responded during licking for fruit juice and/or visually guided reaching for food. Some neurons also responded during a 500 msec. tone which preceeded the presentation of the visual stimuli. It was possible to show that basal forebrain neurons with differential responses in the visual discrimination task projected to the cerebral cortex, using antidromic activation from cortical stimulation electrodes. A second population of neurons (39/2004) responded to novel stimuli in the recognition memory task. These responses could not be attributed to the association of a stimulus with reinforcement, as shown by the visual discrimination task. The differential response to novel as compared to familiar stimuli was maintained between 2-12 stimuli for different neurons. The responses of both populations of neurons could not be attributed to arousal or movements induced by touch to the trunk and limbs. These findings show that in the basal forebrain neurons are found that reflect two types of memory. One population reflects the association of visual stimuli with reinforcement. A second population reflects whether stimuli have been seen recently.

37.9 NEURONAL ACTIVITY IN NUCLEUS BASALIS OF MEYNERT DURING A DELAYED RESPONSE TASK IN MONKEY. R. T. Richardson and M. R. DeLong. Depts. Neurology and Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

The nucleus basalis of Meynert (NBM) is the primary source of cholinergic input to cerebral cortex. In Alzheimer's disease (AD) there is a reduction in cortical cholinergic markers and a loss of NBM neurons. The NBM may play a role in memory processes since memory impairments are characteristic of AD. To explore this possibility, we have recorded the activity of single NBM neurons in a monkey performing a delayed response task, a commonly used test of spatial memory in primates.

The animal was trained to make movements at the elbow which moved a spot of light horizontally on a screen in front of the animal. He first had to move his light into a circle in the center of the screen and then follow the circle as it jumped to a position to the left or right of the center position (cueing) and then returned to the center (recentering). After a variable delay period of 1 to 6 sec., both left and right circles appeared and the monkey had to move his light into the circle to which he had moved previously (choice) in order to receive a water reward.

Of the responsive NBM neurons recorded, most responded during the choice movement and/or the reward delivery. A smaller proportion responded during the cueing and recentering phases. A common response pattern consisted of responses with the cueing and recentering movements followed by an equal or greater response with the choice movement. Changes in firing rate associated with the delay period were rare and were similar for both left and right cues. Several cells had progressive increases in firing through each trial which returned to baseline after reward delivery.

In conclusion, in a delayed response task, NBM neurons respond most often in association with the delivery of a water reward and with the movement made in order to obtain that reward. The same movement in the cueing phase of the task is usually not accompanied by a neuronal response. Many NBM cells show an increase in firing throughout the trial, but few cells have altered activity in the delay period during which a spatial location or response pattern must be retained. These results provide little evidence for a direct involvement of NBM in the memory component of this task. The findings suggest that the NBM may be related to aspects of reward acquisition, but it is also possible that the changes in NBM neuronal activity reflect more general processes such as attention or arousal.

37.10 AMYGDALA UNIT ACTIVITY DURING DISCRIMINATION OF VARIOUS OBJECTS AND FEEDING BEHAVIOR IN MONKEY.
T. Ono, H. Nishino*, M. Fukuda*, H. Nishijo* and K. Yamatani*. Dept. of Physiol., and Neurosurgery, Fac. of Med., Toyama Med. and Pharmaceu. Univ., Sugitani, Toyama 930-01, Japan.

The amygdala (AM) is thought to be important in the processing of complex information to recognize the significance of stimuli. The primary objective of this study was to elucidate functions of the AM in learning to recognize food and non-food. AM unit activity was recorded in chronic monkeys during object discrimination and eating of various foods. To the present six neuron types have been observed : 1) Attention or arousal type indiscriminately responded to food and non-food objects, shutter movement, sound, etc. 2) Specific food dominant type responded at the sight of food, and tended to habituate at the sight of neutral non-food objects. 3) Specific non-food dominant type responded primarily at the sight of aversive-related non-food objects including human faces. Responses of neurons that predominantly responded to human face were stronger at the sight of real human faces than for photographs, and were modulated by changes in facial expression. 4) Attention-evaluation type responded to the introduction of new food, change from preferred to aversive food or from aversive to preferred food, and experimenter's approach to the animal. During these trials background activity also increased. These responses disappeared when the monkey recognized an aversive food and stopped feeding, or when the new food became familiar to the monkey after several trials. 5) Anticipatory food-related type as well as food dominant type responded to the sight of food, and also food anticipation situations. 6) Reward perception type responded to food only in the ingestion phase after putting food into the mouth. This response was attenuated by heavily salting food.

These results agree with the suggestion that the AM is related to the formation of stimulus-affective association for recognizing the significance of stimuli and is also crucial to the discrimination of objects.

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37.11 PREFRONTAL NEURON ACTIVITIES IN A VISUAL DESCRIMINATION REVERSAL TASK WITH GO-NO GO PERFORMANCES IN UNDER-TRAINED MONKEYS. K.KUBOTA. Dept. of Neurophysiol., Primate Res. Inst., Kyoto Univ., Inuyama, Aichi 484, Japan.

To understand processes in the course of task learning, neuron activities of the prefrontal cortex were recorded while under-trained monkeys were performing a visual discrimination reversal task with GO-NO GO performances. Results were compared with those obtained from well trained monkeys (Brain Res., 244:269,'82).

Upon yellow lamps on, the monkey pressed the lever and later, red or green Cue lamp was on. Then, color was changed to yellow, indicating Response period. In schedule A, upon green, the monkey continued to press the lever (NO GO), or, upon red, he released the lever (GO). Correct responses were rewarded. In schedule B, relations between Cue colors and responses were reversed.

Four naive monkeys, with performance levels below 66%, pressed the lever to initial Lamps with 0.6-0.8 s latencies, similar to those of well trained monkeys, and released the lever in Response period with 0.3-0.6 s latencies, similar or slightly longer than those of well trained monkeys.

In under-trained monkeys 27 task-related neurons were obtained by 306 electrode penetrations which were done evenly 1-2 mm apart from each other. Chances to obtain task-related neurons was low, being one at every 8.2 penetrations, compared to that of well trained monkeys (one at every 2 penet.). Except for one, neurons of under-trained monkeys were not activated during more than one period. During Cue period 4 neurons were activated, and 2 were suppressed. During Response period 3 neurons were activated, 2 were suppressed, and 1 was activated prior to lever release. During Reward period 10 neurons were activated after the reward, 2 were after absence of reward and 3 were regardless of reward.

In one monkey, 8 penetrations in a narrow cortical area (4 x 4 mm) did not yield task-related neurons in under-trained state but penetrations of the same tracks at better performance level yielded 6 task-related neurons.

Thus, in under-trained state not many prefrontal neurons are involved in the task performance. Results are in agreement with the hypothesis that, as learning proceeds associations between cue stimuli and responses are established among visually activated and lever release-related neurons in the prefrontal cortex, and more neurons are activated in the task performance.

37.12 ADAPTIVE PLASTICITY IN PRIMATE SPINAL STRETCH REFLEX (SSR): A TWO-PHASE PROCESS. J.R. Wolpaw, J.A. O'Keefe* and R. Dowman. Ctr. for Labs and Research, NYS Dept. of Health and Depts. of Neurology and Anatomy, Albany Med. Coll., Albany, N.Y. 12201.

Monkeys can change the amplitude of the wholly segmental, largely monosynaptic, spinal stretch reflex (SSR) when reward is contingent on such change. SSR increase (SSR↑ mode) or decrease (SSR↓ mode) occurs without alteration in initial muscle length or background alpha motoneuron tone and is relatively specific to the agonist muscle (Wolpaw et al., J. Neurophysiol. 50: 1296-1319, 1983).

SSR amplitude change occurs in two distinct phases. Phase I occurs within 6 hours after imposition of the SSR↑ or SSR↓ mode, producing an 8% change. Phase II begins immediately after Phase I and continues for at least 40 days, producing further change of 1-2%/day. Although Phase I's rate is about 20 times that of Phase II, the change produced by Phase II is much greater because Phase II continues for weeks while Phase I is over within the first 6 hours.

Phase I indicates a nearly immediate change in suprasegmental input on the segmental arc of the SSR. Because stretch onset time is unpredictable and the SSR occurs before any other possible response, this change in descending activity must be tonic; it must be present continually, day after day, for the 5-7 hrs/day the animal spends at the task. Phase I produces a rapid and significant increase in reward probability. Thus it may be readily interpreted as an example of operant conditioning, provoked by the reward contingency (SSR↑ or SSR↓).

Phase II's extremely slow rate, on reversal and redevelopment as well as on initial development, distinguishes it from Phase I and suggests that, unlike Phase I, Phase II represents long-term plasticity, with a persistent structural or biochemical basis. This slow rate makes it difficult to view Phase II as operantly-conditioned: it seems much too slow to provide the animal with detectable reinforcement. Why, then, does it occur at all? One reasonable explanation is that Phase II is a side effect of Phase I, a long-term persistent result of the continual change in descending activity responsible for Phase I. If, as related evidence suggests, part or all of this long-term alteration occurs at the segmental level, it should constitute a technically accessible substrate of memory.

37.13 ABNORMAL MOVEMENTS AND DECREMENT OF MOTOR CONDITIONED RESPONSES BY ELECTROLYTIC LESIONS OF SUBTHALAMIC NUCLEI IN CATS. B. Prieto-Gómez* and H. Brust-Carmona. Depto. de Fisiología, Div. de Investigación, Fac. de Medicina, UNAM. México 04510, D.F., MEXICO

It is generally accepted that motor conditioned responses (MCR) depend on a neural circuity. It has been postulated that the information required for a lever pressing response reaches de caudate nucleus (CN) through the medial thalamic nuclei (Mth), and the response is elicited by entopeduncular (EPN) and subthalamic nuclei (Sth). Previous studies analized the role of Mth and EPN. The present work investigates the possible participation of Sth in a "facilitatory" lever pressing (LP) and an "inhibitory" motor conditioned response (passive avoidance conditioning, (PAC).

Cats were conditioned to LP when a light was on (reinforced with 0.5 ml of milk) every day during 12 min. After 15 sessions, bilateral electrolytic lesions of Sth were performed using conventional stereotaxic techniques. Once recovered, they were observed daily for any motor alteration in an open field, and submitted to 30 additional conditioning sessions. After this period, the acquisition of a PAC was tested in a two compartments chamber.

The lever pressing rate tested 48-72 hrs after the lesions decreased and remained at a significantly lower level ($p < 0.05$) during all the postlesions sessions. The larger Sth lesions abolished completely the MCR (N=3). Twenty to thirty days after the bilateral Sth lesions, fast "shaking" movements, similar to the ballistic limb movements in monkeys, were observed, which persisted until the animals were sacrificed. This ballistic type of movement was observed in 9 cats with lesions localized mainly in Sth, as determined by histologic sections.

The average latency to cross from one compartment to the other during the acquisition session of PAC was of 50 s (N=7), the learning criterion was set at 600 s, tested 24 hrs later. Sth lesions did not impair this learning.

Our results suggest that the Sth nuclei play a role in normal motor coordination, as well as in learned excitatory responses (LP), but not in the inhibitory type (PAC).

37.14 EFFECT OF MILD HEAD TRAUMA ON ENDOGENOUS AUDITORY EVOKED POTENTIALS: APPLICATION OF A CAT MODEL IN STUDIES OF COGNITIVE IMPAIRMENT AFTER BRAIN INJURY. A. DeSalles,* C. Dixon*, S. Reuther*, Y. Katayama*, P. Newlon*, D. Becker and R. Hayes (SPON: J.A. Stevenson). Div. of Neurosurg., Med. Col. of Va., Richmond, VA 23298.

Even mild head injury often results in long-term deficits in attention and memory, the genesis of which is unknown. Electrophysiological studies in humans have shown that a task-relevant stimulus can differentially elicit a late positive potential (P300) in the scalp-recorded auditory evoked potential (AEP) that is believed to reflect neural activity related to cognitive processing and attention. A similar late positive component (LPC) has been reported in cats (Wilder et al., 1981) and monkeys (Arthur and Starr, 1984). Therefore, we have studied the use of the LPC paradigm in the cat to detect impairment in cognitive function after low levels of fluid percussion brain injury. This injury model produces no long-term gross neurologic deficits as assessed by conventional methods of evaluation.

Four cats (2.5-3.6 kg) were prepared for painless head restraint under sodium pentobarbital anesthesia. A screw was implanted for fluid percussion injury, 0.8 mm posterior to bregma. Two stainless steel recording screws were placed in skull at the vertex and frontal sinus to record AEP's. One week later, animals were trained in the paradigm described by Wilder (1981) for obtaining LPC. Pupillary dilatation was conditioned to tones. A random sequence of 2 discriminable tones (3 and 5 kHz) was presented at a rate of 0.5 per sec. The lower tone (target) had a probability of 0.2 and was followed, after 700 msec, by a tail-shock (2-4 mA, 2 msec, 100 Hz) for 250 msec. After 500-1,000 trials, animals differentially attended to the target stimulus as inferred by selective pupillary dilatation. In the AEP, positive waves at 80-150 msec were enhanced, and a positive LPC (250-350 msec) appeared in response to the target tone. Animals were then anesthetized and received a concussive head injury (2.0-2.4 atm).

Although complete neurologic recovery was seen within 2 hours after injury (normal reflexes, motor coordination, orienting response), LPC's were suppressed as long as one month after injury. This preliminary evidence suggests that the AEP model may provide an objective method of assessing post-concussive disturbances in cognitive processing after brain injury in animal models. This work was supported in part by NIH Grant NS 12587.

37.15 A MULTIPLE CHANNEL SYSTEM FOR SEPARATING ACTION-POTENTIALS FROM SINGLE NEURONS IN MULTIUNIT RECORDS. B.S. Drakulic*, G. Heit, E. Halgren (SPON: D. O. Walter). Crump Inst. for Med. Engr., VA Southwest Epilepsy Ctr. and Brain Res. Inst., UCLA, Los Angeles, CA 90024.

Current techniques for separating the action-potentials (APs) generated by particular neurons out of multiunit recordings are subjective, laborious and ineffective. This is especially true for hippocampal pyramidal cells, which often fire in bursts of APs with successively smaller amplitude and longer duration.

We report here the use of intelligent data acquisition units (IDAU) for spike detection and separation. Each IDAU continuously samples the amplified and filtered signal from a single microelectrode. When a threshold (based on the background noise) is exceeded, the IDAU automatically searches for the maximal amplitude of the initial and second peaks of the spike and the time between these two peaks. The IDAU also finds the maximal amplitude of the first peak on an adjacent microelectrode. McNaughton (J. Neurosci. Meth. 8: 391, 1983) has shown that the ratio of amplitudes in adjacent wires is constant for all APs from a given neuron, regardless of their absolute amplitude. The IDAU also notes the elapsed time in msec for later correlation of APs with behavioral events.

An IDAU based upon the 8 MHz Intel 8086 microprocessor is dedicated to each microelectrode pair. Two very low-noise instrumentation amplifiers, followed by a high speed switch, antialiasing filter and sample-and-hold, lead to a very high speed 12 bit A/D converter. Net system throughput, including logical operations necessary for peak detection, is over 100,000 samples per second. The parameters are placed in first-in first-out memory for collection by the host PDP 11/23 microcomputer for statistical processing.

Every AP recorded by an IDAU constitutes a point in the 4D space of peak heights, interpeak interval and interelectrode ratio. Points are clustered using statistical algorithms integrated with graphic displays. A multistage process in which clustering is first applied to the initial APs in bursts is under development. The ability of this system to separate the APs from individual neurons out of actual or synthetic multiple unit recordings will be discussed.

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37.16 HIGHER ORDER DIFFERENCE EQUATION FOR NEURAL NETS WITH CHEMICAL MARKERS. P. A. Anninos, M. Kokkinidis* and G. Thomas*. Department of Physics, University of Crete, Iraklion, Crete, Greece.

A mathematical analysis of probabilistic neural nets is presented here in which the neural activity is given by finite difference equation of higher order than one(Wong and Harth,1973). In this new study the neural connections are set up by means of chemical markers carried by the individual cells (Anninos and Kokkinidis,1974). It is shown that this formalism can take into account any combination of refractory periods and effective delays that may exist in such neural nets. With this new approach we studied again the dynamics of neural nets with and without sustained inputs. Results obtained with this method show that for slowly varying excitatory or inhibitory inputs there exist stationary states which exhibit marked simple and multiple hysteresis effects similar to the behavior of first order neural nets(Anninos et al.,1970 and Anninos and Kokkinidis,1984). Such Hysteresis effects may be considered again to represent the basis for short-term memory.

37.17 THE TRION MODEL: A MODEL OF CORTICAL ORGANIZATION EMBODYING A BASIS FOR A THEORY OF INFORMATION PROCESSING AND ASSOCIATIVE MEMORY. Dennis J. Silverman,* John C. Pearson* and Gordon L. Shaw, Physics Department, University of California, Irvine, CA 92717.

In the spirit of Mountcastle's [V. B. Mountcastle in The Mindful Brain, G. M. Edelman and V. B. Mountcastle, (Eds.) pp. 1-50.] organizational principle for neocortical function, and strongly motivated by Fisher's [M. E. Fisher and W. Selke, Phys. Rev. Lett. 44, 1502 (1980)] model of physical spin systems, we have introduced a new cooperative mathematical model [Shaw, Silverman and Pearson, UCI preprint 84-4] of the cortical column. Our model incorporates an idealized substructure, the trion, which represents a localized group of neurons. The trion model allows for a completely new framework for information processing and associative memory storage and recall: Small networks of trions with highly symmetric interactions are found to yield an enormous number of quasi-stable, periodic firing patterns. Experience or learning would then modify the interactions (away from the symmetric values) and select out the desired patterns (as in the selection principle of Edelman [G. M. Edelman in The Mindful Brain, pp. 51 -100]). Conceptually this suggests a radically different approach from those information processing models which start at the opposite extreme of a randomly connected neural network with no periodic firing patterns, and then (via Hebb-type modifications in the synaptic interactions) reinforce specific firing patterns. Another exciting feature is that our model includes the known statistical fluctuations in the post-synaptic potentials. These fluctuations are essential for having the huge number of patterns. We believe that these phenomena are of interest to the fields of neurophysiology, cellular automata and molecular scale processors, as possibly applied to a future generation of computers. Investigations concerning the learning capabilities of the trion networks will be presented. In addition, simulations relevant to multi-electrode recordings in cortex will be given that suggest new ideas in how to design and analyze these experiments.

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37.18 INFORMATION TRANSFORMS FROM NEURAL LINES TO DYNAMIC RANGE: CONSERVING CONNECTIONS USING ASSOCIATIVE SUBSYSTEMS. R. B. Glassman. Dept. of Psychology, Lake Forest College, Lake Forest, IL 60045.

When a large set of inputs and outputs lend themselves to categorization, brain evolution may accomplish a saving of neural connections by funneling the inputs toward a relatively few associative modules (1983 Abs. #245.8). This presentation further develops the logic of such systems.

Although parts of the brain do not make arithmetic tabulations, we may think about associative modules in terms of discrete approximations: A set of modules may be thought of, equivalently, as (1) a number of units, each representing a "dimension" of input and output and resolving some integral number of values, (2) digit places for counting distinct inputs, or (3) elements across which a unique distribution of activation levels exists for each combination of meaningfully different inputs.

The number of input elements (n) together with the number of possible significantly different levels of activation of each input element (its range; call it g for the special case in which all ranges are equal) suggests a number (g^n) that represents the required capacity of the set of associative modules. A parsimonious convergence to a set of associative modules smaller than the number of input elements can take place only when (1) the range of each module serving a particular associative function is greater than the range of the input modules or the function allows fuzzing some of the input resolution or (2) input combinatorial possibilities are restricted.

Although it is not yet clear how to apply these ideas in detail to empirical cases, examples of (1) might be emotional decisions such as fight or flight, requiring crude perceptual discriminations; examples of (2) might be present in lateral inhibition and other aspects of perceptual systems, which ignore certain details, to yield Gestalt perceptual phenomena such as "closure." Another implication is that a set of associative modules will be specialized to register either local or global, but not intermediate combinations of the input set, because the number of combinations of n things taken r at a time is small for either small r or r close to n. Some evidence suggests that the different visual cortical areas might be characterized in this way (Sprague, in Pompeiano & Ajmone-Marsan; Raven, 1981.)

38.1 FURTHER STUDIES ON THE MORPHOLOGICAL BASIS OF LONG-TERM HABITUATION IN APLYSIA. C.H. Bailey and M. Chen. Ctr. for Neurobiol. & Behav., Depts. Anat., Neurol., & Psychiat., Columbia Univ., P & S, and N.Y.S. Psychiat. Instit., N. Y., N.Y. 10032.

We have used the gill- and siphon-withdrawal reflex of Aplysia californica to explore the morphological basis of the synaptic plasticity that underlies simple forms of learning and memory. In an earlier study (Bailey and Chen, Science, 1983), we examined the ultrastructural correlates of long-term habituation and sensitization by analyzing the active zones of identified sensory neuron presynaptic terminals (a critical site of plasticity for the short-term forms of both types of learning) in control and behaviorally-modified animals. Using HRP to label presynaptic terminals (varicosities) of sensory neurons and complete serial reconstruction to analyze synaptic contacts, we found that the number, size, and vesicle complement of sensory neuron active zones were larger in animals showing long-term sensitization than in control animals, and smaller in animals showing long-term habituation.

In the present study we have extended our structural analysis of learning by examining the effect of long-term training on the total number of varicosities per sensory neuron. Toward this end we have analyzed 18 sensory neurons from two groups of animals, control (untrained) animals and animals trained for long-term habituation (Carew et al., 1972). Following behavioral training and retention testing, a single sensory neuron in each animal was injected with HRP. After a 2 hour incubation period to allow HRP to fill the neuropil arbor of the sensory neuron as well as its axon in the siphon nerve, ganglia were fixed, histochemically processed and embedded in Epon. Sensory neurons were completely reconstructed using serial 20μm slab-thick sections and the total number of HRP-labeled varicosities for each sensory neuron was counted through a blind procedure. We have found that long-term habituated animals have 35% fewer varicosities per sensory neuron (836 $\pm$ 75 S.E.M., N=10) than do control animals (1291 $\pm$ 138 S.E.M., N=8, $\bar{t}$ = 3.05, $p < .01$).

These findings, combined with those of our earlier ultrastructural study, indicate that long-term habituation is accompanied by structural alterations on two levels of synaptic organization: 1) changes in focal regions of membrane specialization (active zones) of the synapse, and 2) more global alterations involving a modulation of the total number of varicosities. By re-embedding 20μm sections for ultrastructural analysis, we plan to directly correlate quantitative aspects of active zone morphology with alterations in synaptic number and thereby gain a better understanding of the complete family of structural changes which accompany long-term memory.

38.2 DIFFERENTIAL EFFECTS OF MEDIAL AND DORSAL CORTEX LESIONS ON SPATIAL REVERSALS IN TURTLES (CHRYSEMYS PICTA). W. Grisham* and A. S. Powers. Dept. of Psychology, Bryn Mawr College, Bryn Mawr, PA 19010.

Previous research in this laboratory has shown that dorsal cortex (cd) lesions produce a deficit in the reversal of a simultaneous pattern discrimination although original learning is unimpaired. Reversal deficits of a similar nature have been found to result from hippocampal (Hc) lesions in several mammalian species. The cd, however, has not traditionally been considered to be the anatomical equivalent of the mammalian Hc. Rather that role usually has been ascribed to the medial cortex (cm). Since the cd is interconnected with the cm, however, it was hypothesized that the reversal deficit found after cd lesions was due to a loss of its connections with the cm. It was further hypothesized that cm lesions would produce even greater reversal deficits than lesions of the cd.

Accordingly, the effects of cd and cm lesions were investigated with regard to spatial reversal, a task which has been shown to produce a robust reversal deficit in several mammalian species. Turtles were pretrained to press keys for food reward and then given lesions of the cd (n=4) or cm (n=3), or sham lesions (n=6). After post-operative recovery, they were trained on a spatial discrimination (response to the key on one side was reinforced) to a criterion of 85% correct for two consecutive days. Then they were given a reversal of the discrimination (the response to the other key was now reinforced). Finally they were reversed back to the original side.

The results showed that turtles with cd lesions were significantly impaired on the first reversal compared to shams but that those with cm lesions were not. Thus, the reversal deficit previously found with cd lesions is not exclusive to pattern discriminations. The lack of an effect of cm lesions suggests that the cm may not be necessary for reversal learning. The dissociation of these effects implies that the cd and cm are functionally distinct.

38.3 CEREBELLAR ANSIFORM CORTEX ASPIRATION DOES NOT ABOLISH TRACE CONDITIONING IN THE RABBIT Diana S. Woodruff-Pak, David G. Lavond, & Richard F. Thompson Department of Psychology, Stanford University, Stanford, California 94305

Previous research in our laboratory has demonstrated that the cerebellar deep nuclei are essential for the acquisition and retention of the classically conditioned nictitating membrane/eyelid response in rabbits using the delay paradigm in which a tone CS and airpuff US overlap during the last 100 msec of the CS presentation. The trace conditioning paradigm in which the CS and US do not overlap requires subjects to retain a "trace" of the CS to associate it with the US. The task is more difficult, requiring about five times the number of trials to criterion as the delay paradigm. The present study was undertaken to determine if: 1) the cerebellar nuclei were essential in trace as well as in delay conditioning, and 2) additional brain structures such as the cerebellar ansiform cortex were involved when CS-US association over longer time intervals was required.

Sixteen young adult male New Zealand white rabbits had recording and in some cases lesion electrodes surgically implanted before training. They were adapted to the running chamber and trained daily with 126 paired trials of 85 dB, 1000 Hz tone of 250 msec duration and 2.1 N/cm^2 airpuff lasting 100 msec. The trace period between the tone CS offset and the airpuff US onset was 500 msec. Mean number of trials to criterion of 8/9 CRs was 466.9 trials. After a day of overtraining the animals had one of three treatments: 1) aspiration of the left ansiform lobe (N=9), 2) aspiration of the left ansiform lobe and underlying cerebellar nuclei (N=4) 3) electrolytic lesion of the left cerebellar nuclei (N=3).

The 9 animals with ansiform lobe aspirations exhibited a transient decrease in CRs but relearned with savings. Histology verified that the entire ansiform lobe (between the primary fissure and the posterior inferior sulcus) was removed. The shape and latency of pre and post aspiration CRs were similar. When the cerebellar nuclei were significantly damaged by aspiration of overlying ansiform lobe or when only the small amount of critical tissue in the nuclei was lesioned, the animals' capacity for CRs on the side ipsilateral to the lesion was permanently abolished. However, the ability to condition was not eliminated in the contralateral eye by cerebellar nuclei lesions.

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38.4 INITIAL IDENTIFICATION OF THE ESSENTIAL BRAINSTEM AUDITORY PATHWAY NECESSARY FOR CLASSICAL CONDITIONING OF THE RABBIT NICTITATING MEMBRANE(NM)/EYELID RESPONSE. D.G. Lavond, D.S. Woodruff-Pak and R.F. Thompson, Department of Psychology, Stanford University, Stanford, CA 94305.

We have demonstrated that the interpositus nucleus of the cerebellum is essential for classical conditioning of the rabbit NM/eyelid response. If the interpositus is the site of plasticity necessary for conditioning then it must receive information about the CS and US. The path for auditory CS information into the cerebellum is unknown except that the inferior colliculus is not essential for conditioning. We report here that the auditory CS passes through the lateral lemniscus rather than through certain reticular intermediates or direct cochlear projections.

Experimental rabbits were implanted with bilateral cannulae at the root of the lateral lemniscus near the superior olive/trapezoid nuclei. Control rabbits were implanted with cannulae in the caudal reticular pontine and the reticular tegmental nuclei. After 2-4 days of recovery, they were overtrained first on an NM/eyelid CR to a tone CS (1 KHz, 85 dB SPL, 350 msec) and a coterminating corneal airpuff US (0.210 kg/cm^2, 100 msec), overtrained second with a light CS, and then overtrained with alternating 8-trial blocks of tone CS and light CS. Training consisted of about 120 trials per day over an average of 6 days.

Each rabbit was tested with 4 baseline blocks of alternating CS blocks and then injected with 1 ul of 3% lidocaine through the implanted cannulae, and then tested on a tone and a light block. This was repeated. A third injection was followed by a light CS block and then a tone CS block. The experimentals were then trained for 5 blocks to a tone CS.

In all instances the experimentals showed CRs to light CS but had no CRs to the tone CS. The experimentals recovered CRs to the tone CS. Controls continued to show good CRs to both light and tone CSs at all times.

The experimentals were then injected with 10 ug of ibotenic acid, allowed 6 days of recovery, and retested. All showed good CRs to both CSs indicating that the lidocaine effect is due to inactivation of fibers of passage.

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38.5 SUPERIOR COLLICULUS LESIONS DISRUPT CLASSICAL CONDITIONING TO VISUAL BUT NOT AUDITORY STIMULI. R. W. Skelton*, N. H. Donegan*, and R. F. Thompson (SPON: E. Smith). Dept. of Psychology, Stanford Univ., Stanford, CA.

Recent experiments have demonstrated the essential role of the deep cerebellar nuclei in short-delay classical conditioning with both auditory and visual conditioned stimuli (CS), and have suggested that the neuronal plasticity subserving the learning develops within this region. However, the afferents to the deep nuclei essential for conditioning remain unidentified. The present study extends the analysis of the neural circuit underlying classical conditioning by investigating the role of the superior colliculus in conditioning with a visual CS.

During each daily test session, male albino rabbits were presented with 108 pairings of either an auditory CS (3 kHz, 85-db tone) or a visual CS (small 6V lightbulb near left eye) with an airpuff delivered to the left eye. The CS began 250 msec before the 100-msec airpuff and co-terminated with it. Animals were trained to criterion (8 consecutive conditioned responses) first with the tone CS, and then with the light CS. Three pre-lesion baseline sessions were then run, with each CS used for 1/2 of each session (order of presentation counterbalanced across days and animals). Responses were evaluated by measuring the integrated EMG recorded from chronic electrodes implanted in the left eyelid. Large, bilateral lesions of the superior colliculi were then made, either by aspiration or by multiple electrolytic lesions, using halothane as the anesthetic.

Tests begun 1 week after surgery revealed deficits in conditioned responses to the visual CS ranging from complete abolition to partial decrements. Because responding to the auditory CS was not affected, the decreased responding to the light CS could not have been due to a generalized disruption of eyelid responses or to damage of the deep cerebellar nuclei or its efferent structures. These data suggest that for classical conditioning with visual stimuli, the superior colliculus and/or adjacent regions of the tectum may be an essential component(s) of the neural circuit afferent to the deep cerebellar nuclei.

This work was supported by a fellowship from NSERC (Canada) to RWS and by NSF grant BNS-81-17115 and Office of Naval Research contract N00014-83 held by RFT.

38.6 EFFECT OF VENTRAL PERIAQUEDUCTAL GRAY (PAG) LESIONS ON LONG-TERM HABITUATION OF THE ACOUSTIC STARTLE REFLEX. G. S. Borszcz, J. Cranney, and R. N. Leaton. Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Animals with ventral PAG lesions demonstrate deficient conditioning on a number of aversively motivated tasks (e.g., Halpern, 1968; Liebman, Mayer and Liebeskind, 1970; Lyon, 1964). Recent work in this laboratory suggests that sensitization during a long-term habituation procedure involves an association between the initially aversive startle-eliciting stimulus and contextual cues. The current study investigated the effects of ventral PAG lesions on long-term habituation of the acoustic startle reflex.

Thirty-six male albino rats received bilateral ventral PAG lesions (n=12), unilateral ventral PAG lesions (n=12), or sham lesions (n=12). Following recovery, animals were exposed to the startle-eliciting stimulus (95 db, 100 ms white noise) during three sessions a day (intersession interval=2.25 h), on four alternate days. In each session, 10 startle-eliciting stimuli were presented (interstimulus interval=60 s). Freezing behavior was monitored during the 10 s prior to each startle-eliciting stimulus. Results indicate that while lesioned and control animals did not differ in their initial responsiveness to the startle stimulus, animals with damage to the ventral PAG habituated more rapidly. In addition, there was a positive correlation between the amount of damage of the PAG and the rate of long-term habituation. Animals with PAG lesions also exhibited significantly less classically-conditioned freezing during habituation training than did controls. These findings are interpreted in terms of the possible role of the ventral PAG in an associatively-based long-term sensitization process.

38.7 LATERALIZED DECREMENT OF HYPOTHALAMIC SELF-STIMULATION AFTER UNILATERAL LESION OF THE PREOPTIC AREA. C. Munoz*, I. Keller* and J.P. Huston. Inst. of Psychology III, University of Düsseldorf, 4000 Düsseldorf, F.R.G.

The intent of this study was to search for structures in the diencephalon which might be critical for self-stimulation (SS) in the lateral hypothalamus (LH) of rats. Since the preoptic area (POA) is a major source of afferents to the descending medial forebrain bundle (MFB) and since previous studies in our laboratory have implicated this region in LH-SS, we examined the effects of unilateral radiofrequency lesions in the POA on LH-SS.

Fifteen rats were bilaterally implanted with bipolar stimulation electrodes in the LH and bipolar lesion electrodes in the POA. They were tested for SS in the LH at three different current intensities and then received a unilateral radiofrequency lesion in the POA. Four hours later they were again tested for SS at the three current intensities and then daily for 14 days or until they recovered their presurgical rates of SS. The lesion severely decreased rate of SS in the damaged hemisphere, whereas the rate of responding for SS in the intact hemisphere increased above the pre-lesion levels. In all but three animals rate of SS recovered to the pre-lesion level during a period from one to two weeks after the lesion. This lateralized decrement of SS after the lesion suggests that the POA might be involved in LH-SS. Neurotoxic lesions with kainic acid in the POA will confirm whether the neurons intrinsic to this structure are critical for maintaining SS in the LH.

The effects of D-amphetamine (1 mg/kg) and apomorphine (2 mg/kg) injections on turning behavior were also studied in an open field and in a rotometer. Apomorphine induced contraversive turning (away from the lesion side) in the open field, and D-amphetamine induced ipsiversive turning in the rotometer.

38.8 AUDITORY FEAR CONDITIONING IS MEDIATED BY PROJECTIONS FROM THE MEDIAL GENICULATE TO, NOT THROUGH, AN ARCHI-NEOSTRIATAL FIELD. J. Iwata, J.E. LeDoux, S. Arneric, M. Meeley, D.J. Reis. Lab. of Neurobiol. Cornell U. Med. Coll. New York, NY 10021

The medial geniculate (MG) in rat projects, in addition to auditory cortex, to a striatal field (STR) involving portions of the neostriatum (caudal caudate-putamen) and the underlying archistriatum (central and lateral nuclei of the amygdala) (LeDoux et al, this volume). Unilateral lesions of MG and the contralateral STR disrupt auditory fear conditioning, presumably by damaging intrinsic neurons or fibers passing through STR (LeDoux et al, ibid). To distinguish between these possibilities we have examined the effects on fear conditioning of STR lesions produced by a selective cellular toxin, ibotenic acid (IBO).

Rats were prepared with unilateral electrolytic lesions of MG. Contralaterally, IBO (0.4ul; 10ug/ul; n=6) was microinjected into STR (n=6) or the rostral caudate (n=4). Controls received unilateral MG lesion with vehicle injections in the contralateral STR (n=6) or were unoperated (n=6). After 10 days the animals were subjected to classical conditioning procedures involving the presentaion of a pure tone in association with footshock. The next day the duration of suppression of exploratory activity or drinking during a 120 sec exposure to the tone was measured.

Lesion of MG and the contralteral STR reduced the druation (in sec) of activity suppression (unoperated control: 100±15; lesion: 45±10; $p<.01$) and drink suppression (control: 100±15; IBO: 50±20; $p<.01$) during the tone. Vehicle injections or lesions placed anteriorly in the caudate had no effect. Histological evaluation revealed extensive neuronal loss and glial proliferation in the caudal caudate/amygdala or rostral caudate in IBO injected rats.

To confirm that IB0 destroyed intrinsic neurons and spared fibers of passage in STR, IBO was unilaterally microinjected into STR. After 5 days: (a) the brain was processed for HRP histochemistry following injection of WGA-HRP (10-15nl) into MG, which projects to and through STR (n=3); or (b) tissue was sampled from the lesioned and unlesioned STR and assayed for choline acetyl transferase (ChAT) or tyrosine hydroxylase (TH) activity, enzymes which are contained in neurons intrinsic to STR (ChAT) or in fibers projecting to STR from the substantia nigra (TH).

HRP labeled fibers from MG terminating in and passing through the lesion were preserved. Relative to the control side, ChAT activity was substantially reduced (33±1%, $p<.01$) but TH activity was not (90±1%, ns). Intrinsic neurons but not fibers passing through were thus destroyed by IBO injections in STR.

We conclude that auditory fear conditioning is mediated by projections from MG to intrinsic neurons of STR.

38.9 LESIONS OF THE AMYGDALA, BUT NOT OF THE CEREBELLUM OR RED NUCLEUS, BLOCK CONDITIONED FEAR AS MEASURED WITH THE POTENTIATED STARTLE PARADIGM. J.M. Mondlock* and M. Davis. Depts. of Psychology and Psychiatry, Yale University, Conn. Mental Health Ctr., 34 Park St., New Haven, CT 06508

Aversive conditioning can result in learning of fear and/or adaptive motor responses. It has been suggested that these two types of learning involve different neural substrates. Considerable evidence indicates that lesions of the amygdala impair fear conditioning, as measured by conditioned heart rate and conditioned emotional response paradigms. On the other hand, lesions of the cerebellum abolish learned motor responses, such as conditioned nictitating membrane and conditioned leg flexion responses, but do not affect heart rate conditioning, a measure of conditioned fear. Potentiated startle (increased acoustic startle in the presence of a light previously paired with shock) is another conditioning paradigm that seems to reflect fear, since it is sensitive to drugs that alter fear and anxiety in humans. It was hypothesized that lesions of the amygdala would block potentiated startle, since it is a measure of conditioned fear, but lesions of the cerebellum or red nucleus (which receives most of the cerebellar afferents) would have no effect on potentiated startle.

Rats were given 10 light-shock pairings on 2 successive days. At 24-48 hrs following training, groups of rats received either bilateral transection of the cerebellar peduncles, bilateral lesions of the red nucleus, or bilateral lesions of the central nucleus of the amygdala. Control animals were sham operated. At 3-4 days after surgery, the rats were tested for potentiated startle. Potentiated startle was blocked by the amygdala central nucleus lesion, but not by transection of the cerebellar peduncles or lesions of the red nucleus. A visual pre-pulse test indicated that the attenuation of potentiated startle observed in the amygdala lesioned animals could not be attributed to visual impairment. Future studies exploring the role of the amygdala in fear conditioning as measured with potentiated startle will investigate the effects of anxiolytic or anxiogenic drugs administered directly into the amygdala, and the anatomical pathway that mediates the involvement of the amygdala in potentiated startle.

LEARNING AND MEMORY: ANATOMY II

39.1 THE SUPERIOR CERVICAL GANGLIA AND LEARNING OF A SPATIAL-MEMORY TASK. L. E. Harrell and S. Barlow*. Department of Neurology, V.A. Medical Center and University of Alabama, Birmingham, AL 35294.

When the cholinergic projection from the medial septum to the hippocampal formation is interrupted, peripheral sympathetic nerves originating in the superior cervical ganglia (SCG) invade the hippocampal formation. Recently, we have demonstrated that prevention of sympathetic ingrowth results in enhancement of learning, suggesting a detrimental influence of peripheral sympathetic fibers on some appetitive spatial/memory task. To more fully elucidate the role of the peripheral sympathetic nervous system in learning and memory processes, we removed the superior cervical ganglia (SCGx) and studied acquisition, performance, and reversal learning of rats on a radial 8 arm maze.

Adult male Sprague-Dawley rats were trained to approach four baited arms and ignore four unbaited arms of an eight arm maze. Daily testing was performed and criterion of learning was defined as selection of four baited arms out of the first five arm choices on each of five consecutive days. Rats were assigned to either an acquisition (A) or performance (P) group. Group A rats underwent either SCGx or sham surgery prior to training, while rats in Group P were treated after mastery of the task. After attainment of either initial criterion (Group A) or after reaching a second criterion (Group P) all animals underwent a reversal procedure in which baited and unbaited arms were exchanged.

SCGx either prior to (Group A) or after mastery of the task (Group P) did not affect animal's ability to either learn or perform in this particular paradigm. Reversal performance was likewise unaffected.

Our data suggest that in normal animals, peripheral sympathetic fibers originating from the SCG do not play a major role in the initial learning or retention of an appetitive spatial/memory task. This suggests that the detrimental effects of these fibers on learning and memory processes observed following cholinergic denervation must be due to some direct effect or interaction within the hippocampal formation.

39.2 MEMORY FOR EGOCENTRIC SPATIAL LOCALIZATION IN AN ANIMAL MODEL OF ADVANCED HUNTINGTON'S DISEASE. D. G. Cook* and R. P. Kesner (SPON: M. E. Ellis). Dept. of Psychology, Univ. of Utah, Salt Lake City, Utah 84112.

A large body of evidence has shown that Huntington's Disease (HD) patients display a wide array of memory deficits. Even though neuronal loss in HD patients involves the caudate nucleus (CN), there is damage to other neural regions making it difficult to determine which loci might mediate specific cognitive dysfunctions.

In order to better understand the role of the caudate nucleus (CN) in the memory disorders of HD patients, rats were tested on an adjacent arm and an 8-arm radial maze task. In the adjacent arm task the animals were placed at the end of a randomly selected arm on an 8-arm radial maze and were reinforced for running to one of the adjacent arms. This task is presumed to emphasize the use of vestibular and kinesthetic feedback to solve the maze (egocentric localization). The second task was a standard 8-arm radial maze task where rats were allowed to find food at the end of each arm. In this task rats are presumed to rely on a cognitive map of the environment (allocentric localization). After training, animals received sham or bilateral electrolytic lesions of CN.

Results show that rats are impaired on the adjacent arm task but show no deficits on the 8-arm radial maze task. Sham lesioned controls showed no deficits on either task.

As a test of the generality of these findings new rats were tested on two different tasks: a Left-Right discrimination test between randomly paired adjacent arms (egocentric) and a place learning task where rats were started at the end of a randomly selected arm on a radial maze and were reinforced for running to a single arm whose position in space never varied (allocentric).

Results indicated that CN lesioned rats are markedly impaired on the Left-Right task but show only a transient deficit on the place learning task.

These data are consonant with the theory that the CN plays a critical modulatory role in the processing of egocentric spatial information. Since only a small component of memory deficits seen in HD patients might reflect a problem with egocentric localization, it appears that the CN plays an important but limited role in the memory dysfunctions of HD patients.

39.3 **SPATIAL BEHAVIOR IN THE RAT: THE ROLE OF THE NEOSTRIATUM.** K.E.Sabol, J.B.Richards, and D.B.Neill (SPON: J.Justice). Dept. of Psychology, Emory University, Atlanta, GA 30322.

Lesions of the neostriatum disrupt performance of spatial tasks such as delayed alternation (Divac et al., Br.Res., 1978,151: 523). This T-maze paradigm, however, does not distinguish between a response strategy (make alternate left and right turns using the body axis as reference) or a spatial strategy (go to alternate locations in the maze relative to specific environmental cues). More recently, 2 tasks have been devised which provide a better measure of spatial behavior in rats: the Olton radial arm maze and the Morris water task.

The purpose of the present experiment was to determine whether cell body lesions of the neostriatum would disrupt performance on either the radial arm maze or water task. We were further interested in determining whether two subregions of this structure, dorsal-medial (DM) and ventral-lateral (VL), were differentially involved in spatial behavior.

After 10 days of training in both tasks, rats were given intracranial injections of quinolinic acid (24ug/2ul) or vehicle (PBS, pH=7.4) into DM or VL striatum. One group of animals was tested immediately after surgery and another group was given a 3 week recovery period. In both cases, post-lesion testing lasted for 10 days.

When testing was conducted either immediately after surgery, or after a 3-week delay, DM lesions disrupted performance in both the maze and the water task. VL lesions resulted in disruption of the water task only when there was no delay between lesion and testing. VL lesions disrupted maze performance only when the 3 week delay was imposed between lesion and testing. Histological analysis showed that the VL injections resulted in cell body loss in the ventral 2/3 of the striatum. The DM lesions were more localized, resulting in cell loss in the upper 1/2 of the striatum. In conjunction with the behavioral result, this indicates that the involvement of neostriatum in spatial behaviors is better associated with the DM compared to the VL striatum. In all animals, performance improved during the second 5-days of the post-lesion test period. This indicates that the DM striatum cannot be said to have a critical role in the spatial behavior of rats.

39.4 A FUNCTIONAL ANATOMICAL STUDY OF NEURAL PATHWAYS INVOLVED IN MEMORY MECHANISMS USING ^{14}C-2-DEOXY-D-GLUCOSE : EFFECTS OF ENTORHINAL CORTEX ELECTRICAL STIMULATION. C. Destrade, J. Sif, M. Gauthier and A. Calas (SPON : D. Beaubaton).CNRS Lab. Neurobiologie : Médiateurs et Comportement, Université de Bordeaux I, 33405 Talence Cedex France.

Previous behavioral results using posttrial electrical stimulation of the brain suggested that the entorhinal cortex (EC) is involved in mnemonic processes (Gauthier et al. Brain Res., 233:255, 1982). In order to characterize functionally the neural pathways activated by EC stimulation, patterns of uptake of ^{14}C-2-deoxy-D-glucose (2-DG) were assessed following stimulation of the lateral (LEC) or medial (MEC) EC in intact animals and after lesion of the perforant path (P-P).

Male BALB/c mice were implanted with a bipolar electrode in the LEC or MEC and a polyethylene catheter in the jugular vein; in addition, 4 animals received an electrolytic lesion of the P-P ipsilaterally to the stimulated side. The EC was stimulated (at subconvulsive intensity) for 5 min before and 30 min following an injection of 2-DG. Stimulation of the EC produced significant increases in 2-DG radioactivity in the hippocampus (dentate gyrus and CA3); however LEC stimulation produced greater labeling of presubiculum than did MEC stimulation. Demonstrable labeling was found in brain areas beyond the hippocampal formation after both LEC and MEC stimulation : piriform cortex and amygdala; moreover, higher densities of labeling were seen within the cingulate cortex (retrosplenial area);less dense but significant increases were noted in the Diagonal Band of Broca, the medial and lateral septal nuclei and the medial forebrain bundle. Finally, the colliculus posterior (ipsilateral following LEC stim; bilateral with MEC stim) also showed evidence of metabolic activation but we were unable to determine the activated pathway. After P-P lesion, the metabolic activity disappeared ipsilaterally in subiculum, hippocampus and in some thalamic nuclei : lateral nucleus and lateral geniculate nucleus, but all extra-hippocampal labeling was unchanged. These data, considered along with our previous behavioral results, suggest that EC stimulation may act on mnemonic processes by the recruitement of extra-hippocampal structures (particularly the cingulate area) directly or indirectly connected with the entorhinal cortex.

39.5 SPATIAL LOCALIZATION DEFICITS AFTER INTRAHIPPOCAMPAL INJECTIONS OF NEUROTOXINS: ON THE CONTRIBUTIONS OF LOCAL AND DISTANT DAMAGE. R. J. Sutherland, Dept. of Psychology, The University of Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4.

Evidence from unit recording, behaviour after ablations, and behavioural disruption produced by electrical stimulation suggests that hippocampal (HPC) circuitry plays a central role in spatial mapping. In previous work with neurotoxins which preferentially destroy HPC granule cells or pyramidal cells we found qualitatively similar deficits in spatial mapping in the Morris water task. IntraHPC injections of kainic acid produce an extensive loss of neurons from CA3-CA4, in addition, there is a loss of neurons in distant structures. This study determines whether the deficit in the Morris water task is correlated with the degree of HPC or non-HPC damage.

Following pre-operative training in the Morris water task rats received: 1. sham surgery, or 2. intraHPC kainic acid (0.1 µg/0.5 µl per site), or 3. intraHPC colchicine (2 µg/0.5 µl per site), or 4. multiple HPC electrolytic lesions, or 5. kainic acid injections with diazepam pretreatment, or 6. bilateral electrolytic lesions in claustrum, neocortex in the dorsal bank of the rhinal fissure, amygdala, pyriform cortex, and midline thalamus, and aspiration of cortex overlying the HPC (each structure shows cell loss after intraHPC kainic acid). After a 7-10 day post-operative interval all rats were retested in the Morris water task.

All groups with HPC damage were deficient in time taken to reach the hidden platform, directionality of the swim path, length of the swim path and preference for the sector of the pool containing the platform. Histological reconstruction of the HPC damage using cresyl violet, Timm's and dithizonate stains indicated that the magnitude of the navigational deficit was proportional to the loss of granule cells or pyramidal cells. Lesions in claustrum, neocortex surrounding the HPC and in the dorsal bank of the rhinal fissure, amygdala, pyriform cortex, and midline thalamus did not affect place navigation.

Although these results cannot establish the relative importance of individual components of the HPC system for spatial processes, nor do they address non-spatial consequences of "distant" damage from neurotoxin injections, they do emphasize the importance of transmission through the HPC trisynaptic route for normal place navigation.

39.6 MULTIGENERATIONAL EFFECTS OF MILD, PERINATAL ZINC DEFICIENCY ON BEHAVIOR AND HIPPOCAMPAL MORPHOLOGY IN ADULT RATS. E.S. Halas and C.D. Hunt*. USDA, ARS, Grand Forks Human Nutrition Research Center & Dept. of Psychology, University of North Dakota, Grand Forks, ND 58202.

It was hypothesized that a mild zinc deficiency (ZD) induced during gestation and lactation would permanently impair brain function and behavior. Furthermore, the mild ZD may adversely affect development and function of various organ systems such that zinc metabolism is permanently impaired. Thus, subsequent generations raised under similar conditions may exhibit a cumulative, ZD induced injury.

Three generations of rats were fed a mildly ZD diet (10.0 µg Zn/g diet) during gestation and lactation. One group of 10 dams (ZD) was fed the diet starting on the first day of pregnancy and continued until their pups were weaned at 23 days. This group was given distilled, deionized drinking water. A second group of 10 dams (PF) was given the same quantity of the diet as consumed by their ZD mates. A third group of 10 dams (AL) was fed the diet ad libitum. The PF and AL were given zinc supplemented water (25.0 µg Zn/ml). After weaning, all pups (F_1) were fed Purina Chow ad libitum. At 100 days of age, they were reduced to 85% of their normal weight and trained on a 17-arm radial maze. ZD rats were significantly inferior in learning and short-term memory when compared to PF and AL rats. PF rats suffered some learning deficits. Long-term memory was not impaired. The female offspring were bred for F_2 and then F_3 generation studies. The dietary treatment was constant for all generations. Analysis of data showed that the behavior deficits were the same for the F_1 and F_2 generations. At 300 days, following behavior experiments, 21, F_2 generation, female rats (8, ZD; 8, PF; 5, AL) were selected for histological analysis. All rats were perfused with a buffered aldehyde solution. The right hippocampi were embedded in Epon/Araldite, sectioned at 3 µm in a horizontal plane that intersected with the anterior commissure and the anterior tip of the cerebral aqueduct, and stained with toluidine blue. Mild ZD affected the hippocampi of F_1 and F_2 generation rats in a similar fashion. ZD increased the incidence of vacuolated and dark cells in the endal and ectal limbs of the dentate gyrus respectively. The behavioral and anatomical data for the F_3 generation are currently being collected and analyzed.

39.7 **FETAL BRAIN LESIONS CAN INDUCE DIFFERENTIAL IMPAIRMENTS IN THE ACQUISITION OF MEMORY TASKS IN RATS.** J. Pevsner*, Dept. of Neurosci., Johns Hopkins School of Med., Baltimore, MD, 21205, M.L. Shapiro, Dept. of Psychol., Johns Hopkins Univ., Baltimore, MD, 21218, P.R. Sanberg, Beh. Neurosci. Lab., Dept. Psychol., Ohio Univ., Athens, OH, 45701, J.T. Coyle (SPON: R.Gould) Depts. of Psychiatry & Neurosci., Johns Hopkins School of Med., Baltimore, MD, 21205.

Different memory impairments result from lesions of different neural systems. The present experiment examined the effects of in utero treatment with the mitotic inhibitor methylazoxymethanol (MAM) on performance in memory tasks. On day 15 of gestation, pregnant rats were given a single i.p. injection of saline vehicle or MAM (20 mg/kg). MAM causes selective destruction of cells undergoing mitosis, and when given on day 15 induces lesions in cortex and hippocampus, but not subcortical structures (e.g. cerebellum) (Johnston, M.V. & Coyle, J.T., TINS, 5:153, 1982).

Male and female rats (n=40) treated in utero with saline or MAM were tested in adulthood (8-30 weeks of age) on a T-maze which included two memory tasks. One was a left/right discrimination on the stem of a T-maze which tested reference memory, and the other was a discrete-trial, rewarded alternation discrimination on the arms of the maze which tested working memory.

Saline treated rats of both sexes acquired the reference and working memory tasks rapidly. MAM treated rats were impaired in the acquisition of both the reference ($p < .05$) and working ($p < .01$) memory tasks relative to those given saline. MAM treated rats of both sexes were equally impaired in the acquisition of the reference memory task compared to the saline treated rats. Female MAM treated rats were also impaired in the acquisition of the working memory task ($p < .01$). Male MAM treated rats acquired the working, but not the reference memory task, almost as quickly as saline treated rats.

These results, together with those showing that lesions of the hippocampal system impair performance in working and not reference memory tasks provide evidence that these processes can be doubly dissociated. Hence these processes may require different neural systems. **(Supported by MH26654 Pratt HD grant, OURC, Tourette Syndrome Assn, and NIMH P316123.)**

39.8 DISSOCIATION OF EXPECTANCY-BASED AND DATA-BASED INFORMATION PROCESSING OF A LIST LEARNING TASK FOLLOWING PARTIAL HIPPOCAMPUS LESIONS. J. A. Laylander,* D. R. Beers,* R. P. Kesner, Dept. of Psychology, Univ. of Utah, Salt Lake City, Utah 84112.

It has been suggested that the hippocampus codes temporal-spatial attributes of a memory requiring data-based information processing in tasks where critical information is variable from trial to trial (working memory). However, the hippocampus does not code temporal-spatial attributes of a memory requiring expectancy-based information processing in tasks where critical information is invariant from trial to trial (reference memory). To test this possible differential role of the hippocampus in processing of data- rather than expectancy-based aspects of spatio-temporal information, a single task was developed in which both processes can be measured.

Animals with electrolytic partial dorsal hippocampus or sham lesions were trained on a constant 5-arm sequence followed by a variable multiple discrimination test on an 8-arm radial maze. On each trial each animal is presented with an unchanging 5-arm constant sequence of doors (study phase). The memory measure for the exact temporal-spatial sequence is noted by measuring an anticipatory response in front of the door appropriate for the sequence. After the study phase each animal is tested for discrimination between a sequence arm that contains food and a non-sequence arm that does not contain food using a win-stay rule. For each trial two arms are pseudo-randomly selected for the test; one each from the non-sequence and sequence arms. After 50 trials (one trial per day) the results indicated that both dorsal hippocampus lesioned and sham-operated animals make the same pattern and frequency of anticipatory responses. In contrast, dorsal hippocampus lesioned animals show marked deficits in performance of the variable multiple discrimination test, while sham-operated animals are near perfect in test performance. Also, when dorsal hippocampus lesioned animals made appropriate anticipatory responses to arms that were subsequently selected for a discrimination test, errors were made on at least one third of the tests. No such errors ever occurred for sham-operated animals. These results suggest that there is a dissociation between expectancy-based and data-based information processing and that the hippocampus primarily mediates data-based information processing.

39.9 EFFECTS OF MAMMILLARY BODIES LESIONS ON SPONTANEOUS ALTERNATION IN DISCRETE AND SEQUENTIAL TEST PROCEDURES: EVIDENCE FOR INCREASED VULNERABILITY TO INTERFERENCE. R. Jaffard and D. Beracochea (SPON: F. Clarac) Lab. Psychophysiologie, Université Bordeaux I, 33405 Talence Cedex France.

Spontaneous alternation (S.A.) of male mice of the BALB/c strain was examined under various conditions, namely (i) intertrial intervals (ITIs) of 30 sec and 2 min; (ii) discrete vs. sequential test procedures, respectively consisting of either only one alternation test (two trials) or six succesive trials within a session. The results showed that while there were no significant differences between S.A. rates at 30 sec and 2 min with the discrete test procedure, sequential testing with an ITI of 2 min, but not 30 sec, resulted in a progressive decay of S.A. rates as the number of trials increased. This phenomenon was interpreted as resulting from a gradual build-up of interference accrued over successive trials. Accordingly, the same procedures were used to investigate whether, as postulated for Korsakoff patients, mammillary body (MM) lesions would produce an increased vulnerability to such interference. Thus, MM-lesioned mice (kainic acid: 0.5 µg/2 µl and electrolytic lesions) were compared to sham-operated animals on discrete and sequential tests with an ITI of 30 sec. There were no differences in S.A. rate in the discrete test procedure (pooled lesioned: 77.5%; controls: 76.9%) while repetition of trials progressively impaired S.A. in lesioned (from the 2nd to the 6th trial: 77.5%; 69.7%; 64.1%; 49.4% and 55.6%) but not in control subjects (all rates above 75.4%). In the 3rd experiment we tried to reverse the observed impairment by adding an intramaze cue on the 5th trial. Such cueing resulted in a dramatic improvement of S.A. rate on this (5th) trial (from 49.1 to 80.6%, $p < 0.001$) but additional trials with the cue (5th to 8th) resulted once again in a decrease in S.A. rates (from 80.9 to 62.2%) which was again reversed by removing the cardboard on trial 9 (81.8%). Our results resemble those reported for Korsakoff patients who exhibit an increased vulnerability to proactive interference which can be partially reversed by cueing (Winocur and Kinsbourne, Neuropsychologia, 1978, 16:671-682).

The poster will also compare these results with others we have obtained in the 8-arm radial maze after MM lesions.

39.10 LESIONS IN THE NUCLEUS BASALIS MAGNOCELLULARIS AND MEDIAL SEPTAL AREA OF RATS IMPAIR SPATIAL WORKING MEMORY IN A T-MAZE TASK. D. Hepler, D. Olton, and G. Wenk, Dept. of Psychology, The Johns Hopkins Univ., Baltimore, MD 21218; J. Coyle, Dept. of Psychiatry and Behavioral Science, The Johns Hopkins Sch. of Med., Baltimore, MD 21205.

The functional contribution of the nucleus basalis magnocellularis (NBM) and medial septal area (MSA) to memory was evaluated in two different spatial discriminations. Preoperatively, rats were trained to a criterion level of performance in a simultaneous left/right discrimination on the stem of a T-maze, and a discrete-trial, rewarded alternation (win-shift) discrimination on the arms. Each rat then received a bilateral lesion in either the NBM or MSA made by the the microinfusion of ibotenic acid (IBO) or the application of radiofrequency current (RF). An additional group of rats received IBO lesions in both the NBM and MSA. Control rats received operations in which no current was passed or neurotoxin injected. Lesions in the NBM decreased choline acetyltransferase levels (ChAT) in frontal cortex but not hippocampus, while lesions in the MSA decreased ChAT in hippocampus but not in frontal cortex. Lesions in both the NBM and MSA decreased ChAT in frontal cortex and hippocampus. Lesion size and location was assessed in nissl-stained histological material. Rats in all three lesion groups performed similarly in three behavioral tasks relative to controls: postoperative reacquisition of the arm discrimination was impaired, while postoperative reacquisition and reversal of the stem discrimination were not impaired. The MSA lesion groups had a greater impairment than the NBM lesion groups in the arm discrimination. The NBM-IBO and NBM-RF groups had quantitatively similar behavioral impairments, while the MSA-RF group had a greater impairment than the MSA-IBO group. The NBM-MSA group had behavioral impairments similar to those of the MSA-RF group. All rats eventually reached normal criterion performance. These results may have implications for memory disorders associated with pathological changes in the cholinergic systems. (Supported by grant NS184 from NINCDS and by grant DAMD 17-C-8225.)

39.11 MEDIAL SEPTUM AND NUCLEUS BASALIS MAGNOCELLULARIS LESIONS PRODUCE ORDER MEMORY DEFICITS IN RATS MODELING ALZHEIMER'S DISEASE. M. O. Measom,* M. G. Robinson,* R. P. Kesner, and K. A. Crutcher, Departments of Psychology and Anatomy, Univ. of Utah, Salt Lake City, Utah 84112.

Patients with Alzheimer's disease have a cell loss within medial septum (MS) and nucleus basalis magnocellularis (NBM) and a subsequent depletion of their cholinergic projection systems (e.g., hippocampus and cerebral cortex). In the early stages of Alzheimer's disease, these patients have poor memory for early items and a somewhat reduced memory for late items of a list. In the later stages of Alzheimer's disease, there is a deficit for all items of a list. In order to test whether comparable deficits can be found in animals following damage to MS and NBM, rats were tested for order memory for a list of items (places on a maze).

Rats were trained on an eight arm radial maze using Froot Loop reinforcement. After extensive training each animal was allowed on each trial (one per day) to visit all eight arms in an order that was randomly selected for that trial. Thirty seconds after the animal had received reinforcement from the last of the eight arms, the test phase began. Only one test was given for each trial and consisted of opening two doors simultaneously. On a random basis, either the first vs the second, fourth vs the fifth, or seventh vs the eighth doors that occurred in the sequence were selected for testing. The rule to be learned was to choose the arm that occurred earlier in the sequence. After a large number of tests, all rats displayed excellent order memory for the early and late items within the list.

Animals then received electrolytic lesions of MS or ibotenic acid lesions of NBM. Following recovery from surgery all animals were retested on the order memory task. Results indicated that animals with MS lesions and partial depletion of dorsal hippocampal AchE had an order memory deficit for the early items, but not the late items of the list. Animals that had NBM lesions with extensive bilateral cortical depetion of AchE and animals that had MS lesions with complete depletion of dorsal hippocampal AchE showed deficits for all items of the list. Animals that had NBM lesions with unilateral cortical depletion of AchE showed no clear deficits. These data are remarkably similar to memory performance deficits of Alzheimer's patients with presumed MS and/or NBM damage.

39.12 POSTERIOR PARIETAL CORTEX: A PART OF THE COGNITIVE MAP? B. V. DiMattia* and R. P. Kesner (SPON: B. I. Grosser), Dept. of Psychology, Univ. of Utah, Salt Lake City, Utah 84112.

The most salient symptoms exhibited by humans with damage to the posterior parietal cortex (PPC) are spatial disorientation and topographical amnesia, which may involve the loss of long-term geographic knowledge, as well as the loss in the ability to learn new spatial environments.

Even though there are such spatial deficits in PPC damaged humans, little emphasis has been placed on the possibility that PPC might play a role in "cognitive mapping." Rather, it is the hippocampus (HC) that has been hypothesized to be the neural substrate of the cognitive map (O'Keefe & Nadel, The hippocampus as the cognitive map, 1978).

Support for this hypothesis has been provided by the demonstration that rats with HC lesions are impaired on certain "cognitive mapping" spatial tasks. One such task is the Morris milk tank task (Morris et al., Nature, 1982, 297, 681-683). In this task the rat must learn the fixed location of a submerged (hidden) platform in a circular tank of milky water. From trial to trial the rats are placed into the pool at varying locations. Normal rats learn to escape from the water very quickly, so that, by 10-15 trials, they can find the platform within a few seconds, usually with very little navigational heading error. HC rats, however, are impaired in this task in that they show a longer latency to find the platform and are less accurate in their initial swim heading.

In the present study separate groups of hippocampus and posterior parietal lesioned rats were compared to sham-operated and unoperated controls in acquisition of the milk tank task. The results indicated that in comparison to the control groups both lesioned groups were significantly impaired in latency to find the platform. Importantly, however, the PPC lesioned animals were significantly more impaired than the HC lesioned animals. Comparable deficits were also found on other performance measures such as swim distance and initial swim heading errors. In addition, when the location of the platform was changed the animals in both lesioned groups were no more likely to swim in the previously correct quadrant of the tank than in any other quadrant.

We suggest the possibility that PPC is an integral part of the cognitive map substrate, perhaps interacting with HC.

39.13 FURTHER EXAMINATION OF LEARNING AND RETENTION OF VISUAL DISCRIMINATIONS WITH ANTERIOR TEMPORAL LOBE COOLING. Mary Lou Voytko, Dept. of Anatomy, Upstate Medical Center, Syracuse NY 13210.

Learning, but not retention, of object discriminations is severely disrupted by temporal pole cooling in monkeys (Behav. Neurosci.,98:310,1984). The present investigation examined further the ability of monkeys to learn and retain object discriminations under various combinations of cooling and noncooling of the anterior temporal lobe. Four chronic cooling probes were bilaterally placed on the dura overlying the anterior temporal lobe. Two of the probes covered the temporal pole and the anterior extreme of the inferotemporal cortex (anterior probes) and the other two probes covered middle inferotemporal cortex (posterior probes). During the cooling trials the temperature of the probes was set at 0°C. The subjects faced 3 rear projection screens. A white light projected to the center screen started a trial, and a response to it extinguished the light and exposed the discriminative stimuli on the two side screens. Randomly paired photographs of objects were used as stimuli and the correct stimulus alternated sides randomly. Four combinations of learning and retention of object discriminations with and without the cold were then examined: I. learning a discrimination without the cold and testing its retention without the cold,II. learning a discrimination without the cold and testing its retention with the cold,III. learning a discrimination with the cold and testing its retention without the cold,IV. learning a discrimination with the cold and testing its retention with the cold. The animals were allowed a maximum of 1000 trials to learn each discrimination and retention was tested for 100 trials 48 hours after learning criterion was reached. Cooling the anterior set of probes produced a retardation in learning,however the animals were able to learn the discriminations. Posterior probe cooling had very little effect upon learning. Retention was not severely affected in the learning-retention combinations I to III; however there was a slight retention deficit in IV. Thus in agreement with our previous findings, anterior temporal lobe cooling was found to affect the learning of object discriminations but not the retention of discriminations learned prior to cooling. In addition it was found that despite these learning deficits the animals are capable of learning object discriminations and that once learned they are retained fairly well under noncooling conditions, but less so if retention is tested with the cold. (Supported by NINCDS NS18291).

39.14 FURTHER EVIDENCE OF A SEVERE IMPAIRMENT IN ASSOCIATIVE MEMORY FOLLOWING COMBINED AMYGDALO-HIPPOCAMPAL LESIONS IN MONKEYS. R. R. Phillips*, and M. Mishkin. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20205.

Combined damage to the amygdaloid complex and hippocampal formation in monkeys was previously found to produce a seemingly permanent loss of one-trial associative memory on each of two tasks. Both tasks, however, involved a difficult conditional reaction. In the acquisition phase of one (Spiegler and Mishkin, Soc. Neurosci. Abst. 5:323, 1979), two novel objects were presented successively with or without bait in random order. In the test phase, each object was paired separately with a constant alternative (gray card). Animals were required to choose the object if it had been baited in acquisition, but to choose the constant alternative if not. Because of its ambiguous reward value, the constant alternative may have contributed substantially to the impairment (Jones and Mishkin, Exp. Neurol. 36:362-377, 1972). The second task (Malamut and Mishkin, unpublished) was identical to the first except that the constant alternative was replaced by a novel alternative (i.e., in the test phase, a totally new object was used for each pairing). In this case, on half of the trials, the monkeys were required to make a choice contrary to their natural tendency to explore unfamiliar objects (Mishkin and Delacour, J. of Exp. Psycnol. 1:326-334, 1975). To eliminate all such complicating factors from the present study, we used a nonconditional stimulus-reward association task designed by Gaffan (Learn. and Motiv. 10:419-444, 1979).

In acquisition, as before, two novel objects were presented successively, one with bait and the other without in random order. On the test, however, the two objects were paired with each other, and the monkeys were required to choose the previously baited object. Postoperatively, animals with combined amygdalo-hippocampal lesions failed to regain criterion within 1000 trials. This impairment is as severe as that found in the studies cited above and far more severe than that of animals with amygdalectomy alone (300 trials to criterion) or hippocampectomy alone (10 trials to criterion) which were tested previously on the task used here (Phillips and Mishkin, Soc. Neurosci. Abstr. 9:638, 1983).

The results indicate that, as with one-trial recognition memory (Mishkin, Nature 273:297-298, 1978), (a) one-trial associative memory is dependent upon the medial temporal limbic region, and (b) combined damage to the amygdala and hippocampus is neccessary to show this.

39.15 VISUAL RECOGNITION IN INFANT RHESUS MONKEYS: EVIDENCE FOR A PRIMITIVE MEMORY PROCESS. M. Brickson* and J. Bachevalier. Lab. of Neuropsychology, NIMH, Bethesda, MD. 20205.

The development of visual recognition memory in infant monkeys was traced with a preferential looking task (Fagan, Child Develop., 48:68, 1977) which measures how fixation time is distributed between a familiar and a novel visual stimulus. The task was also used to assess the effects of amygdalo-hippocampal (AH) damage, which is known to affect visual recognition memory as measured by traditional problem-solving tests (Mishkin, Nature, 273:297, 1978).

Four infant and 3 adult rhesus monkeys served as unoperated controls, and 3 infants and 3 adults received bilateral AH lesions. The infants received their bilateral ablations in two stages, at 7 and 21 days of age, while the adults sustained a one-stage bilateral ablation. Performance on the preferential looking task was assessed in all infants at the age of 5, 15, and 30 days and in all adults at the age of 3-4 years. A set of 10 trials, each with new objects, was presented as follows. On each trial, 2 identical objects were first shown simultaneously for a 30 sec familiarization period; then, after a 10-sec delay during which the monkey's view of the objects was blocked, one object of the pair was presented with a novel object in two successive recognition tests of 5 sec each with a 10-sec intertest interval. On these tests the left-right positions of the two objects were interchanged. The 10 trials were presented successively at 20 sec intervals, and, for each trial, percent of fixation time (PFT) spent on the novel object was calculated. Separate sets of objects were shown to the infants at 5, 15, and 30 days of age, while only the first set was shown to the adults.

The results indicated that normal infants of 15 and 30 days of age, like normal adults, spent more time fixating the novel stimuli (mean PFT: 63%, 72%, and 83%, respectively). By contrast, 5-day-old normal infants did not (PFT: 47%). There was also an absence of preference for novelty in 15-day-old infants with unilateral AH lesions, 30-day-old infants with bilateral AH lesions, and adult monkeys with bilateral AH lesions (PFT: 43%, 55%, and 58%, respectively).

These findings indicate that visual recognition is a primitive memory process, which is present in rhesus monkeys by at least 15 days of age. Furthermore, the development of this process appears to depend on the integrity of limbic structures.

39.16 SOURCES OF TELENCEPHALIC INPUTS TO THE MEDIODORSAL NUCLEUS OF THE THALAMUS (MD) IN THE MONKEY. F.T. Russchen* and J.L. Price. Dept. of Anatomy & Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Since the 1960's it has been known that the medial part of MD receives inputs from the basal forebrain, especially the olfactory cortex and amygdala, and is interconnected with orbital and medial parts of the prefrontal cortex. More recently, observations in both human patients and monkeys have suggested that lesions of the medial thalamus, including MD, produce deficits in declarative memory and learning tasks, similar to those produced by lesions of the limbic structures in the medial temporal lobe. As part of an attempt to define the relation between MD and the basal forebrain more fully, we will report here on the distribution of cells in the telencephalon which project to MD.

In addition to the cells in layer VI of the prefrontal cortex, injections of retrograde tracers involving the medial, magnocellular part of MD label cells scattered throughout a wide range of structures in the basal forebrain (the nucleus of the diagonal band, the nucleus basalis of Meynert, the ventral pallidum, the olfactory cortex and the amygdala) and in adjacent parts of the temporal lobe (the entorhinal and perirhinal cortices, the rostral temporal cortical areas, and to a lesser degree the hippocampal formation). The cells in the region of the diagonal band and nucleus basalis do not double label with acetylcholinesterase, and presumably do not provide a cholinergic input to MD. In all of these areas, the density of labeled cells is remarkably small (especially as compared with layer VI of the prefrontal cortex) and the cells are diffusely distributed (often along fiber pathways). The cells are relatively large, multipolar neurons, which appear to have long radiating dendrites.

This wide distribution and sparse density of the cells which project to the medial part of MD suggest that they are not relaying "specific" sensory or other information to the thalamus. An alternative possibility is that the projection may be "alerting" MD to activity in the basal forebrain. Both the amygdala and the olfactory cortex, as well as the rostral temporal cortex, have direct projections to the prefrontal cortex, which might provide more specific information by a non-thalamic route. Interactions between MD and the prefrontal cortex might then be essential for the process of committing that information to memory.

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39.17 EVIDENCE FOR MEMORY SPECIALIZATION WITHIN THE MESIAL TEMPORAL LOBE IN MAN. R.Rausch* and T.L. Babb (Spon: M.Nuwer). Depts. of Psychiatry and Neurology. Univ. of Calif., Los Angeles, CA 90024.

Ten epileptic patients with focal, left temporal lobe seizures were examined with a battery of memory tests prior to surgical treatment of their epilepsy. Their epileptic condition results in varying degrees of verbal memory deficits, and have as their pathological substrate varying degrees of selective loss of neurons in the temporal lobe (Babb, T.L., et al., Epilepsia, in press). Light-microscopic analyses of the resected language-dominant temporal lobe were related to preoperative memory functioning. Volumetric cell counts of different anatomical fields within the left mesial temporal lobe disproportionately related to scores on two types of verbal scores. The CA1 subregion of the archicortex positively correlated more with the learning of rote verbal associations, while a periallocortical region , the multilayered gyrus hippocampi, correlated more with learning semantically complex information. The cell counts of one area, the subiculum, correlated negatively with selective verbal scores. Nonverbal memory scores did not relate to cell densitites of the left temporal lobe indicating the specificity of these findings. These data strongly indicate memory differentiation within mesial temporal lobe structures.

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NO ABSTRACT

41 SYMPOSIUM. AUTORECEPTORS AND MODULATION OF NEUROTRANSMITTER RELEASE. L.X.Cubeddu, Univ. of North Carolina, and M.L. Dubocovich, Northwestern Univ. Med. Sch. (Co-chairmen); N. Weiner, Univ. of Colorado; J. Tepper*, Univ. of California, San Diego; M.J.Bannon, Yale Univ.

This symposium will focus on the modulation of neurotransmitter release through activation of autoreceptors and presynaptic receptors located on nerve terminals and soma-dendritic region of peripheral and central nervous system neurons. We will discuss the possible physiological role of these receptors in modulating the release of norepinephrine from postganglionic sympathetic nerve terminals and the release and activity of dopaminergic and cholinergic neurons of brain and retina, as studied using pharmacological, biochemical and electrophysiological techniques.

L. Cubeddu will briefly introduce the concept and history of modulation of transmitter release by presynaptic receptors. N. Weiner will focus on the role of alpha-2 adrenoceptors in modulating norepinephrine and dopamine-beta-hydroxylase release and in regulating tyrosine hydroxylase activity. L. Cubeddu will present evidence of modulation of dopamine and acetylcholine release from corpus striatal slices through activation of dopamine and muscarinic receptors. He will discuss how the frequency of stimulation determines drug efficacy on presynaptic receptors. The consequences of chronic treatment with neuroleptics and dopamine agonists on dopamine autoreceptor sensitivity will also be discussed. The modulation of dopamine release from the mammalian retina through activation of dopamine D-2 autoreceptors and alpha-2 presynaptic receptors will be discussed by M. Dubocovich. She will emphasize the role of the potent inhibitor of dopamine release in retina, melatonin and related indoleamines in modulating dopamine release in vitro and in regulating dopaminergic retinal activity in vivo. J. Tepper will discuss the electrophysiological consequences of activation and blockade of autoreceptors in vivo. He will present evidence suggesting that the level of autoinhibition increases as a function of firing rate and that the receptors involved in changes in excitability in the nigrostriatal dopaminergic neuron are, in fact, located on the afferent terminal region of the neuron. M. Bannon will discuss the regional distribution of dopamine autoreceptors and the autoreceptor function and sensitivity following pharmacological treatments, as assessed in vivo using biochemical and electrophysiological techniques. M. Dubocovich will summarize the symposium in order to open a final general discussion.

PROCESS OUTGROWTH AND GUIDANCE MECHANISMS II

42.1 AXONAL GUIDANCE IN CAENORHABDITIS ELEGANS STUDIED WITH ANTIBODIES TO HORSERADISH PEROXIDASE. Shahid S. Siddiqui* and J. G. Culotti* (SPON: V. M. CARR). Dept. of Biochem., Molecular Biol. and Cell Biol, & Neurobiol. and Physiol., Northwestern Univ., Evanston, IL. 60201

Antibodies to neural components could reveal the morphology of identified neurons at the level of light microscopy. Such probes may aid in the characterization of nerve growth abnormalities in mutants of the nematode Caenorhabditis elegans, since the structure and wiring of all of its 302 neurons is completely known(J.G. White, unpublished). Using indirect immunofluorescence we have earlier shown that affinity purified antibodies raised against horseradish peroxidase(HRP Abs) label specific neurons and sensory support cells in C. elegans. Mutations in 5 unc (uncoordinated) genes which result in foreshortened PHA and PHB axons as determined by FITC uptake(E.Hedgecock,N.Thomson,J. Culotti & L.Perkins,unpublished) were analyzed immunocytochemically for their effect on the growth of nerve processes through the pre-anal ganglion(PAG). Mutants of genes, unc-33 IV, unc-44 IV, unc-51 V, unc-76 V, and unc-(ev411) V, when labeled with anti-HRP Abs show that while PHA and PHB axons are bolcked in their growth through the PAG, other neuron types are not. These data show that the short axon phenotype of some neurons in these mutants is not due to a non specific block of neurite growth within the PAG. This precludes certain interpretations concerning the nature of these defects, e.g. the mutations do not result in some kind of a physical barrier to axon growth within the PAG per se, nor are they alterations in the overall organization of the PAG which might block the growth of all axons through the ganglion. Besides the premature termination of PHA and PHB axons in mutants of unc-44 and unc-51 staining with anti-HRP Abs revealed that in a fraction of these animals nerve fibers abnormally emanate from the lumbar ganglia, fail to reach the ventral nerve cord and instead run in various lateral positions. Therefore, although the PHA and PHB axons show normal growth to the posterior end of the PAG, other neurons in the lumbar ganglia are misguided in these mutants. Moreover, in some unc-51 (e369) animals, nerve fibers could be seen to exit from the ventral cord, wander aimlessly, and terminate randomly. These results are consistent with the finding that the post-deirid neuron (PDE) in unc-51 mutants can also show guidance defects (E. Hedgecock, J. Culotti and Liz Perkins, unpublished results).

42.2 MUTANT SENSORY AXONS IN CAENORHABDITIS ELEGANS. Joe Culotti*, Ed Hedgecock*, and Liz Perkins*. (SPON. B.P.M. Menco) Dept. of Biochem., Molec. & Cell Biology, Northwestern Univ., Evanston, IL 60201 and Roche Institute of Molecular Biology, Nutley, NJ 07110

We have identified mutations in 9 unc (uncoordinated) genes that lead to foreshortened or misguided PHA and PHB axons in C. elegans. PHA and PHB are bilaterally symmetric pairs of sensory neurons with cell bodies in the right and left lumbar ganglia of the tail which are able to concentrate fluorescein dyes. They have dendrites that end in the phasmid sensilla and axons that extend forward into the pre-anal ganglion at the posterior end of the ventral nerve cord. In mutants unc(ev404)I, unc(ev416)I, unc(ev411)V, unc-33 IV, unc-44 IV, unc-51 V, and unc-76 V the phasmid axons accurately find the ventral nerve cord but terminate prematurely, stopping just as they enter the posterior end of the pre-anal ganglion. The terminals of these neurons appear to be abnormally enlarged. In two mutants unc(ev400)X and unc(ev410)I the phasmid axons frequently fail to reach the ventral nerve cord and instead run in various lateral positions. In cases where the phasmid axons do reach the ventral nerve cord, however, the axons appear to grow forward into the pre-anal ganglion normally. Thus, the two classes of mutants appear complementary and divide the growth of phasmid axons into two steps, finding the ventral nerve cord and growing into it.

Another class of sensory neurons named the post-deirids (PDE) have been examined by fluorescein staining in mutants unc-33 IV, unc-44 IV, unc-51 V, unc-76 V, and unc(ev400)X. In all of these mutants the PDE neurons have more axons and branches than the wild type, many of which run in various abnormal positions.

We have also constucted strains carrying two mutations: one which by itself leads to phasmid guidance errors [e.g., unc(ev400)X] and one which by itself leads to premature termination (e.g., unc-76 V). Preliminary results suggest that the premature termination phenotype of unc-76 V may not always express in axons that are mispositioned or misguided.

42.3 DEPENDENCE OF CENTRAL AXON OUTGROWTH ON PERIPHERAL ECTODERMAL CELLS IN THE LEECH. Jochen Braun*. SPON: G.S. Stent. Dept. of Biophysics, U. of California, Berkeley, CA 94720

During embryogenesis of the leech Helobdella triserialis axons exiting the segmental ganglia grow into one of three major nerve roots, termed AA, MA, and PP. Ectodermal cells (including neurons) on either body side arise from four clonal groups, named the n-, o-, p-, and q-bandlets.

I labelled individual bandlets by injecting their parent blastomere with a fluorescent lineage tracer I had prepared: fluorescein dextran preparation 12 (FDX_{12}). This tracer is sufficiently bright to label growing axons. In addition, it photosensitizes labelled cells and allows their ablation by brief illumination with a laser microbeam (5mW, 488nm).

The central neurons contributed by the n-bandlet send axons into all three contralateral nerve roots. Outgrowth of these 'n-axons' begins at approximately 130 hours of development (hd). At this time, peripheral neurons, contributed by the contralateral o-, p-, and q-bandlets together with central neurons, epidermal cells, and six so-called cell florets, occupy defined positions along the contralateral roots. These cells were first described by M. Shankland.

I labelled the right n-bandlet and the left o-, and/or p- or q-bandlet(s). At 105hd, I ablated all labelled cells in 3 to 4 segments of the left body half, taking care to spare the n-bandlet in the right body half. At 175hd, I compared n-axons in the lesioned region with n-axons in an intact region of the same embryo. When both o- and p-bandlet cells were ablated, no n-axons were present in any root. This is consistent with a dependence of n-axon growth on contralateral cells of the o- and/or the p-bandlet. When only p-bandlet cells were ablated, n-axons in the MA root consistently reached, but did not extend beyond, the nephridial pore (cell floret 3). This is consistent with a dependence of n-axon growth in the distal MA root on the one peripheral neuron situated there: the p-bandlet cell pz8. N-axons in the AA and PP roots showed variable deficiencies, ranging from normal (in about half the cases) to shortened or missing. Damage to n-bandlet cells by scattered laser light or debris from ablated cells could account for this. When either o- or q-bandlet cells were ablated, I did not observe consistent deficiencies. The observation that the deficiency resulting from simultaneous ablation of o- and p-bandlet cells is greater than the combined effects of separate o- and p-ablation is consistent with redundancy within a set of o- and p-bandlet cells required for normal n-axon outgrowth.

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42.4 AXON GUIDANCE IN SURGICALLY MANIPULATED WING DISCS OF DROSOPHILA. S.S. Blair, L.M. Nagy*, and J. Palka, Dept. of Zoology, NJ-15, Univ. Washington, Seattle WA 98195

The wings of the fruit fly Drosophila melanogaster are formed from the wing imaginal discs during metamorphosis. These epithelial sacs evert in the initial stages of pupariation and then flatten, apposing the originally separate dorsal and ventral epithelia. During this process the sensory neurons of the wing arise and grow. These neurons can be stained and identified using antiserum generated against horseradish peroxidase; in this way it was shown previously that the neurons of the wing arise in a stereotyped sequence, laying down a characteristic pattern of nerve bundles which project proximally into the CNS.

It is possible to remove wing discs from prepupal flies and rear them in culture; the sensory neurons in such cultured discs develop largely normally. This technique allows the surgical manipulation of wing discs before the period of neural outgrowth. In this way different mechnisms of neural guidance have been tested. It was previously shown that neural outgrowth was normal in proximal and distal wing disc fragments; this finding eliminated guidance based on the presence of proximal "guidepost" neurons. In a second type of experiment, obstructions were placed along normal neural pathways, and it was shown that neurons could be deflected from their normal pathways but would not return to these pathways. This last result argues against a wing-wide system of guidance cues; guidance cues seem localized to the normal pathways.

Since nerve bundles in adult wings are found only in the veins, developing veins might act as mechanical guides for axon outgrowth. Veins develop during the early stages of axonogenesis, appearing as restricted gaps between the dorsal and ventral epithelia. To eliminate these veins as guidance cues, an experiment was performed in which dorsal and ventral epithelia were separated before vein formation and reared in culture. While the outgrowth of one ventral neuron was abnormal in ventral fragments, the neurons in dorsal fragments grew normally. Plastic sections indicate that developing veins were indeed absent from such dorsal fragments. Thus veins are not neccessary for the correct guidance of dorsal neurons. Substrate guidance or neuron-intrinsic cues are both likely candidates for the mechanisms of axon guidance in the dorsal wing.

42.5 CHOICE OF CENTRAL PATHWAYS BY SENSORY NEURONS IN DROSOPHILA. D. J. Wigston, R. L. Ellison* and J. Palka. Zoology Dept., Univ. of Washington, Seattle, WA 98195.

The wings of Drosophila carry a small number of campaniform sensilla that monitor cuticular deformation. Ten of these can be uniquely identified and the sensory neurons associated with them send their axons into three distinct tracts within the CNS. We have studied these projections to determine if the axons of identified sensilla choose specific pathways within the CNS. Individual sensory axons were stained by injecting cobalt into single sensilla with a micropipette. Central tracts were identified with reference to a cobalt-fill of the entire sensory projection from the contralateral wing, since projections from the two wings show strong bilateral symmetry.

We find that the axons of identified sensilla project into specific tracts with a probability >90%. The choice of tract is not governed by the peripheral location of a sensillum. For example, the twin campaniform sensilla on the anterior wing margin (TSM) are separated by only about 20 μm but their sensory axons choose different central tracts. Clustered with the two TSM neurons is a third neuron of unknown function; it innervates no distinctive cuticular structure, but nevertheless sends an axon into a third central tract. Thus three neurons that are almost in contact in the periphery project into three different central tracts. Further, each of the three tracts contains axons from different locations. For example, one TSM and a sensillum on the anterior cross-vein that links the third and fourth veins project into the same tract.

We occasionally found flies in which only one TSM was present and have developed lines in which this phenotype predominates. Normally, both TSM appear identical and are so close together that it is difficult to know which one is missing in the genetic variants. However, in the variants the remaining TSM consistently projects into the tract preferred by the distal TSM in normal flies, suggesting, as does independent evidence, that it is the distal TSM that remains. This shows that the choice of tracts by TSM axons in normal flies is not a consequence of interactions between the two axons.

We conclude that sensory neurons in Drosophila wings choose different central pathways depending on the identity of the sensillum they innervate. Their pathway choice does not depend simply on the peripheral location of their parent sensillum or on competition for access to particular tracts within the CNS.

42.6 PROXIMAL POLARITY (LIMB AXIS) GUIDANCE CUES FOR PATHFINDING BY PERIPHERAL PIONEER NEURONS IN GRASSHOPPER LEGS. Michael Caudy and David Bentley. Biophysics Group and Department of Zoology, University of California, Berkeley, CA 94720.

The first growth cones to pioneer a peripheral nerve route in embryonic grasshopper legs are from a pair of afferent neurons born at the limb tip. These growth cones navigate along the inner surface of the epithelium, initially growing proximally along the limb axis near the anterior edge of the leg. Along this anterior pathway, the dominant cues for growth cone steering are two nonadjacent, immature (guidepost) neurons. Filopodial contact with these neurons reorients growth cones in their direction.

In addition to orientation by guidepost cells, other cues might be present which influence the routes taken by pioneer growth cones. Several observations indicate that cues are present which can orient growth cones proximally along the limb axis: (1) before filopodia extended from pioneer neuron cell bodies have made contact with any guidepost neuron, the pioneer growth cones usually emerge from the proximal side of the soma. Moreover, the axon often grows for a short distance in a direction which is proximal, but not directly toward the first guidepost neuron, before the first filopodial contact with the guidepost neuron is made. Thus, information is present which can direct proximal axonogenesis. This infomation could be intrinsic, due to polarization of the pioneer cell body, or extrinsic, due to an environmental cue. Details of pioneer morphogenesis indicate that at least an intrinsic cue is operative. (2) Embryos have been observed where the two pioneer axons run proximally along separate but parallel routes, and where each has independently reoriented proximally along the limb after an initial segment of non-axial growth. This suggests the presence of a distributed proximal polarity cue which is independent of intrinsic pioneer cell polarity and of guidepost cells. (3) That one such cue might be a proximally increasing adhesion gradient is suggested by differences in the morphology of pioneer growth cones in proximal and distal regions of the anterior path. These differences are analogous to those seen in a variety of cells cultured on high versus low adhesion substrates.

Our observations suggest that both intrinsic and extrinsic cues are present which can orient pioneer growth cones in the proximal direction along the limb axis, and that one extrinsic cue appears to be a proximally increasing adhesion gradient.

42.7 CIRCUMFERENTIAL GUIDANCE CUES FOR PATHFINDING BY PERIPHERAL PIONEER NEURONS, LIMB SEGMENT BOUNDARIES, AND EPITHELIAL CELL DOMAINS IN EMBRYONIC GRASSHOPPER LEGS. David Bentley and Michael Caudy. Department of Zoology and Biophysics Group, University of California, Berkeley, CA 94720.

In embryonic grasshopper legs, the growth cones of the first afferent pioneer neurons navigate across the inner surface of the epithelium. The predominant guidance cues for these growth cones are specifically located, immature (guidepost) neurons. When filopodial contact has been made with a guidepost neuron, this cue dominates others in steering pioneer growth cones.

As the pioneer growth cones progress proximally down the limb, they encounter, at a particular axial location, an additional guidance cue which reorients them from axial to circumferential growth (unless they have already made filopodial contact with even more proximally placed guidepost neurons). Although pioneer filopodia readily cross this circumferential cue, the growth cones normally do not. Rather, they often spread symmetrically at this boundary, and can then turn in either direction and grow in a straight line (with respect to the epithelial surface) circumferentially around the limb. Their behavior suggests a circumferential cue which is continuous and extends around the limb. Certain early arising neurons are born in close spatial proximity to this cue and their axonogenesis is oriented circumferentially rather than axially.

Circumferential cues with similar properties are found at other specific locations along the limb axis.

We have examined the characteristics and develoment of the epithelium at these sites. Certain circumferential cues are closely related spatially to the subsequent appearance of limb segment boundaries within the epithelium. Cells in the boundary region have distinctive morphology. Histochemical staining reveals domains of epithelial cells whose borders are coincident with these boundaries. 3H-thymidine autoradiographs of embryonic legs demonstrate circumferential domains of recently divided epithelial cells which alternate with bands of older cells.

We conclude that very early in leg morphogenesis, a pattern is present in domains of epithelial cells. These domains are manifest in differential cell division, histochemical properties, and cell morphology. Various domains are correlated with limb segementation, the spatial pattern of neurogenesis, the orientation of axonogenesis, and the routes of migrating pioneer neuron growth cones.

42.8 **SELECTIVE FASCICULATION IN THE GRASSHOPPER EMBRYO: EXPERIMENTAL TEST OF THE LABELED PATHWAYS HYPOTHESIS.** Sascha du Lac* and Corey S. Goodman (SPON: M. Adams). Dept. of Biol. Sci., Stanford University, Stanford, CA 94305.

Extensive studies on identified growth cones in the grasshopper embryo led to the labeled pathways hypothesis (e.g. Raper, Bastiani, and Goodman, 1983) which proposes that early axonal pathways are differentially labeled on their surfaces, and later growth cones are differentially determined to follow specific labeled axon fascicles. The first three longitudinal fascicles contain only 7 identified axons and thus provide an ideal model system with which to test this hypothesis. The MP1/dMP2 fascicle contains the axons of MP1, dMP2, and pCC; the vMP2 fascicle contains the vMP2 axon; and the U fascicle contains the axons of the U1, U2, and aCC. Here we focus on the divergent pathway choices made by the sibling aCC and pCC neurons (anterior and posterior corner cells, first progeny of NB 1-1).

The pCC sends its growth cone anteriorly and fasciculates with the MP1 and dMP2 axons. Although both the aCC and pCC growth cones have equal filopodial access to the MP1/dMP2 fascicle, the aCC filopodia appear uninterested in these two axonal surfaces. Rather, its growth cone remains pointed anterior for about another 12 hours. By this time the sibling U1 and U2 cells extend growth cones which pioneer the U fascicle. As the U axons come within filopodial grasp, the aCC growth cone turns towards them, passing just over both the vMP2 and MP1/dMP2 bundles as it fasciculates with the U axons. TEM serial sections confirm these observations.

Thus, the pCC appears to specifically recognize the MP1/dMP2 axons, whereas the aCC appears to specifically recognize the U axons. We tested the specificity of these recognition events by laser ablations of the U cells before axonogenesis. Embryos were cultured in vitro for 48-56 hours following ablations. The contralateral hemisegment served as an internal control. The aCC on the control side fasciculated with the U axons as in ovo. In contrast, in the absence of the U axons, the aCC on the experimental side (n=19) neither extended an axon posteriorly nor fasciculated with the remaining vMP2 or MP1/dMP2 bundles. Instead the aCC growth cone remained pointed anteriorly.

These results support the labeled pathways hypothesis. Moreover, they demonstrate the high degree of specificity underlying the selective fasciculation by these 7 neurons as they form the first three longitudinal axon bundles.

42.9 **NEURONAL DEVELOPMENT IN THE DROSOPHILA EMBRYO: CELL RECOGNITION AND SELECTIVE FASCICULATION BY NEURONAL GROWTH CONES.** Michael J. Bastiani, John B. Thomas, and Corey S. Goodman. Dept. of Biol. Sci., Stanford University, Stanford, CA 94305.

Whereas the grasshopper embryo has been an ideal system for a cellular analysis of neuronal development, the Drosophila embryo has obvious attributes for a molecular genetic analysis. Our results show that the early Drosophila embryo is a miniature replica of the grasshopper embryo in terms of its identified neurons, their growth cones, and their selective fasciculation choices.

We have used Nomarski optics observations of dissected embryonic nervous systems, intracellular dye injections, and TEM serial section reconstructions to describe the cellular events of neurogenesis between hours 10-13 of Drosophila development. The events of selective fasciculation that occur over a three day period in the grasshopper embryo occur over a three hour period in the fly embryo. The first two longitudinal axon bundles, the vMP2 fascicle and the MP1/dMP2 fascicle, are pioneered by the interactions of the MP1, dMP2, vMP2, and pCC neurons. The aCC growth cone pioneers the intersegmental nerve; the RP1 and RP2 growth cones fasciculate on the aCC axon.

By hour 12, the G growth cone turns anteriorly in a lateral bundle. As in the grasshopper embryo, the G growth cone joins the A/P fascicle, and in particular is associated with the P and not the A axons. Thus, the patterns of cell recognition by growth cones has remained highly conserved throughout insect evolution.

The fact that we can now work with identified neurons, their growth cones, and their patterns of selective fasciculation in the Drosophila embryo means that a variety of genetic and molecular approaches can be applied to unraveling the mechanisms of cell recognition during neuronal development.

42.10 **NEURONAL DEVELOPMENT IN THE DROSOPHILA EMBRYO: MONOCLONAL ANTIBODIES AS PROBES FOR POTENTIAL RECOGNITION MOLECULES.** Stephen L. Helfand*, John B. Thomas, and Corey S. Goodman. Dept. of Biol. Sci., Stanford University, Stanford, CA 94305.

Extensive studies on neuronal growth cones in the grasshopper embryo are consistent with the notion that (i) many different molecules are differentially expressed on the surfaces of fasciculating embryonic axons, and (ii) that these cell recognition molecules guide growth cones to their appropriate targets. Monoclonal antibodies (MAbs) generated against the grasshopper embryo CNS have revealed cell surface antigens whose expression in the embryo correlate with these predictions; small subsets of neurons whose axons fasciculate together share common surface antigens (Kotrla and Goodman, 1983, 1984).

The next step is to isolate these potential recognition molecules, and then test their function during neuronal development. To this end, we have generated MAbs against the Drosophila embryonic CNS in order to take advantage of the advanced molecular genetics. Between hrs 10-13, cell recognition by neuronal growth cones occurs amongst a small number of neurons in relative isolation of the later expression of mature neuronal phenotypes. We used a 'mash' technique to isolate up to 10^5 10-13 hr CNS's (80-90% pure); mice were immunized and hybridomas were screened using these isolated CNS's. We generated several MAbs which recognize surface antigens expressed on subsets of fasciculating axons during development.

The SOX MAb appears to recognize a surface antigen on a small subset of neurons whose axons fasciculate. Between hours 10-11, only three neurons express the SOX antigen in each hemisegment: aCC, RP1, and RP2. Their axons fasciculate to form the intersegmental nerve (aCC's sibling, pCC, fasciculates in a different bundle and does not express the SOX antigen). Later in development, additional neurons express the SOX antigen; their axons also fasciculate in the intersegmental nerve.

We would now like to isolate and map the genes encoding for these surface antigens using cDNA expression cloning, and test their function by a genetic analysis.

42.11 **NEURONAL DEVELOPMENT IN THE DROSOPHILA EMBRYO: SUBTRACTED cDNA AS PROBES FOR POTENTIAL RECOGNITION MOLECULES.** John B. Thomas, Phil Patten*, and Corey S. Goodman. Dept. of Biol. Sci., Stanford University, Stanford, CA 94305.

We have characterized the cell recognition events between identified neurons in the Drosophila embryo which give rise to the stereotyped patterns of selective fasciculation. We would now like to begin identifying the surface molecules implicated by these cellular studies. The well characterized cell recognition events take place between hours 10-13 of embryonic development. This is before the appearance of neurotransmitters, electrical excitability, or synaptogenesis.

We have developed techniques for purifying 100,000s of CNS's from this stage of development, and have constructed a lambda gt10 cDNA library (10^6 clones) using poly A^+ mRNA isolated from this material. The library is thus enriched for cDNA clones representing 10-13 hour nervous system mRNA. By exhaustively hybridizing radiolabeled 10-13 hr nervous system cDNA with 30-fold excess mRNA from 3-8 hr embryos, and selecting for single stranded cDNA, we have stripped from the cDNA probe those sequences common to both time periods. Using this subtracted cDNA probe, we have isolated approx. 100 putative nervous system-specific cDNA clones from the library which are expressed at 10-13 hours but not earlier.

We are currently hybridizing the clones in situ to the embryonic nervous system in order to identify those mRNAs which are differentially expressed by subsets of identified neurons. Of particular interest will be genes expressed in subsets of neurons whose axons fasciculate with one another. These genes may encode neuronal surface molecules mediating cell recognition events during embryonic development.

DEVELOPMENT AND PLASTICITY: SYNAPTIC CONNECTIONS

43.1 REARRANGEMENTS IN THE RETINO-GENICULATE PROJECTIONS OF RATS FOLLOWING NEONATAL ABLATION OF THE SUPERIOR COLLICULUS OR NEONATAL RETINAL LESIONS

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The present communication describes changes in the retino-geniculate projections which occur subsequent to early lesions of the SC and compares this with the effect of an early retinal lesion. Neonatal rats were given a unilateral lesion of the SC or a small retinal lesion. When adults, most rats received unilateral eye injections of 5µl of 25% HRP. Remaining rats which had received unilateral SC lesions received a retinal lesion in adulthood, placed in the eye ipsilateral to the lesion at varying locations along the temporal crescent. Processing for HRP-TMB reaction product or for degeneration argyrophilia was conventional.

In the normal adult rat, ipsilateral and contralateral retinal terminal fields are segregated in the rostral three quarters of the dorsal lateral geniculate nucleus (dLGN), the ipsilateral field residing ventro-medially, with only the contralateral terminal field present in the caudal quarter of the nucleus. Following an early SC lesion, the contralateral field displayed a vacancy in the far caudal quarter of the nucleus just ventral to the optic tract. This vacancy is filled by an aberrant ipsilateral retinal terminal field. These rearrangements occurred only in the dLGN ipsilateral to the ablation. That part of the contralateral projection which is vacated corresponds to the representation of upper temporal retina near the optic disk. The ipsilateral retinal projection which aberrantly fills this vacancy arises from upper temporal retina at greater eccentricities: Although retinal lesions placed anywhere along the temporal crescent produced a column of degeneration in the rostral three quarters of the ipsilateral dLGN, as in normal rats, only upper crescent lesions resulted in an extension of that column caudo-dorsally into the region which is aberrantly vacated by the contralateral field. Similar rearrangements could be induced to form in the dLGN contralateral to a neonatal retinal lesion. If the lesioned eye was later injected with HRP, the contralateral field displayed an aberrant vacancy which traversed the nucleus caudo-dorsally to the optic tract. If the opposite eye was injected, an aberrant ipsilateral retinal terminal field formed which progressed caudo-dorsally to the optic tract through regions normally occupied by contralateral retinal termination only. Determinants of these rearrangements, and acuity deficits following early SC lesions are discussed.

43.2 TIME OF ORIGIN OF GANGLION CELLS GIVING RISE TO CROSSED AND UNCROSSED PROJECTIONS IN THE MOUSE RETINA. Ursula C. Dräger. Dept. Neurobiol., Harvard Med. Sch., Boston, MA 02115.

In the mouse, as in most mammals, the crossed optic projections originate from the entire extent of the retina, whereas ganglion cells giving rise to the uncrossed (ipsilateral) projection are restricted to the temporal and inferior retina. The nasal border of this bilaterally projecting region in the retina corresponds to the midline of the visual field. Only for the subpopulation of ganglion cells that provide the main input to the lateral geniculate is the midline representation reflected in a line of decussation in projections to one or the other side of the brain. Here the questions were asked: when are ipsi- and contralaterally projecting ganglion cells born in the embryo, and when is the midline representation formed?

Pregnant C57BL/6J and C3H mice were injected with ^{3}H-thymidine at selected times during gestation, from embryonal age E11 to E18. When the progeny had grown up, the ganglion cells giving rise to the crossed and uncrossed projections were marked by HRP injections into one optic tract. The retinas were then processed for autoradiography and were screened for cells containing both HRP and thymidine label. Contralaterally projecting ganglion cells were found to be generated throughout the test period in a crude concentric fashion, with the oldest cells in central and youngest ones in peripheral retina. Ipsilaterally projecting cells were born from E11 to E16, i.e. during the earlier part of the period in which the contralateral projection was born. In ipsilaterally projecting cells there was no obvious topographical pattern of birth dates comparable to the concentric pattern of contralaterally projecting cells. At the earliest time of ganglion cell generation (E11-12) ipsi- and contralaterally projecting cells were born within separate retinal regions, with the future midline representation forming the border between the two zones. This distinction became lost after E13, when both ipsi- and contralaterally projecting cells were born in the bilaterally projecting region. Hence it appears that at E11-12 the retina has a bipartite organization, allowing the specification of the two maps of opposite topographical polarity in which the crossed and uncrossed projections are known to be organized. Since in the adult retina this bipartite organization is preserved only in the large ganglion cells that project to the geniculate, and since large cells are known to be the earliest ones formed, these cells may be the ones that establish the early and bilateral projections of the retina. (NIH grant EY 01938)

43.3 POSTNATAL DEVELOPMENT OF GENICULOSTRIATE AXONS IN GALAGO. S.L. Florence and V.A. Casagrande. Dept. Anatomy, Vanderbilt Univ., Nashville, TN 37232.

In adult Galagos we previously identified two distinct types of geniculostriate axons (types I and II) (Florence, et.al., 1983). Both axon types, although restricted in distribution to the width of an ocular dominance column, differ in terms of axon and arbor size. Based upon sublaminar distributions in layer IV of striate cortex, types I and II fibers likely represent axons from cells in magno- and parvocellular LGN layers, respectively. The purpose of the present study was to compare the morphology of developing (2-4 weeks postnatally) axons with mature ones. As in our previous study, we identified individual axons by injections of HRP into the white matter below striate cortex. Successful filling of axons was obtained in 2, 3, and 4 week old Galagos. Thus far we have only been able to fill axons terminating within the superficial tier of cortical layer IV, the zone of termination of adult type I fibers.

At 2 weeks, the terminal arbors have very few branches and appendages compared with adults, and often terminate in structures resembling growth cones. At 3 weeks, the terminal arbors are still sparse but have many bouton-like appendages with irregular shapes and protuding spikes, which are not seen in mature animals. At 4 weeks, boutons are more mature with respect to size and arrangement, although many have a crenulated shape as opposed to the smooth, round shape of mature boutons. A comparison of the axon diameter (mean = 3.1μm) and arbor width (ranging from 0.12mm to 0.6mm) of adult type I fibers with the axon diameter (mean = 0.9μm) and arbor width (ranging from 0.16 mm to 0.7mm) of axons at 3 weeks of age suggests that the majority of the immature axons arbors are as big as arbors in adult cases. Additionally, since the ocular dominance columns (labelled by transneuronal transport of 3H-proline or WGA-HRP) are not visible at 4 weeks of age, it is likely that the individual immature axonal arbors actually occupy proportionally more area in cortex than corresponding axonal arbors of adults. This would suggest that the final columnar organization in Galago, as has been reported in cat and monkey (Rakic, 1976, 1977; LeVay, et.al., 1978, 1980; LeVay and Stryker, 1979) may result from a loss or change in the distribution of terminal arbors with maturation.

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43.4 ULTRASTRUCTURE OF THE DEVELOPING TREE SHREW LATERAL GENICULATE NUCLEUS. J. K. Brunso-Bechtold, A. J. Sweatt, D. Moore Smith and V. A. Casagrande. Depts. of Anatomy, Bowman Gray Medical School, Wake Forest University, Winston-Salem, NC 27103 and Vanderbilt University, Nashville, TN 37232.

The tree shrew dorsal lateral geniculate nucleus (LGN) contains six cell layers that segregate postnatally from a homogeneous cell group. Because of our interest in the developmental mechanisms related to the process of laminar formation, we prepared for EM the LGN from tree shrews sacrificed on postnatal day (P) 0, prior to the formation of cell layers; on P7, when all six layers are first evident; and on P15, when the segregation of cells into layers is essentially complete. At P0, neuronal somata are grouped tightly with much of their plasma membranes contiguous. These neurons appear immature, having little cytoplasm and relatively few organelles. In addition, there are numerous large profiles containing only flocculent material that are similar in appearance to dendritic growth cones. Synaptogenesis is underway by P0, but despite the presence of retinal axons throughout the LGN at this time, the synaptic profiles do not resemble those of mature retinal synapses. Instead they are small elements with round vesicles and restricted contacts. By P7, synaptic profiles are not only more numerous, but some profiles now resemble mature retinal terminals. In addition, there are fewer groups of juxtaposed neurons. Individual somata are more mature and frequently have large concentrations of organelles adjacent to a dendritic outgrowth. The growth cone-like profiles containing flocculent material seen at P0 are present but appear to comprise less of the neuropil. By P15, there are more mature dendrites and axons in the neuropil. Furthermore, synaptic profiles are more numerous as well as more mature in appearance. However, even at this stage, synaptic profiles do not yet have the complexity present in the adult and some growth cone-like profiles are still evident. This suggests that several features of the LGN are immature even after cell layers have segregated.

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43.5 DEVELOPMENT OF GAD IMMUNOREACTIVITY CORRELATES WITH ONSET OF INHIBITION IN CAT LATERAL GENICULATE NUCLEUS. S.L.Shotwell, M.B.Luskin and C.J.Shatz, Department of Neurobiology, Stanford Univ. Sch. Med., Stanford, CA 94305.

In the visual system of the cat, excitatory signals from retinal ganglion cells to principal relay cells of the lateral geniculate nucleus (LGN) are modified by inhibition thought to be mediated by gamma amino butyric acid (GABA). This inhibition has two known sources - LGN intrinsic interneurons, and perigeniculate nucleus (PGN) cells. During development, in-vitro electrophysiological studies show that inhibition does not appear until shortly before birth, at least two weeks after excitatory inputs begin to function (Shatz and Kirkwood, J.Neurosci 4, 1984). To learn more about the development and sources of inhibition, and to confirm the physiological results, we carried out an immunohistochemical study of the developing thalamus using an antiserum for glutamic acid decarboxylase (GAD), the synthetic enzyme for GABA. D. Schmechel and W. Oertel generously provided the serum (Neurosci. 6:2689, 1981).

In the adult thalamus, the anti-GAD staining pattern was similar to that reported by Fitzpatrick et al. (Neurosci. Abst. 8:261, 1982). LGN laminar zones contained many small cells with stained somata and proximal dendrites. The laminar zones also contained a complex array of punctate terminal staining. Inter-laminar zones were relatively free of stain. In PGN, most somata and proximal dendrites were heavily stained; there was very little punctate staining.

In development, the first staining was seen two weeks before birth and was different from that seen in the adult. Staining was confined to fascicular structures within LGN, and to somata within PGN. In contrast, LGN somata did not stain until around birth. Punctate staining became prominant a few weeks after birth. LGN and PGN staining patterns became progressively more adult-like up to 3 months of age, when they were indistinguishable from adult patterns.

Our results demonstrate a good correlation between the onset of inhibition seen physiologically and the appearance of anti-GAD staining in LGN. In addition, they suggest that cells of the PGN may provide the first source of GABA-mediated inhibition to the LGN, with intrinsic inhibition from LGN arising later, around the time of birth. Finally, the relatively late appearance of the adult-like pattern suggests that intrageniculate inhibitory circuitry continues to develop well after birth, and consequently may be modified by visual experience. (Supported by NIH grants EY-05632-1, EY-02858, NS-07158, and NSF grant BNS-8317228).

43.6 MORPHOLOGICAL CHANGES IN PHYSIOLOGICALLY IDENTIFIED RETINOGENICULATE AXONS AFTER UNILATERAL ENUCLEATION IN CATS. P.E. Garraghty, M. Sur, R.E. Weller and S.M. Sherman, Section of Neuroanatomy, Yale Medical School, New Haven, CT 06510 and Department of Neurobiology and Behavior, SUNY at Stony Brook, NY 11794.

We have examined the terminations of single retinal X- and Y-cell axons in the lateral geniculate nucleus of adult cats reared with one eye enucleated at 1-2 days of age. Optic tract axons from the remaining eye were recorded, classified as X- or Y-cell, and then impaled and intracellularly injected with horseradish peroxidase. To date, we have recovered 4 X- and 15 Y-cell axons. The X-cell axons did not differ from normal adult X-cell axons either in terminal volume or in numbers of terminals. The Y-cell arbors, however, were larger than normal, and the numbers of boutons on the Y-cell axons were consistently at the high end of the normal range. Furthermore, for 5 Y-cells, pronounced expansion into deafferented laminae was observed. In some cases, up to 50% of the terminations of a Y-cell axon were found to be in a "wrong" lamina.

At the microscopic level, we observed profound degeneration in deafferented lamina A or A1. Concurrently, lamina A or A1 innervated by the intact eye was found to be over 50% larger than normal. An analysis of cell size and density indicated that this expansion arose from an increase in the number of cells in laminae innervated by the intact eye and not from hypertrophy of existing cells or a decrease in their packing density. Since cell genesis has ceased long before birth, this increase in laminar volume and cell number must reflect an acquisition of deafferented geniculate relay cells by the afferents (largely Y-cell) of the intact eye.

Our conclusions must be qualified because the number of X-cell axons in our sample is low. At least Y-cell retinogeniculate axons from the intact eye show considerable sprouting after unilateral enucleation at birth. This may be related to other evidence of prolonged plasticity among Y-cell retinogeniculate arbors (Sur et al., Nature, 300:183, 1982; and in press).

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43.7 RETINOTOPIC TERMINATION AND ABNORMAL PATHWAYS OF REGENERATING OPTIC AXONS IN THE GOLDFISH TECTUM. U.Egert, E.Kalko and C.A.O.Stuermer, MPI fuer Entwicklungsbiologie, Tuebingen, FRG.

Earlier experiments demonstrated gross devia - tions from the normal spatial order in the regenerated retinotectal pathway of goldfish(JCN,'84). After insertion of HRP through Stratum Opticum (SO) and the synatic layer SFGS into rostral dorsomedial hemitectum the normal pattern of retrogradely labeled ganglion cells in whole-mounted retinae(i.e.partial annulus, retinotopic cluster and straight group of cells between both)was absent. Instead, labeled cells were scattered over the whole retina.

Our aim was to determine the origin of the pathway mistakes. First(see these abstracts) we revealed that the fascicular path of regenerated axons in SO and their exit from their fascicles is relatively normal, although less precize. Here we test the order in SFGS in two experiments. Do regenerating axons terminate retinotopically ?

1.Minute pledgets of HRP were placed stereotaxically into SFGS of regenerates. Two days later the retina was whole-mounted and reacted to show the retrogradely labeled ganglion cells. With rostral sites most ganglion cells were in a cluster retinotopically located in the ventrotemporal retina. Few scattered cells were present in addition. With caudal sites all cells were confined to a cluster in the ventronasal retina.

2.Crystals of HRP were placed intraretinally onto sectioned axon bundles in ventronasal retina close to the optic disc. After 3 days the anterogradely labeled axons and their terminal arbors were traced in DAB reacted tectal whole-mounts. Most terminal arbors were in a sector in retinotopical locations in the caudal dorsomedial hemitectum.Many extrafascicular axons between the fascicle and the terminal arbors coursed rather straight others,however in aberrant routes. Axons branched frequently which is never observed in normals. Thus, these experiments confirm that regenerating retinal axons ultimately terminate retinotopically. However, they course through abnormal pathes.

43.8 MORPHOLOGY AND INTERACTIONS OF EMBRYONIC CEREBELLAR AXONS. C.A. Mason and E. Gregory, Dept. Pharmacology, N.Y.U. School of Medicine, New York, NY 10016.

In mature cerebellum, climbing and mossy fibers have distinct morphology and cellular targets, climbing fibers synapsing with Purkinje cells, and mossy fibers synapsing with granule neurons. Our studies have shown that during postnatal development, both of these types of axon display dual morphological characteristics and connections (J. Neuroscience, in press). Here we address earlier stages of development to determine the shapes of axons and to correlate these with cell interactions. To do this we have used 1) HRP filling of axon bundles in slices of cerebellum, and 2) immunocytochemistry with antisera to the 160 kd component of the neurofilament triplet (gift of R.K.H. Liem) to map axonal projections, and to synapsin I, a nerve-terminal specific phosphoprotein found in most CNS synapses (J. Cell Biol. 96:1355; gift of P. DeCamilli).

Axons are seen entering the cerebellar anlage as early as embryonic day (E) 13, and at this early phase of growth through the emerging tracts, have enlarged growth cones. As they leave the tracts to enter the cellular regions that have yet to segregate into Purkinje and granule layers (E15-postnatal day (P) 3), their shapes become more simple, with fine diameters and minute bud-shaped growing tips. Midway through this stage, at E17-18, punctate but sparse synapsin staining is first seen, suggesting axon-cell interactions. Between E18 and birth, a few cells are encased by synapsin-positive foliate endings. Finally, after P5, when granule cells arrive and Purkinje cells align, morphological differentiation of axons begins, the cell layers form, and synapsin staining reveals both the characteristic shape and topography of synapses, even as the "combination" axons with dual features persist (until P21).

These findings indicate that synaptic contacts are formed at very early stages of axon development, prior to formation of cortical layers, and possibly prior to axon target cell selection. The axons with dual morphology and connections may be a remnant of these early interactions. Studies are in progress (1) to determine when growing tips have synapsin and if its presence correlates with typical morphological synapses, and (2) to identify the target cells contacted by these immature axons before cellular layers form. (Supported by NIH grant NS-16951).

43.9 DEVELOPMENT OF A CHOLINERGIC PATHWAY TO THE RAT OLFACTORY BULB: AChE APPEARS TWO WEEKS AFTER CHOLINERGIC FIBERS REACH THE BULB. S. Van Ooteghem, S. Schumacher and M.T. Shipley. University of Cincinnati College of Medicine, Cincinnati, OH 45267-0521.

The development of a pathway involves the growth of fibers to a target structure, the formation of synaptic contacts and the expression of molecular mechanisms necessary for synaptic transmission. Little is known about the degree to which these events are temporally correlated or interdependent. We report results which suggest that biochemical maturation of cholinergic (Ch) synapses occurs long (14-20 days) after the fibers reach the bulb.

Specific AChE was localized in pre-, postnatal and adult brains using a copper thiocholine method. Cholinergic afferents to the bulb were retrogradely labeled at the same ages by WGA-HRP injections in the main olfactory bulb (MOB).

The source of cholinergic input to MOB is from neurons in the nucleus of the diagonal band (DB). Neurons in DB are retrogradely labeled in abundance at birth and the number of labeled neurons increases steeply in the first week of life. At the same time, DB neurons are intensely AChE positive. There is a close correspondence among AChE+ and choline acetyltransferase ChAT + neurons in DB (Eckenstein and Sofroniew, '83). By contrast, MOB is devoid of AChE until nearly two weeks after birth, yet our method does stain AChE terminal sites from birth onwards in other brain areas. The AChE pattern in MOB is not fully mature until around 50 days.

These results provide evidence for the idea that cholinergic terminals do not become biochemically "mature" until several weeks after they have grown into the bulb. In autonomic ganglia, AChE appears slightly ahead of ChAT and synaptic transmission. Thus, in the brain, synaptic function may lag considerably behind connectional formation. It will be of interest to learn what events regulate synaptic biochemical maturation and to determine whether other, transmitter specific projections to the bulb develop similarly to the Ch system.

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43.10 REGENERATION STUDIES OF A SINGLE IDENTIFIABLE MOTONEURON IN THE CRAYFISH, PROCAMBARUS CLARKII. William P. Hunt. Department of Biological Sciences, Dartmouth College, Hanover, NH 03755.

The superficial flexor muscle of the crayfish Procambarus clarkii is innervated by five excitatory motoneurons. The four smallest of these connect across the muscle in a specific pattern of innervation (Velez and Wyman, J. Neurophysiol. 41, 75-96). Previous experiments have examined how these motoneurons react to axotomy and changes in target area (Velez and Hunt, Soc. Neurosci. Abstr. 6, 1980; Clement, Hunt and Velez, Soc. Neurosci. Abstr. 7, 1981; Hunt, Worden and Velez, Soc. Neurosci. Abstr. 8, 1982). These experiments all concentrated on the plasticity of motoneurons 1-4 because of their high rate of spontaneous activity. Experiments are now being conducted on the largest excitor using suction electrode stimulation techniques to determine its ability to regenerate specific connections. Manipulations include normal regeneration, limited target regeneration and partial target rotation. Preliminary results indicate that the largest excitor is able to regenerate a normal connectivity map by eight to ten weeks in a whole muscle. Limited target operations show a faster rate of regeneration when the motoneuron is limited to its main target area in the muscle. This may imply some sort of recognition of the lateral head of the muscle by the nerve. Long term regeneration data is now being collected to see if these connections are stable.

43.11 ORGANIZATION OF MOTOR UNITS FOLLOWING CROSS-REINNERVATION OF ANTAGONISTIC MUSCLES IN THE CAT HINDLIMB. C. Thomas*, T. Gordon and R.B. Stein. Depts. of Physiology and Pharmacology University of Alberta, Edmonton, T6G 2H7.

We have studied the reorganization of motor units according to size following misdirection of motoneurons to muscles with antagonistic functions. Misdirection was ensured experimentally by surgically cross-uniting the tibial and peroneal nerves to one hindlimb at knee level in nine 2-6 months old cats. Flexor and extensor muscle activation patterns during locomotion were recorded from the cross-reinnervated and normally innervated hindlimb muscles using bipolar EMG electrodes. In normal locomotion, extensor muscles fire in extension while flexor muscles show a double burst pattern with a short burst in both flexor and extensor phases. The cross-reinnervated flexor muscles only fired during extension of the limb. The cross-reinnervated extensor muscles were active in the extensor and sometimes in the flexor phase of locomotion. The flexor burst was frequently absent where the cross-reinnervation was less than 90% pure. The timing of the muscle activity during locomotion was therefore inappropriate, indicating that the muscles were activated according to the nerves that now supplied them.

In acute experiments 18-24 months after suture, compound action potentials on the L6, L7 and S1 dorsal and ventral roots were measured in response to stimulation of the CP, LGS, and MG nerves in the cross-reinnervated and contralateral control hindlimbs. The success of the motor nerve cross was between 60-90% as determined by comparing the charge contribution of the lumbar and sacral roots to flexor (CP) and extensor (MG and LGS) nerves. The peripheral organization of the cross-reinnervated extensor muscles was studied by dissecting and stimulating the appropriate ventral root filaments and by recording the evoked nerve and motor unit potentials simultaneously with muscle tension from either the MG or LG and soleus muscles. Muscle unit force and contractile speed were directly correlated with the size of the innervating nerve as found in the same normal muscles from cats of comparable age. The size relationships could be accounted for by the differences in neural size and force development of the different motor unit types classified according to their fatiguability and "sag" property. These data show that peripheral reorganization of nerve and muscle properties according to size occurs even when motoneurons are misdirected to antagonistic muscles and produce inappropriate movement patterns.

ACTION POTENTIALS AND ION CHANNELS I

44.1 CALCIUM SENSITIVE TRANSIENT POTASSIUM CURRENT INFLUENCES RHYTHMIC MOTOR OUTPUT IN BARNACLE NEURONS. B.A. Premack*, P. Ruben*, and S.H. Thompson* (SPON: C. Beck). Dept. of Zoology, Univ. of Alberta, Edmonton, ALTA T6G 2E9, and Hopkins Marine Station of Stanford Univ., Pacific Grove, CA 93950.

Outward currents of barnacle motor neurons were analyzed in order to determine their contribution in shaping centrally driven spike trains. Identified motor neuron cell bodies of Balanus nubilus were voltage and current clamped in the isolated CNS using two microelectrodes. Cells were followers of the interneuronal oscillator which drives the cirral appendages, either hydrostatic extensor (HEN) or cirral flexor (CFN) motor neurons.

Depolarizing voltage steps from -40 mV generated outward currents similar in voltage dependency and activation kinetics to those of mollusc cell bodies. A TEA sensitive (50 mM) voltage dependent rectifier (I_K) was predominant, with a small amount of Ca^{++} activated K^+ current. No significant differences were noted between HENs and CFNs. Hyperpolarizing prepulses followed by depolarizing voltage steps often activated a transient potassium current (I_A). Large (600 nA), fast (peak 4 msec, to decay 10-50 msec) I_A was present in HENs, but absent or greatly reduced in CFNs. Replacing external Ca^{++} with Mn^{++} has demonstrated that 45-80% of the I_A in HENs is Ca^{++} dependent for its activation. The remaining portion is 4-aminopyridine (2 mM) but not TEA sensitive. Cells were current clamped with square pulses and sinusoidal waves (0.3 Hz) to analyze the contribution of Ca^{++} sensitive I_A during spike trains (since treatments that reduced I_A also disrupted normal synaptic input). In cells with I_A present Ca^{++} replacement had several effects, 1) decreased spike latency after hyperpolarizing pulses, 2) increased spike frequency to short depolarizing pulses and sinusoidal waves, 3) increased action potential duration. Similar protocol in CFNs (I_A lacking) generated higher control spike frequencies, which were relatively unaffected by 0 Ca^{++} saline. The possibility that spike changes observed in Ca^{++} free saline are due in part to reduction of the late Ca^{++} activated K^+ current exists, but seems unlikely due to the small proportion present in these neurons.

These results suggest that a Ca^{++} sensitive I_A contributes to action potential repolarization, and burst firing frequency in neurons that extend barnacle cirri. This hypothesis is currently being tested by Ca^{++} chelator injection into neurons during normal, rhythmic synaptic drive.

44.2 DEPRESSION OF CALCIUM-ACTIVATED POTASSIUM CURRENT IN INTERNALLY DIALYZED HELIX NEURONS IS DUE TO ACCUMULATION OF FREE INTRACELLULAR CALCIUM. Edwin S. Levitan* and Irwin B. Levitan (Spon: L. Levine). Biochemistry Dept., Brandeis Univ., Waltham, MA 02254

A number of workers have demonstrated that internal dialysis of molluscan neurons and bovine chromaffin cells produces a rundown of calcium current with time. It has been thought that this might result from the loss from the cell of some soluble factor(s) necessary for the maintenance of calcium current. We report here that internal dialysis of Helix neurons also produces a rundown of calcium-dependent potassium currents, elicited by depolarizing voltage steps presented under voltage clamp. This rundown is prevented for as long as 2 hours when cells are dialyzed with an internal solution strongly buffered to about 0.1 μM free calcium (112 mM K^+ aspartate, 2 mM $MgCl_2$, 0.5 mM Na_2ATP, 0.25 mM $CaCl_2$, 0.5 mM EGTA, 5 mM K^+-Hepes, pH 7.1). In fact in cells in which the calcium-dependent potassium current is initially small, dialysis with the above solution can cause this current to increase for as long as 60 minutes. Dialysis with internal solutions in which the free calcium is only weakly buffered to 0.1 μM (25 μM calcium, 50 μM EGTA), or strongly buffered to about 1 μM (0.95 mM calcium, 1 mM EGTA), depresses the calcium-dependent potassium current. Addition of 1 μM calmodulin to the internal solution, with either 0.1 μM or 1 μM free calcium, does not affect the current. From these results it is concluded that depression of the calcium-dependent potassium current in dialyzed Helix neurons does not result from the loss of calmodulin or some other cytoplasmic factor, but rather is caused by the accumulation of intracellular calcium. Although calcium currents have not been measured directly in these experiments, it seems possible that the depression results from calcium-dependent inactivation of calcium current, and thus the present results may have some bearing on the rundown of calcium currents reported by other workers.

44.3 EFFECT OF VENOM FROM CONUS STRIATUS ON THE DELAYED RECTIFIER POTASSIUM CURRENT FROM MOLLUSCAN NEURONS. T.J. Chesnut, D.O. Carpenter and G.R. Strichartz. Ctr for Labs & Res, NYS Dept. of Health, Albany, NY 12201 and Anesth. Res. Labs., Harvard Med Sch, Boston, MA, 02115

The delayed rectifier potassium current (I_k) was recorded from voltage clamped cell bodies of both abdominal and pleural ganglia isolated from the central nervous system of Aplysia californica. I_k was isolated from the calcium dependent potassium current through the use of low Ca^{++}, Co^{++}seawater to reduce calcium influx. The fast transient potassium current, I_A, was inactivated through the use of holding potentials of -40 mV. Exposure of these cells to an extract from venom ducts from Conus striatus (0.2-1.4 µg total protein/ml of bath volume) resulted in a large increase in the leakage current (I_L) often followed by death of the cell. That the increase in I_L was caused by phospholipase action on the cell membrane was supported by direct biochemical assay indicating that the venom contained phospholipase activity. Use of the phospholipase inhibitor chloroquine to control I_L enabled three different effects on I_k to be measured during exposure times of 5-45 min. Effect I (7/30 cells) was an increase in the peak amplitude (I_p) of I_k, effect D (23/30 cells) was a decrease in I_p, while effect K (all 30 cells) was a slowing of both the activation and the inactivation kinetics of I_k. Some cells exhibited a small, transient increase in I_p followed a steady state net decrease. Both the effects on I_p were more pronounced at more depolarized voltage steps. All three effects were dose dependent. That the three effects were due to separate components of the venom was suggested by dose-response curves indicating no correlation between the occurrence of the three effects at all venom doses. Further support for the contention that the effects were due to separate components was provided by experiments using venom filtered through an Amicon Centriflo filter (50K Dalton cut-off). Cells treated with the filtered venom exhibited significantly different ratios of the three effects. No decrease in I_p was observed in 9 cells, an increase in I_p occurred in all 9 cells while effect K was observed in 4/9 cells. Venom components producing K and I were heat stable at 100°C for 60 min while about 60% of the activity of component D was lost during the heat treatment. (Supported by NIH Postdoctoral Fellowship NS06930 to TJ Chesnut and NIH grants NS18435 to D.O. Carpenter and GM15904 to G.R. Strichartz.)

44.4 **INWARD CURRENT AND CALCIUM DEPENDENT POTASSIUM CURRENT ARE INCREASED AND OTHER POTASSIUM CURRENT DECREASED BY SEROTONIN IN PEDAL NEURONS OF HERMISSENDA.** **Jon Jacklet, Juan Acosta-Urquidi and Daniel Alkon. Neural Systems, NINCDS-NIH, Marine Biological Laboratory, Woods Hole, MA. 02543.**

The large pedal neurons (LP1-3) of Hermissenda exhibit outward potassium (K) currents, IA, IK(Ca), and IK(V) and inward sodium (Na) and calcium (Ca) currents, INa, and ICa under voltage clamp. IA is suppressed by influx of Ca during prolonged depolarization and recovery follows the reduction in intracellular Ca (Acosta-Urquidi etal. Soc. Neurosci. Abst. 1983). Serotonin and 8-BT-cAMP depolarize these neurons and increase Rin in low Ca- high Mg seawater and RO-1724, a phosphodiesterase inhibitor, causes spike broadening (Jacklet etal. Soc. Neurosci. Abst., 1983). We have investigated the serotonin effect further. In 0 Na, 100 mM TEA, 200 mM TMA, 10-20 mM Ca, 10 mM K, 5 mM 4-AP, TRIS, pH 7.8 seawater, depolarization in current clamp evokes an 80 mV, 50 mS Ca spike, blocked by 5 mM cadmium (Cd). Spike fails with repeated depolarization at > 0.3/S. The absence of pronounced after-spike hyperpolarization suggests the failure is due to ICa inactivation. Serotonin does not change the duration or amplitude of the spike but does increase Rin and allow repeated firing. Under voltage clamp, a large voltage dependent inward Ca current and late outward current is observed when membrane is depolarized from holding potential near -40 mV. In twin pulse paradigm, 800 mS duration, 800mS interval, the Ca current inactivates but recovers fully in 5-30 S. The inward and late outward currents are blocked by Cd, indicating the inward current is ICa and the outward current is IK(Ca). Serotonin (10 uM) increases the inward and late outward currents, while the membrane conductance (Gm) at and below holding potential is reduced. The serotonin effect to increase Rin in current clamp and decrease Gm in voltage clamp suggest that increased inward current is due to reduction of a steady IK sensitive to serotonin and cAMP. An additional direct effect on ICa is also possible.

44.5 CYCLIC AMP-DEPENDENT PROTEIN PHOSPHORYLATION MODULATES THE ACTIVITY OF SINGLE POTASSIUM CHANNELS IN ISOLATED MEMBRANE PATCHES AND IN RECONSTITUTED PHOSPHOLIPID BILAYERS. Douglas A. Ewald* and Irwin B. Levitan (Spon: M. Woodruff). Biochemistry Dept., Brandeis Univ., Waltham, MA 02254

The membrane properties of some excitable cells, including several different molluscan neurons, have been shown to be regulated by cAMP-dependent protein phosphorylation. For example in certain Helix neurons intracellular application of the catalytic subunit (CS) of cAMP-dependent protein kinase causes a selective increase in the calcium-activated potassium current. In order to determine whether this results from phosphorylation of the potassium channel itself, the activity of single channels has been examined, either in cell-free membrane patches or following channel reconstitution into artificial phospholipid bilayers. In isolated membrane patches from Helix neurons CS + ATP causes an increase in the activity of a small (20 pS) K^+ channel. This channel resembles the Ca^{++}-activated K^+ channel described by Lux, Neher and Marty in cell-attached patches in Helix neurons, but appears to be more sensitive to calcium in the isolated patch. In several patches CS + ATP has caused a decrease in the activity of a somewhat larger (30-50 pS) K^+ channel, which may be similar to the S channel described by Siegelbaum, Camardo and Kandel in Aplysia sensory neurons. When crude membrane vesicles from Helix ganglia are fused with an artificial phospholipid bilayer, a number of different outward current channels can be seen. At least one of these channels (80-100 pS in 200 mM KCl) is Ca^{++}-dependent, although its sensitivity to calcium varies from one experiment to another. The activity of this channel in the bilayer is increased by CS + ATP. Although the relationship of the channels observed in the isolated patches and the reconstituted system remains to be determined, the results indicate that the activity of individual K^+ channels can be regulated by cAMP-dependent protein phosphorylation, and support the hypothesis that the phosphorylation target is the channel itself or some protein intimately associated with the channel.

44.6 MODULATION OF THE SEROTONIN-SENSITIVE POTASSIUM CHANNEL BY cAMP-DEPENDENT PROTEIN KINASE. M. J. Shuster*, J. S. Camardo*, S. A. Siegelbaum, C. M. Eppler* and E. R. Kandel (SPON: L. P. Rowland). Howard Hughes Institute of Molecular Biology, Ctr. for Neurobiol. & Behav., Columbia Univ., and N.Y.S. Psychiatric Institute, New York, N. Y. 10032.

Serotonin (5-HT) produces a slow EPSP in sensory neurons of Aplysia by causing prolonged closure of a specific class of potassium channels ('S' channels; Siegelbaum et al., Nature, 299:413-417, 1982). While the action of 5-HT has been shown to be mediated by cAMP-dependent protein kinase (Castellucci et al., PNAS, 77: 7492-7496, 1980), the nature of the link between phosphorylation and closure of the S channels remains unknown. To determine whether S channels can be modulated in the absence of cytoplasmic constituents, we have studied the effects of the catalytic subunit of cAMP-dependent protein kinase (cAMP-PK) on cell-free patches of membrane in which the cytoplasmic membrane surface faces the bath solution (inside-out patch) (for preliminary results see Camardo et al., Soc. Neurosci. Abstr., 9:22, 1983).

Purified catalytic subunit of cAMP-PK (0.1 uM) was applied to inside-out patches in the presence or absence of 1 mM MgATP, the source of phosphate for the enzyme. In the presence of ATP, cAMP-PK produced prolonged periods of channel closure in 78% of experiments (N=36). A total of 38.0% of channels closed in response to cAMP-PK (including both successful and unsuccessful experiments). The mean latency to closure of the first channel following addition of kinase is 4.5 + 4.6 min. cAMP-PK is much less effective in the absence of ATP, producing closure in only 23.5% of experiments and resulting in the closure of a total of 9.7% of all channels (N=17). MgATP by itself (1.0 or 10.0 mM) also had only a small effect, producing closure in 20.7% of experiments and affecting a total of 4.7% of all channels (N=29).

The effects of kinase plus ATP in the isolated patch are qualitatively similar to the effects of serotonin in cell-attached patches (5-HT produces channel closures in 76% of experiments (N=36) and closes a total of 45% of all channels). However, the 5-HT induced closures tend to be longer lasting than those produced by cAMP-PK. Preliminary results show that addition of 50 mM KF (a nonspecific phosphatase inhibitor) potentiates the kinase effects and prolongs the closures, suggesting the presence of active phosphatase in the isolated patch. While our results do not allow us to conclude that the channel itself is phosphorylated, they do provide evidence that cAMP-PK can modulate channel activity in the absence of cytoplasmic proteins and that its primary substrate is either the S channel or a protein closely associated with the channel.

This work was supported in part by a grant from the Systems Development Foundation.

44.7 PRESYNAPTIC FACILITATION OF TAIL SENSORY NEURONS OF APLYSIA CALIFORNICA INVOLVES A DEPRESSION OF THE "S" POTASSIUM CHANNEL. J. D. Pollock and J. S. Camardo*. H. Hughes Med. Instit. for Molec. Neurobiol. & Behav., Columbia Univ., and the N.Y.S. Psychiat. Instit., N.Y., N.Y. 10032.

Serotonin decreases the net outward current in voltage clamped tail sensory neurons of the pleural ganglia in Aplysia californica (Pollock et al., Soc. Neurosci. Abstr., 8:523, 1982). These neurons constitute the afferent pathway of the tail withdrawal reflex. The net outward current decreased by serotonin appears to have similar characteristics to the "S" current, the serotonin-sensitive K^+ current of the siphon sensory neurons in the abdominal ganglion. Like the "S" current, the net outward current modulated by serotonin in the tail sensory neurons: (1) is active close to the resting potential; (2) contributes to the repolarization of the action potential; (3) does not inactivate with prolonged depolarization; (4) it is not blocked by substitution of barium for calcium; (5) is cAMP-dependent. The decrease in the net outward current by serotonin in both the tail and siphon sensory neurons mediates presynaptic facilitation, the process underlying sensitization of the gill and tail withdrawal reflexes.

We report here evidence that the net outward current decreased by serotonin in the tail sensory neurons is also a K^+ current. The I.V. relation of the serotonin-sensitive current is altered by changes in extracellular K^+ concentration. In normal sea water, 10 mM K^+ ASW, serotonin decreases the net outward current at potentials more positive than -40 mV. In 70 mM K^+ ASW, serotonin decreases the outward current at potentials more positive than -20 mV. In 120 mM K^+ ASW, the serotonin-sensitive current reverses at -20 mV, close to the predicted Nernst potential of 15.3 mV for 120 mM K^+ with an assumed internal K^+ concentration of 220 mM. The I.V. relation is unaffected by changes in external chloride concentration. These results are similar to those reported by Walsh and Byrne (Soc. Neurosci. Abstr., 9:458, 1983).

Using the patch clamp technique of Hamill et al. (Pfluger Arch., 391:85-100, 1981), we have also examined the kinetics of single K^+ channels and found that they share the characteristics of the serotonin-sensitive K^+ channel (Siegelbaum et al., Nature, 299:413-417, 1982) in the abdominal sensory neurons. The predominant outward channel observed in the pleural sensory neurons has a mean elementary slope conductance at 0 mV of 73.6 ± 18.0 picosiemens ($\bar{X}$ ± S.D., n=10), and shows outward rectification. The channels are active at the resting potential and do not inactivate with maintained depolarization. These channels are closed by bath application of serotonin. These features suggest that the same K^+ channel underlies the serotonin-sensitive currents in both the abdominal siphon sensory neurons and pleural,tail sensory neurons.

44.8 A Comparison of the Interburst Hyperpolarizing Current and the Ca Activated K Current in Aplysia Neuron R15. William B. Adams. Biocenter of the University of Basel, 4056-Basel, Switzerland.

The interburst hyperpolarizing current (I_H) is an outward current with slow kinetics that is activated incrementally by each action potential in a burst. When the summated I_H is large enough to make the net membrane current outward, the burst is terminated and the cell hyperpolarizes. It has long been assumed that this current is a Ca activated K current (I_C). Ca has been shown to enter the cell with each action potential and to accumulate during the burst (Stinnakre & Tauc, Nature New Biol. 242:113,1973). In addition, the presence of a slowly decaying I_C has been demonstrated in R15, and many other cells, by intracellular injection of Ca (Meech, Comp. Biochem. Physiol. 42A:493,1972). I_C reverses at E_K (Gorman & Hermann, J. Physiol. 296:393,1979) and is blocked by low concentrations of TEA (Hermann & Gorman, Neurosci. Lett. 12:87,1979). Removal of Ca from the bathing medium, or addition of Ca channel blockers, reduces the K current that is elicited by long depolarizing pulses. I_H, elicited by action potentials or by brief depolarizing pulses under voltage clamp, is also sensitive to removal of Ca and to addition of Co, Cd, Ni or La. However, I_H does not reverse at E_K, but remains an outward current between -20 and -120 mV. Moreover, I_H is unaffected by changes in K concentration and by addition of K channel blockers, including high concentrations of TEA. A direct comparison of I_H and I_C, in the same cell, shows that while I_C is blocked completely by 10 mM TEA, I_H is in fact increased slightly. The present data suggest that I_H arises from a Ca dependent decrease in a resting Ca current.

44.9 COMPARISON OF CONVENTIONAL MICROELECTRODE AND WHOLE-CELL PATCH CLAMP RECORDINGS FROM CULTURED BULLFROG GANGLION CELLS. M. Galvan*, L.S.Satin*and P.R.Adams. (SPON:N.E.Kremer) Dept. Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

Cultured adult bullfrog ganglion cells show essentially the normal gamut of voltage-dependent membrane currents when studied with conventional microelectrode voltage clamps, but offer the advantage over fresh cells of allowing gigohm seal patch clamp recording. We initially made whole cell recordings using pipettes containing EGTA, HEPES, KCl and 5 mM Mg, and observed very high input resistances (∿1GΩ) and membrane potentials (-70 mV after correction for tip potentials) together with large robust action potentials. However, some of the outward currents normally seen with microelectrodes are small or absent in such patch clamp recordings. In particular the M-current relaxations normally seen when holding at -30 mV and stepping for 600msec to more negative potentials were insignificant, and only tiny M-like currents could be obtained by holding at more positive potentials. Application of 10 μM muscarine to such cells had no effect though 4 mM barium did reversibly inhibit some of the sustained outward current. Because the patch clamp and microelectrode methods may select different cell populations, we combined the 2 on the same cells. A single microelectrode clamp was used to demonstrate the presence of a stable, large M-current. Whole cell recording was then established with a separate pipette, while continously monitoring the M-current via the switch clamp. Within a few seconds a substantial function of M-current was lost, and after several more minutes little remained. To test whether a pipette fluid component was inhibiting M-current we varied calcium in the range 0-1 μM, the nature of the anion, or magnesium. By omitting Mg from the fluid we could recover substantial M-like current. This M-like current was sensitive to barium but not to muscarine. One interpretation of this is that whole cell recording may disrupt the link between muscarine receptors and M-channels. However since Jones (see abstract this volume) has shown that many C-cells are insensitive to muscarine, we cannot exclude the possibility that we coincidentally recorded from such C-cells in the explant cultures. In preliminary experiments using a single microelectrode voltage clamp and a separate intracellular 0.5 M $MgCl_2$-filled microelectrode in fresh ganglion cells we have observed that Mg-iontophoresis can reduce the size of M-current. Determination of normal intracellular magnesium activity would thus be useful in concocting suitable patch clamp pipette solutions. Supported by NS18579 (P.R.A.) the D.F.G. (M.G.), and NS07186 (L.S.S.)

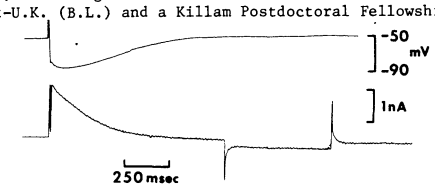
44.10 MINIATURE OUTWARD CURRENTS IN AUTONOMIC NEURONS. L.S. Satin and P.R. Adams.•(SPON:L.M. Mendell) Dept. Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

Cultured bullfrog sympathetic ganglion cells display discrete spontaneous miniature outward currents (or 's.m.o.c.s') under 2 electrode voltage clamp. S.m.o.c.s are generally <1 nA, have a sharp rising phase and a slower, voltage-dependent approximately exponential decay. Both s.m.o.c. frequency and amplitude increase upon depolarization. We have suggested that a s.m.o.c. is due to the synchronous activation of 10-5000 I_C channels by the release of an intracellular packet of Ca^{2+} (Biophys. J. 37, 308a). Similar events have been described recently in cultured DRG neurons (Mathers & Barker, Brain Res. 293, 35). We now report that s.m.o.c. amplitude histograms are exponential in shape, possibly because the "packets" reflect the random open time of calcium channels in an internal membrane compartment. As s.m.o.c. amplitudes vary greatly even at a constant holding potential, mean amplitudes were determined and were found to be a linear function of potential. The least squares regression line intercepted the zero current potential at -92 mV (in 2.5 mM K). The spontaneous hyperpolarizations due to s.m.o.c.s are reversibly blocked by 3 mM TEA; at this concentration I_C would be profoundly reduced in amplitude (Nature 296, 746). These events can be seen even if 1μM TTX or 100-200μM $CdCl_2$ are included in the bathing solutions. Preliminary analysis suggests that the inter-event interval distribution can be adequately described by assuming that s.m.o.c. occurrence is a Poisson process.

Spontaneous hyperpolarizations are also seen in fresh mudpuppy parasympathetic ganglion cells (Hartzell et al., J. Physiol. 271, 817). These events are increased in amplitude by depolarization. Using a single-electrode voltage clamp, we are examining the outward currents that underlie these hyperpolarizations. These currents superficially resemble the events seen in sympathetic cells in both amplitude and waveform. The spontaneous hyperpolarizations are reversibly blocked by 3 mM TEA. Perfusion of 200μM $CdCl_2$ failed to inhibit and may even facilitate them. Supported by NIH Grant NS18579 and the Klingenstein Fund. L.S.S. is an NIH Postdoctoral Fellow (NS 07186).

44.11 HYBRID CURRENT/VOLTAGE CLAMP OF THE BULLFROG SYMPATHETIC NEURON SLOW AFTERHYPERPOLARIZATION. P.R.Adams, P.Pennefather B. Lancaster and R.A.Nicoll. Dept. Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

Minimally damaged ganglion cells exhibit a 2-component afterhyperpolarization (AHP) following single action potentials. The early component is present even in severely damaged cells and decays back to rest passively. It reflects the sum of I_K and I_C as previously discussed (Nature 296 746). Lightly damaged cells show an additional slowly decaying component, which may be separated from the early component by a period of constant or even increasing membrane potential (see Fig.). We switched on a single electrode chopping voltage clamp near the peak of the early AHP to determine what membrane current underlies the late AHP. A small (1 nA at -50 mV holding potential) approximately exponentially decaying (time constant ∿250 msec) outward current, I_{AHP}, was observed (see Fig.). In 2.5 mM K reversal of I_{AHP} as the clamp potential was made negative to -90 mV was sometimes observed, though reversed I_{AHP} was small when visible. Reversal of I_{AHP} at -60 mV in 10 mM K was easily obtained. Thus I_{AHP} is a K-current. However its properties do not correspond with previously described K-currents in these cells (I_K,I_C,I_M, or I_A). Thus the time course of I_{AHP} tails was largely potentially insensitive in the range -50 to -130 mV. I_{AHP} was abolished by 500 μM cadmium, or zero calcium EGTA Ringer, suggesting that it is calcium activated. It was about as sensitive to barium (0.5 mM) as I_M, but much less sensitive to muscarine (20% reduction in 10 μM muscarine compared to 70% for I_M). I_{AHP} was clearly reduced by 5 mM TEA, but much less so than I_K or I_C. This insensitivity could partly reflect spike prolongation and enhanced calcium entry in TEA. However enhancing calcium entry by increasing spike number prolonged the decay of I_{AHP} with only a small amplitude effect. We conclude that the slow AHP of bullfrog ganglion cells is due to a previously undescribed K-current, I_{AHP}. Supported by NS 18579, the Klingenstein Fund (P.R.A.&R.A.N.), the Wellcome Trust-U.K. (B.L.) and a Killam Postdoctoral Fellowship (P.P.)



44.12 TWO-ELECTRODE CLAMP ANALYSIS OF I_{AHP} IN BULLFROG GANGLION CELLS. P. Pennefather, B. Lancaster and P.R. Adams, Dept. of Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

In the accompanying abstract we described a Ca-activated K-current that underlies the slow component of the afterhyperpolarization of bullfrog ganglion cells. Its properties (low sensitivity to TEA and muscarine, voltage insensitivity, slow kinetics and small amplitude) distinguish it from other previously described K-currents in these cells (I_K, I_C, I_M, or I_A). We have used a 2 electrode voltage clamp system, which allows the imposition of short (1-5 msec) spike-like depolarizations, to further study the properties of I_{AHP}, particularly with respect to the other calcium-activated K-current I_C. Double electrode impalement is sufficiently damaging that a clear slow component to the AHP is often absent. In such cells I_{AHP} may already be fully activated by inward calcium leakage. If a slow AHP is present, then brief depolarizations to zero mV elicit rapidly activating, large (40-100 nA) outward currents that are blocked by zero calcium, cadmium or TEA and thus reflect I_C. The calcium-dependent outward currents on returning to the holding potential are composed of a large fast (msec) tail followed by a very slow and small (200-500 pA, 100-500 msec duration) tail, whose amplitude as a function of postpulse potential in normal and high K was consistent with expectations for a K-current. This slow tail thus corresponds to I_{AHP} measured in hybrid clamp experiments. It was clearly less sensitive to muscarine than I_M recorded in the same cell, and was much less sensitive to TEA than the large fast I_C component. The slow tail current was maximally activated by depolarizing pulses as short as 3 milliseconds, suggesting that it can be fully turned on by minimal calcium influx. Even 1 msec pulses are quite effective. Although calcium entry should be reduced for pulses much positive to zero, the slow tail current (unlike the fast I_C component) did not show a corresponding reduction, possibly because the calcium tail current at pulse break is adequate to elicit it. Thus the previously described outward K-currents elicited by iontophoretic calcium injections (Nature, 296, 746) resemble I_C rather than I_{AHP}. In further support of this distinction, we found that apamin (10-100 nM) blocked the slow AHP and the slow Ca-dependent tail current. (c.f. Brown, Constanti & Adams, Cell Calcium 4;408 and Romey & Lazdunski, BBRC 118, 669). Supported by NS18579 and the Klingenstein Fund. B.L. was supported by a travel grant from the Wellcome Trust (U.K.), and P.P. by a Killam Postdoctoral Fellowship.

CIRCUITRY AND PATTERN GENERATION I

45.1 LEECH FEEDING: INITIATION AND TERMINATION. C. Lent and M. Dickinson* Biology & Medicine, Brown University, Providence, RI 02912.

Hungry medicinal leeches bite repeatedly upon warm surfaces, and when fed blood through Parafilm, they increase their weight by 860% over 30 min ingestive periods. Satiated leeches lose 50% of this increased weight within one week, but they do not bite or feed again for 12 to 18 months. In order to determine the chemical and/or mechanical sources of feedback in the short and long term regulation of feeding, we first cannulated the crops of 1-2g leeches. Removing crop contents while feeding prolonged their ingestive periods by 4-8 times. Adding fluid to their crops always produced an immediate behavioral cessation of ingestion. Next, blood was removed from the crops of 10 satiated leeches and their biting behavior was restored immediately. Finally, Ringer (5-8 ml) was injected into crops of 10 hungry leeches whose biting frequency of 0.5/min was reduced immediately to zero. Removing this added Ringer from their crops restores biting. Thus distension of the body wall is the major sensory determinant for the initiation and termination of leech feeding behavior.

We have shown (J. Comp. Physiol. 154,1984) that serotonin has a mandatory role in the integrated expression of leech feeding behavior. Intracellular stimulation of anterior Retzius cells (RZ) evokes salivation and the large lateral (LL) cells induces pharyngeal peristalsis. Thermal stimulation of 2-3°C at the lip synaptically excites both 5-HT cells into phasic bursts of impulses at high frequencies. Stretching the body wall of semi-intact preparations, by injecting Ringer into their crops, generates a tonic hyperpolarization of RZ and LL. Further, body wall stretch terminates or reduces the synaptic thermal excitation of these serotonin-containing neurons. Therefore, stretch, which inhibits leech feeding behavior, both hyperpolarizes and reduces the synaptic excitation of those cells whose serotonin is necessary for the expression of feeding behavior. Supported by NIH grant NS14482.

45.2 SYNAPTIC ACTIVATION OF IDENTIFIED SWIM CENTRAL PATTERN GENERATOR NEURONS IN THE LEECH CNS BY A SEROTONIN-CONTAINING NEURON, CELL 61. M.P. Nusbaum, Dept. of Biology, UCSD, La Jolla, CA 92093.

Intracellular stimulation of a single serotonin-containing neuron, cell 61, causes the expression of the swim motor program in the hirudinid leech Macrobdella decora. In the absence of swim-initiation, cell 61 stimulation often causes a response in the swim central pattern generator (CPG) neuron designated cell 208, in nearby ganglia, whose characteristics are similar to a single cycle of swimming. In such cases, cell 208 responds with an initial depolarization, accompanied by a high frequency burst of impulses, followed by a relatively large hyperpolarization. A second burst of impulses usually follows the hyperpolarization.

The excitatory phase of this membrane potential oscillation results from cell 61 having a strong, apparently monosynaptic, excitatory connection onto cell 208 in nearby ganglia. This excitation is voltage-sensitive, becoming larger as cell 208 is depolarized and smaller as it is hyperpolarized. As a result of this voltage sensitivity, the tonic excitation that cell 208 receives during swimming from the non-serotonergic swim-initiator neuron, cell 204, increases the efficacy of the excitatory effect of cell 61 onto cell 208.

The swim-like response recorded in cell 208 occurs only in cells 208 whose somata are in ganglia nearby the one in which cell 61 is being stimulated. Cells 208 in more distant anterior ganglia do not respond to cell 61 stimulation, whereas those in more posterior ganglia respond with only a tonic excitatory response. This tonic excitation results from the posteriorly directed disynaptic excitatory connection between cells 208, via a newly identified cell pair designated cell 18. This pathway enables local excitation on cells 208 by cell 61 activity to be extended to more distant posterior cells 208. Cell 61 also causes both a depolarization and a barrage of IPSPs in cells 18 of nearby ganglia.

The delayed hyperpolarization of cell 208 by cell 61 is caused by the activation of another swim CPG neuron, cell 60, by cell 61. Cell 60 strongly inhibits cell 208 and is responsible for much of the phasic inhibition to cell 208 during swimming.

The synaptic connections described herein indicate a means whereby cell 61 is able to initiate patterned activity in a swim CPG neuron, which is part of the pathway by which cell 61 stimulation causes the initiation of the leech swim motor program. Supported by PHS NS14410.

45.3 SWIM INITIATION BY NEURONS IN THE LEECH BRAIN OCCURS BY INDEPENDENT PATHWAYS. P. D. Brodfuehrer and W. O. Friesen. Dept. of Biology, University of Virginia, Charlottesville, VA 22901.

Investigations of the mechanisms by which central pattern generators are controlled by neurons from higher order ganglia are an integral aspect of the study of animal movement. We have identified two pairs of neurons in the leech subesophageal ganglion (SubEG), cells Tr1 and Tr2, which can initiate and modulate swimming activity in the segmental nerve cord. These cell pairs are found in the most anterior division of the SubEG. The cells are characterized by large somata (40-60 um diameters) and large neurites with relatively few processes, which cross the midline and project posteriorly into the ventral nerve cord via the contralateral connective.

Stimulation of either cell Tr1 or Tr2 with a short (1-2 s) current pulse triggers swimming activity in brain-nerve cord preparations. Swimming ensues approximately 3 s after the onset of the stimulus pulse. Neither Tr1 nor Tr2 is rhythmically active during swimming episodes; therefore, these cells are neither part of the swim oscillator nor do they receive feedback from it. However, stimulation of either cell Tr1 or Tr2 can phase shift the ongoing swimming rhythm.

To investigate the pathways by which cell Tr1 and Tr2 initiate swimming, we obtained pairwise intracellular recordings from these neurons and either segmental swim-initiating neurons (cells 204 and 205), or serotonin-containing neurons (Retzius cells and cells 61: Kristan, TINS, 6:84,1983). Cell Tr1 is strongly excitatory to these two cell types. Two physiological tests, constant latency postsynaptic potentials and persistence of synaptic potentials with elevated levels of divalent cations, indicate that these interactions are monosynaptic. Cell Tr2, in contrast, does not excite these segmental neurons; however, it does directly (constant latency postsynaptic potentials) inhibit cell 60, a candidate swim oscillatory neuron (Friesen, Neurosci. Abstr. 8:161,1982). In addition, we have observed indirect excitatory drive of swim oscillatory neurons (cells 28 and 208) by cell Tr2. Thus cells Tr1 and Tr2 initiate swimming activity by independent pathways: Tr1 via cells 204, Retzius cells and cells 61; and Tr2 via direct inhibitory and excitatory input to swim oscillator interneurons. Supported by BNS 81-0243.

45.4 THE PHYSIOLOGICAL EFFECTS OF PROCTOLIN AND FMRFAMIDE ON THE STOMATOGASTRIC GANGLION OF PANULIRUS INTERRUPTUS AND CANCER IRRORATUS. S. L. Hooper & E. Marder. Biol., Brandeis Univ., Waltham, MA 02254.

The pyloric system of the stomatogastric ganglion (STG) of decapod crustaceans is a well-studied central pattern generator. Bath applied proctolin and FMRFamide induce characteristic and different changes in the motor output of the STGs of two species of decapod crustaceans, Panulirus interruptus and Cancer irroratus.

In both species proctolin application induced rhythmic motor output of the pyloric system in quiescent ganglia and generally increased the pyloric cycle frequency of slowly cycling STG. Proctolin application also changed the pattern of the motor output. A concentration dependent (10^{-9}M to 10^{-6}M) increase in the length of the LP neuron burst and the number of LP neuron action potentials/burst was seen in both Panulirus and Cancer. In Panulirus, proctolin application also elicited rhythmic activity in the motor neurons of the gastric system.

Bath application of 10^{-5}M FRMFamide also produced alterations of motor output in isolated STG of both species. Like proctolin, FMRFamide was capable of eliciting pyloric activity in quiescent ganglia, and increased the pyloric cycle frequency of slowly cycling preparations. The effects of proctolin and FMRFamide on the burst lengths and phase relationships of the neurons which comprise the motor pattern output were very different. In both species FMRFamide application primarily resulted in increased PY neuron activity, which sometimes resulted in the LP neuron no longer generating action potentials. Since both peptides are capable of inducing cycling in quiescent ganglia it is likely that they modify voltage dependent conductances. Immunohistochemical data (Marder, Hooper, & Siwicki, this meeting) indicate the presence of proctolin and FMRFamide-like fibers in the input nerve to the STG, as well as neuropilar processes in the STG. These data, together with the physiological data reported here suggest that different input neurotransmitters can elicit different motor output patterns from the same neuronal circuit. Research supported by NS-17813 to E.M.

45.5 CHOLINERGIC ACTIVATION OF BURST GENERATING OSCILLATIONS MEDIATED BY OPENING OF CA^{2+} CHANNELS IN LOBSTER PYLORIC NEURONS. F. Nagy*, J.A. Benson and M. Moulins* (SPON: B. Gähwiler). Lab. de Neurobiologie Comparee, CNRS, Place Peyneau, F-33120 Arcachon, France.

The pyloric neurons of the cape lobster (Jasus lalandii) stomatogastric ganglion (STG) produce rhythmic, regenerative depolarizations (plateau potentials) by which their firing is organized into periodic bursts. The ability of pyloric neurons to produce plateau potentials depends upon central inputs; one of these inputs is cholinergic and involves muscarinic receptors (Nagy, F. and Dickinson, P.S., J. exp. Biol., 105:33, 1983). We show that this activition is mediated by opening of Ca^{2+} channels in pyloric neurons.

After deafferentation of the STG, the rhythmic pyloric output disappears. Deafferentation can be achieved by application of TTX on the STG. In this situation, intracellular injection of either brief or prolonged pulses of depolarizing current cannot trigger any active depolarization in pyloric neurons. In the continued presence of TTX, superfusion of the STG with oxotremorine, a cholinergic muscarinic agonist, induces in the pyloric neurons rhythmic slow regenerative depolarizations which are similar to the plateau potentials displayed by the same neurons in the TTX-free saline. We hypothesize that the slow TTX-resistant regenerative depolarizations underlie the plateau potentials of pyloric neurons. These regenerative events are suppressed by the calcium channel blockers Mn^{2+} and Cd^{2+}. In zero Ca^{2+} saline, Ba^{2+} and Sr^{2+} can carry the inward current responsible for production of the regenerative depolarizations. This indicates that Ca^{2+} channels are involved in these TTX-resistant regenerative events.

The induction of regenerative depolarizations by oxotremorine cannot be associated with the closure of K^+ channels. In the absence of the muscarinic agonist, blockade of K^+ channels by either intracellular injection of TEA or superfusion of 4-aminopyridine or cesium, cannot evoke regenerative depolarizations in pyloric neurons.

From these observations, we propose that acetylcholine released from central afferents binds to muscarinic receptors and allows activation of a calcium current. This in turn causes the production of burst-generating plateau potentials in the pyloric neurons.

45.6 OCTOPAMINE INDUCES SLOW PACEMAKER POTENTIALS. M. Wadepuhl* and A. I. Selverston. Dept. of Biol. B-022, UCSD, La Jolla, CA 92093.

The neural pattern underlying the movements of the gastric mill in the lobster (Panulirus interruptus) is essentially produced within the stomatogastric ganglion. The pattern is considered to rely on network properties, though rhythmic activity restricted to one subdivision of the network - that driving the medial tooth - gave rise to speculations about endogenous bursting properties in some members of this circuit.

We discovered that bath applied octopamine (OA) (10^{-4}M) produces slow membrane potential oscillations in DG, a cell resetting the medial tooth. The response reflects endogenous properties of this cell, for 1st) bursting cannot be explained by known circuitry, 2nd) no PSPs are recorded, 3rd) oscillations can be evoked in 0-Ca^{++}, which abolishes synaptic interactions and 4th) the main excitatory input to DG (Int 1) is silenced by 10^{-4}M OA. OA induced bursts are prolonged in comparison to the control. The response seems to be specific for OA since dopamine, histamine and serotonine failed to induce bursts. Concentrations of $10^{-3}$and 10^{-5}M OA were not effective.

OA is almost certainly present in the ganglion according to other authors (Barker D.L., Kushner P.D., Hooper N.K.: Brain Research, 161, 99, 1979; Sullivan R.E., Friend B.J. and Barker D.L.: J. Neurobiol., 8, 581, 1977).

45.7 AMINERGIC MODULATION OF THE PYLORIC RHYTHM IN THE STOMATOGASTRIC GANGLION OF Panulirus interruptus. R.M. Harris-Warrick and R.E. Flamm*, Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14853.

The pyloric motor pattern produced by the stomatogastric ganglion (STG) of the lobster Panulirus interruptus is an extensively studied and relatively simple central pattern generator (CPG). Because of the detailed knowledge of the neuronal components of the pyloric CPG, it is an excellent system to investigate the cellular mechanisms of modulation of centrally patterned motor activity. We have studied the effects of three amines, dopamine, octopamine, and serotonin on the pyloric motor pattern and its neurons.

Experiments were performed on the isolated stomatogastric nervous system. A long-term sucrose block was applied to the stomatogastric nerve to block all modulatory descending input to the STG. This reduced or eliminated much of the pyloric activity and increased the cycle period of the remaining active neurons (usually AB, PD, and VD). Intracellular and extracellular recordings were made from all pyloric neurons. Amines (10^{-4}-10^{-5}M) were bath applied for 10 minutes.

Changes in pyloric activity were qualitatively different for each amine. Dopamine was the most generally excitatory of the three amines. It initiated or enhanced bursting in several neurons (LP, AB, PY's, IC) but decreased or eliminated activity in the PD neurons. Dopamine had variable effects on the cycle frequency. Octopamine was also generally excitatory, enhancing or initiating burst activity in the AB, PD's, LP, and PY's. VD activity was often enhanced, but could be inhibited by simultaneous LP activation. Octopamine had variable effects on the cycle frequency; this depended on the activity state of the LP. Serotonin had more restricted effects than dopamine and octopamine, enhancing activity of the PD/AB group and abolishing VD activity. In some experiments the IC was activated. The cycle frequency was increased.

In conclusion, (1) the three amines each have a unique pattern of alteration of the pyloric rhythm and are therefore candidate neuromodulators of the STG; and (2) the alterations produced by these amines are changes in quantity (neurons activated and inactivated) and quality (changes in cycle frequency) of patterned output. Supported by NIH Grant NS-17323.

45.8 NEURONAL TARGETS OF DOPAMINE, OCTOPAMINE, AND SEROTONIN IN THE PYLORIC CENTRAL PATTERN GENERATOR OF THE STOMATOGASTRIC GANGLION OF THE LOBSTER, Panulirus interruptus. R.E. Flamm* and R.M. Harris-Warrick (SPON: R.R. Hoy), Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

In an accompanying abstract (Harris-Warrick and Flamm), we described the effects of three amines, dopamine, octopamine, and serotonin, on the neurons generating the pyloric rhythm in the stomatogastric ganglion (STG) of Panulirus interruptus. To investigate the detailed cellular mechanisms of aminergic modulation within the pyloric CPG, one must know which neurons are directly affected by each amine. Therefore, we performed experiments to identify the target neurons of dopamine, octopamine, and serotonin.

Experiments were performed on the isolated stomatogastric nervous system with a long-term sucrose block on the stomatogastric nerve. All synaptic connections to each identified pyloric neuron were blocked using a combination of lucifer yellow photoinactivation and pharmacological antagonism of the pyloric neurotransmitters (glutamate and acetylcholine). Amines (10^{-4}-10^{-5}M) were bath applied for ten minutes, and activity of each synaptically isolated neuron was monitored.

Each of the amines produced effects on at least three of the six neuronal classes in the pyloric CPG. Dopamine initiated or enhanced activity in the AP, IC, LP, and PY neurons and inhibited spike activity in the PD's. Octopamine excited the PD and LP neurons. Serotonin increased AB and IC activity, and had no significant effect PD, LP, and PY activity. The VD neuron was inhibited by serotonin.

These experiments show that the target neurons for modulatory inputs to a simple CPG can be identified. As expected, the subsets of directly affected neurons are different for each amine. The effects of each amine on each synaptically isolated neuron are consistent with its effects on the synaptically intact CPG. With the target neurons identified, we are now able to investigate the detailed cellular effects of these three amines on the pyloric rhythm. Supported by NIH Grant NS-17323.

45.9 CENTRAL CONTROL OF SWIMMING IN A PTEROPOD MOLLUSC. R. A. Satterlie. Department of Zoology, Arizona State University Tempe, AZ 85287.

Swimming in the pteropod mollusc Clione limacina results from alternating dorsal and ventral flexions of a pair of laterally-projecting wing-like parapodia (wings). A detailed cine analysis of hovering swimming indicates that the two wings move in synchrony with alternate dorsal and ventral bending movements. Morphological upstroke and downstroke are roughly symmetrical with regard to distance of travel, curvature, pronation and supination.

The central pattern generator for swimming was isolated by sequentually removing central ganglia while observing swimming activity. Removal of pleural, intestinal and buccal ganglia had no effect on swimming. Removal of the cerebral ganglia resulted in a decrease in swim frequency and regularity, but the wings continued to produce synchronous swimming movements. Section of the pedal commissure destroyed coordination of the wings although each wing was capable of independent swimming flexions. These results suggest that each pedal ganglion contains pattern generating circuitry for the ipsilateral wing, and that the pattern generators are coupled via the pedal commissure.

Activation of swim musculature of each wing is via two pools of pedal motorneurons, one each for upstroke and downstroke. Each motorneuron branches repeatedly in the wing and has a wide innervation field. The firing pattern of motorneurons consists of complex synaptic inputs resulting in alternating depolarizations and hyperpolarizations, with action potential bursts superimposed on the depolarizing phases. Activity in upswing and downswing motorneurons is exactly anti-phase. Current injection experiments suggest that motorneurons do not participate in pattern generation and do not synaptically interact with one another.

A class of pedal interneurons has been identified in which the firing patterns are in phase with swimming movements. Interneuronal firing activity is very similar to that of motorneurons except that a single broad action potential occurs with each depolarization. Injected currents can modify interneuron firing frequency, and can similarly modify swimming activity. Each interneuron sends an axon to the contralateral pedal ganglion through the pedal commissure, and provides axon branches in both pedal ganglia. The results suggest that the pedal interneurons are directly involved in pattern generation and may be responsible for bilateral coordination of wing movements.

45.10 MORPHOLOGICAL AND PHYSIOLOGICAL IDENTIFICATION OF PARAPODIAL MOTONEURONS FOR SWIMMING IN APLYSIA BRASILIANA. D.W. Parsons* and H.M. Pinsker. Mar. Biomed. Inst., Univ. TX Med. Br., Galveston, TX 77550-2772.

Chronic recordings from parapodial nerves in A. brasiliana indicated a population of efferent neurons only active during swimming. Backfills of these nerves revealed two discrete pedal cell clusters that we hypothesized to be parapodial opener (with large axons and soma) and closer (with small axons and soma) motoneurons respectively. We now characterize these cells intracellulary with lucifer-filled electrodes using cerebro-pedal connective (CPC) stimulation to elicit the swimming motor program (SMP) in isolated preparations.

In the quiescent preparation, cells that fire in phase with parapodial opening are generally silent. During tonic CPC stimulation these neurons fire rhythmically, in phase with the large unit activity seen during parapodial opening in intact animals. Typically the cell depolarizes tonically and the membrane potential oscillates with the burst of spikes riding on the depolarizing swing of the membrane potential. When stimulation ends, the SMP terminates, the cells stops firing and the membrane potential returns to baseline. Lucifer fills showed that these are neurons from the large soma cluster in the pedal ganglia. From the medio-lateral soma, the neurite loops rostrally in the pedal neuropil before exiting via the parapodial nerves. The neurite typically branches into 2 or 3 axons that project into the anterior, middle and posterior parapodial nerves. No other major neurites branches are seen, though the neurite has many short and fine processes. These large-diameter and heavily-pigmented cells are easily identified morphologically.

During quiescence, cells that fire in phase with parapodial closing fire tonically at low frequencies. During CPC stimulation these cells are briefly inhibited, and then burst in anti-phase to the previous group of cells, i.e., during parapodial closing in intact animals. When stimulation ceases these cells show rebound excitation, and eventually return to pre-stimulus firing rates. Lucifer fills showed that these neurons belong to the small soma cluster. From the soma at the caudal region of the ganglion the neurite loops shallowly in the neuropil. Again, the neurite branches within the neuropil are very small diameter and short. Some of the 'closer' cells identified physiologically project out pedal rather than parapodial nerves.

Supported by NIH and NSF grants to HMP.

45.11 BAG CELL ACTIVITY AND EGG LAYING IN APLYSIA. G.P. Ferguson*, D.W. Parsons*, A. ter Maat* and H.M. Pinsker. (SPON: J.E. Blankenship). Mar. Biomed. Inst., Univ. TX Med. Br., Galveston, TX 77550-2772.

The critical event that determines when egg laying occurs in reproductively mature Aplysia is the "spontaneous" discharge of the neurosecretory bag cells which release an ovulatory hormone. The bag cell discharge causes the release of ripe eggs which move through the oviduct where they are formed into a long string that is eventually deposited on the substrate by rhythmic movements of the head and neck (undulations, weaves and tamps). However, it remains unclear what environmental and internal events cause the bag cells to fire and what behaviors depend exclusively on bag cell hormone.

Because egg laying is seasonal in nature, we examined the effect of increasing temperature on egg laying frequency in two winter-caught species (cold-water A. californica and warm-water A. brasiliana). When the temperature was increased from 15° to 20°C, both species showed a marked increase in egg laying frequency. Increased egg laying in A. californica was attributable both to facilitated oogenesis in previously reproductively immature animals and to increased bag cell activity whereas increased egg laying in A. brasiliana was attributable primarily to increased bag cell activity. Thus, the spontaneous rhythm of bag cell activity across days is highly temperature-dependent.

Because the bag cells in A. brasiliana do not appear to fire unless ripe eggs are available (Pinsker & Dudek, 1977), we examined the effect of gonadectomy on the occurrence of spontaneous bag cell discharges in otherwise intact animals. Chronic recordings showed that bag cells in gonadectomized animals fired as often as they did in mock-operated animals in the first few days after surgery. Thus, spontaneous bag cell discharges do not depend upon the availability of ripe eggs in the ovotestis.

Because the behavioral effects of bag cell extract injections depend on movements of eggs (Cobbs & Pinsker, 1982), we examined the effects of selectively elicited bag cell discharges in gonadectomized animals. Preliminary video analysis indicated a normal increase in undulations following the elicited bag cell discharge in the absence of eggs. However, relatively few weaves and almost no tamps were seen compared to control animals. Thus, in keeping with previous reproductive tract ligations, some of the behavioral consequences of bag cell activity depend upon egg movement. Supported by NIH and NSF grants to HMP.

45.12 NEURONAL MECHANISMS OF A SIPHON MOTOR PROGRAM INDUCED BY PEPTIDERGIC BAG CELL NEURONS IN APLYSIA. P.H. Brownell and M.E. Schaefer. Dept. of Zoology, Oregon State University, Corvallis, OR 97331

We have been investigating the neuronal mechanisms through which the peptide-secreting bag cell (BC) neurons activate long-term changes in motor activities in Aplysia californica. In dissected preparations, stimulated bursts of BC spike activity induce several long-lasting and stereotyped motor responses. One of these "programs" involves facilitation of siphon and gill contractions during respiratory pumping (RP), a periodic strong contraction of branchial organs coordinated by a group of interneurons (Int II) that drive siphon and gill motorneurons. The BC effect on siphon behavior is of interest because it lasts 20 to 60 min and involves 3 distinct components: (1) tonic relaxation of the siphon, (2) increased amplitude of the siphon contraction during RP, and (3) increased frequency of RP. Usually all 3 components develop simultaneously beginning 3-5 min after onset of BC activity.

To determine the cellular mechanisms that mediate each component of the response, we recorded intracellularly from all 7 of the identified siphon motorneurons. BC activity evoked two types of responses among these cells: Long-lasting excitation was observed in the 4 motorneurons ($LDs_{1,2,3}$, and RDs) that mediate contraction of the siphon during RP. The onset and duration of BC effects on these cells were closely correlated with the period of facilitated siphon contraction suggesting a causative relationship. This was confirmed by hyperpolarizing LDs_1 and reversibly reducing the facilitated amplitude of siphon contraction. The second type of neuronal response, long-lasting inhibition, was observed in 3 motorneurons ($LBs_{1,2,3}$) that normally fire tonically and partially contract the siphon. The amplitude and time course of BC-induced inhibition of LB_6 cells was closely correlated with relaxation of the siphon.

Thus, two components of the siphon response to BC activity - increased amplitude of siphon contraction during RP and tonic relaxation - can be accounted for by differential actions of the BC system on 7 motorneurons innervating this organ. The third component of the response - increased frequency of RP - may be an indirect consequence of these neuronal responses or a direct action of the bag cell system on the Int II network of cells. Supported by NIH grant #NS18681.

45.13 CENTRAL CONTROL OF UNDERWATER FLIGHT IN A MOLLUSC. A.N.Spencer. Dept. of Zoology., University of Alberta, Edmonton, Alberta, Canada T6G 2E9.

The pteropod mollusc, Cavolinia inflexa maintains its position in the water column by vertical hovering flight. Flapping of a pair of well-muscularised wings at a frequency of 2 - 6 Hz is centrally controlled. The essential elements of the neuronal machinery for generating the rhythm are contained in the paired pedal ganglia. The majority of neurons in the ganglia display activities that are phasically synchronised with wing elevation or depression as recorded by wing electromyograms. Motorneurons and interneurons can be grouped as either wing elevators or depressors. Fourteen flight motorneurons and seven interneurons have been identified in each ganglion. Motorneurons give a burst of 1-8 spikes on each synaptically driven compound epsp. A burst of spikes in a motorneuron normally never occupies more than 0.3 of a cycle, thus an effective stroke involves serial firing of a population of motorneurons. Although motorneurons display a tendency to spontaneous, rhythmic bursting, precise phase timing is the result of post-inhibitory rebound from large ipsps which appear to come from antagonistic interneurons. Passing long duration hyperpolarizing current pulses into any one of four specific motorneurons slows the rhythm. Motorneurons are not electrically coupled. Interneurons never produce bursts of spikes but characteristically experience large amplitude epsps and ipsps that are synchronised with the flight rhythm. Sometimes a broad spike appears on the depolarizations but it is difficult to determine if they are regenerative. From their morphology it is apparent that interneurons are responsible for bilateral coordination. Passing current into any interneuron alters the flight frequency thus they must also play a critical rôle in rhythm generation. Although the exact mechanism of pattern generation has not yet been determined, bursting in motorneurons corresponds to ipsps in opposite phase interneurons while the peak of depolarization in interneurons is synchronised with ipsps in opposite phase motorneurons. With one exception interneurons are not electrically or dye-coupled.

45.14 INTERNEURONS INITIATING AND MAINTAINING FLIGHT IN LOCUSTS. D.N. Reye*, D.W. Parsons*, G. Bicker* and K.G. Pearson. Department of Physiology, University of Alberta, Edmonton, Alberta, Canada.

Flight activity in locusts usually begins within 30ms following the triggering of a jump, and it is maintained by by an air stream on the head. We are interested in the neuronal mechanisms linking flight initiation to jumping and for the subsequent maintenance of flight activity. In this communication we describe a set of interneurons with characteristics appropriate for their involvement in the initiation and maintenance of flight. We refer to these interneurons as 404s. Their structure has been described by Watson and Burrows (J. Comp. Neurol., 214: 154).

The 404s form a small set of structurally similar neurons in each mesothoracic hemiganglion. To date we have distinguished 3 types by correlating slight but consistent structural differences with differences in physiological properties. Despite these differences all the 404 neurons share a number of properties: 1) they are strongly activated by wind directed towards the head and their discharge rate is correlated with wingbeat frequency, 2) depolarizing currents (5 to 15nA) applied to single 404s initiates flight activity, 3) hyperpolarizing currents either slows or stops flight activity, and 4) they are briefly excited immediately prior to the triggering of a kick of the ipsilateral hindleg. These findings indicate that the 404s function to maintain flight activity. Moreover, their ability to initiate flight activity and their activation during a kick (which uses a motor program similar to a jump) indicates that the 404s may be part of a central pathway linking the jumping system to the flight oscillator.

How the 404s mediate their influence on the flight oscillator has not been established. Watson and Burrows reported numerous output sites from the dendritic processes of these neurons so one interesting possibility is that flight initiation and maintenance involves transmission via these dendritic output sites to mesothoracic flight interneurons.

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46.1 FMRFAMIDE-LIKE SUBSTANCES IN THE LEECH: IMMUNOCYTOCHEMICAL LOCALIZATION. J.R. Kuhlman*, R.L. Calabrese, and C. Li. (SPON: G. Carrow) The Biological Laboratories, Harvard University, Cambridge, MA 02138.

The neuropeptide FMRFamide (Phe-Met-Arg-Phe-NH_2), first characterized in molluscs (Price and Greenberg, Science, 197:670-671, 1977), has since been described in a variety of invertebrates and vertebrates. With immunocytochemistry on whole mount preparations, we have mapped the distribution of FMRFamide-like immunoreactivity (FLI) in central ganglia of the leech Hirudo medicinalis. FLI was localized to approximately 50 cell bodies in a typical midbody ganglion, as well as to processes within the neuropil, roots, and connectives. Deviations from the typical distribution pattern were observed in subsets of the ganglia, particularly ganglia 5 and 6, ganglia 7 and 14, and the most anterior and posterior ganglia of the nerve cord. FLI was also detected in the head and tail brains.

Using both Lucifer yellow intracellular dye injection and indirect immunofluorescence antibody labelling, many of the neurons showing FLI have been positively identified. Identified neurons showing FLI include the rostral and lateral penile evertor motor neurons, the annulus erector motor neurons, the anterior pagoda neurons, and the swim initiating interneuron, cell 204. Of particular interest, the neurons innervating the hearts, the heart motor neurons (HE neurons) and the heart modulatory neurons (HA neurons), showed strong FLI. Immunocytochemical labelling of isolated hearts revealed processes showing FLI running longitudinally along the heart tubes. HRP label was injected into the HE and HA somata and allowed to diffuse to the periphery prior to antibody staining. Double-labelling of the processes showing FLI on the heart confirmed that they were peripheral branches of the HE and HA neurons. These findings have led to investigations of the bioactivity of FMRFamide on the leech heartbeat system (Calabrese et al., this meeting).

We wish to thank W. Watson and T. O'Donohue for their generous gift of anti-FMRFamide antiserum. Supported by NSF grant BNS 81-21551.

46.2 FMRFAMIDE-LIKE SUBSTANCES IN THE LEECH: BIOACTIVITY ON THE HEARTBEAT SYSTEM. R.L. Calabrese, J.R. Kuhlman* and C. Li. The Biological Laboratories, Harvard Univ., Cambridge, MA 02138.

The molluscan cardioactive peptide FMRFamide (Phe-Met-Arg-Phe-NH_2) (Price and Greenberg, Science, 197:670-671, 1977) produces both chronotropic and inotropic effects on a variety of molluscan hearts. The heart motor (HE) neurons and the heart modulatory (HA) neurons which innervate the hearts of the leech, Hirudo medicinalis (Maranto and Calabrese, J. Comp. Physiol., in press, 1984a), show FMRFamide immunoreactivity (Kuhlman et al., this meeting). Encouraged by these observations we investigated the effects of FMRFamide application on the leech's hearts and heartbeat central pattern generator.

Normally, the myogenic hearts are entrained by rhythmic activity in the HE motor neurons that is programmed by a central pattern generator (Maranto and Calabrese, J. Comp. Physiol., in press, 1984b). The HA neurons regulate the myogenic period and the beat tension of the hearts (Calabrese and Maranto, J. Comp. Physiol., in press, 1984). Application of FMRFamide (10^{-7} M) mimics the effects of HA cell activity; it decreases the myogenic period, and it increases the beat tension of a normally entrained heart. Experiments are currently underway to determine if HE cell activity has FMRFamide-like effects on the heart when its phasic interaction with the heart (thought to be cholinergic) is blocked by curare.

Application of FMRFamide (10^{-9} M) to the isolated nerve cord of the leech accelerates the rhythm of the heartbeat central pattern generator. Thus FMRFamide has both central and peripheral effects on the heartbeat system of the leech.

Supported by NSF grant BNS 81-21551.

46.3 NEURAL RELEASE AND PHYSIOLOGICAL ACTION OF AN IDENTIFIED PEPTIDE CONTAINED IN CRAYFISH MOTOR NEURONS. C.A. Bishop, F. Nagy*, J.J. Wine and M. O'Shea. Dept. of Psychol., Stanford Univ., CA 94305 and Dept. of Pharm. Physiol. Sci., Univ. of Chicago, IL 60637.

Proctolin (H-Arg-Tyr-Leu-Pro-Thr-OH) coexists with 'conventional' neurotransmitters in tonic skeletal motor neurons of the cockroach (Adams, M. and O'Shea, M., Sci., 221:286-289, 1983) and the crayfish (Bishop, C. et al., J. Neurosci., in press). In the cockroach, proctolin is released by nerve stimulation and acts postsynaptically to cause a prolonged tension increase, without changing the membrane conductance or transmembrane potential of the muscle fibers (Adams, M. and O'Shea, M., ibid, 1983). We have now shown that proctolin is also released by crayfish motor neurons, and that it acts postsynaptically to increase tension. However, in contrast to the results with insects, proctolin did not itself cause a tension increase in resting crayfish muscle fibers, and it potentiated the amplitude but not the duration of tension evoked by nerve stimulation.

We used the tonic flexor motor system of the crayfish abdomen, which consists of 6 identified motor neurons and about 40 muscle fibers per hemisegment. Release of proctolin was demonstrated by use of a sensitive bioassay. Proctolin could be detected in the 75 µl bath of the target muscle after stimulation of a single motor neuron at 10 Hz for 5 min. Release was specific to the three motor neurons previously shown to react histochemically with proctolin antibody, and was absent from the largest motor neuron, which also does not react with antibody.

The physiological effect of proctolin was assessed by superfusing neuromuscular preparations. In the absence of nerve stimulation, proctolin caused no measurable tension at concentrations up to 10^{-6}M. Proctolin also had no affect on the amplitude or time course of synaptic potentials, nor on the resting transmembrane potential or input resistance of the muscle fibers. However, when superfusion was paired with electrical stimulation of single motor neurons, tension was increased by as little as 10^{-9}M proctolin, and with 10^{-8}M tension was increased by 20% to 300%, with the higher value occurring for stimulation of a non-proctolinergic motor neuron. The duration of tension increase was not changed by proctolin.

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46.4 NEW CLASSIFICATION OF INSECT MOTONEURONS: EXPRESSION OF DIFFERENT PEPTIDE TRANSMITTERS. J. Witten, M.K. Worden*, M.H. Schaffer, and M. O'Shea. Department of Pharmacological and Physiological Sciences, The University of Chicago, Chicago, IL 60637.

Proctolin (Arg-Tyr-Leu-Pro-Thr) is a co-transmitter of the identified slow coxal depressor or Ds motoneuron of the cockroach (Adams and O'Shea, 1983. Science 221:286). Here we show it is associated with a subset of the slow motoneurons in cockroach and locust. We establish proctolin as a transmitter with multiple neuromuscular functions and as a co-transmitter of the slow extensor tibialis or SETi motoneuron of the locust. We also provide evidence that many peptides may serve as transmitters for insect motoneurons. This suggests that insect motoneurons are heterogeneous with respect to peptide transmitter type. The functional implications of this are being investigated.

At least two peptide types other than proctolin are implicated as putative neuromuscular transmitters in our studies. These are the newly discovered myoactive peptides MI(pGlu-Val-Asn-Phe-Ser-Pro-Asn-Trp-NH_2) and MII(pGlu-Leu-Thr-Phe-Thr-Pro-Asn-Trp-NH_2) (O'Shea, Witten and Schaffer, 1984. J. Neurosci. 4:521) and peptides related to gastrin/CCK which contain the sequence Trp-Met-Asp-Phe-NH_2. Our immunohistochemistry shows that each subgroup of peptide-containing motoneurons we stain represents only a small proportion of the total motoneuronal pool indicating to us that the number of different peptides associated with motoneurons may be surprisingly high. Thus we suggest a classification system based on peptide transmitter type that cuts across the more traditional system which classifies motoneurons into four types: 1) fast excitatory (glutaminergic), 2) slow excitatory (glutaminergic), 3) inhibitory (GABAergic) and 4) modulatory (octopaminergic).

What are the functional implications for the diversity of peptide neuromuscular effectors? Stimulation of the SETi motoneuron releases proctolin which produces a prolonged catch-like contraction and also activates a myogenic rhythm in the target muscle. Peptides MI and MII increase lipid metabolism, cause more persistent tonic muscle contraction and also activate cardiac myogenicity. A reason, therefore, for the existence of so many different types of peptide neuromuscular effectors may be the need to produce a diverse array of effects on metabolism and muscle performance in insects.

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46.5 MOLECULAR BIOLOGICAL STUDIES OF THE SEQUENCED INSECT NEUROPEPTIDES. M.H. Schaffer, B.E. Noyes*, and M. O'Shea. Departments of Psychiatry and Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637.

There are four neuropeptides which have been isolated from insects and sequenced. They are proctolin, and the three structurally related peptides, adipokinetic hormone (AKH), MI, and MII. We have recently sequenced the latter two cockroach peptides by FAB Mass Spectroscopy. The sequence for MI is pyro-Glu-Val-Asn-Phe-Ser-Pro-Asn-Trp(NH_2) and the sequence for MII is pyro-Glu-Leu-Thr-Phe-Thr-Pro-Asn-Trp(NH_2) (O'Shea, M., Witten, J. and Schaffer, M. J. Neurosci., 4:521, 1984; Rinehart, K., Hemling, M., Cook, J., Schaffer, M., O'Shea, M. and Witten, J., manuscript submitted, JACS, 1984).

Using organ culture and ^{3}H-labeled amino acids we have shown that locust AKH and cockroach MI and MII are synthesized in the corpora cardiaca (CC) and that homogenates of cockroach CC are also capable of synthesizing fully processed MI and MII. These systems will be used to investigate synthetic intermediates and processing activities employed in the synthesis of the AKH family peptides.

To study the mRNA coding for AKH we had synthesized a mixture of four undecadeoxynucleotides, 5'-d [GT(T/G)CCCCA(A/G)TT], designed to include an oligonucleotide capable of specifically hybridizing to the AKH mRNA. This mixture was separated by HPLC into two pools of two oligonucleotides each, which were then used to prime the synthesis of cDNA from locust CC RNA using reverse transcriptase. This gave a complex mixture of products. To reduce the number of cDNA products requiring analysis, syntheses were performed in the absence of one or two of the four deoxynucleotide triphosphates and products were separated by size. Inspection of possible AKH mRNA sequences predicted from the peptide sequence indicated which size cDNAs were possible candidates for AKH cDNA. These were subjected to nucleotide sequence analysis which identified an AKH specific cDNA of 23 nucleotides. From this, we had synthesized a 22 nucleotide oligodeoxynucleotide complementary to the AKH mRNA and have used this probe to obtain additional sequence from the AKH message. This oligo also provides a specific probe for in situ hybridizations and screening of recombinant DNA libraries.

This same approach, applied to cockroach ganglion poly A$^+$ RNA, yields a partial sequence for proctolin mRNA.

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46.6 **MONOCLONAL ANTIBODIES THAT RECOGNIZE CARDIOACTIVE PEPTIDES IN THE MOTH, MANDUCA SEXTA.**
P.H. Taghert, N.J. Tublitz, J.W. Truman and C.S. Goodman Dept. of Biol. Sci., Stanford Univ., Stanford, CA 98305 and Dept. of Zoology, Univ. of Wash., Seattle, WA 98195.

We are interested in the developmental regulation and physiological functions of neuropeptides. In order to facilitate our studies, we have generated a set of 29 monoclonal antibodies (Mabs) that individually recognize specific subsets of identified peptidergic neurons in abdominal ganglia of the moth (Taghert et al, 1983, Neurosci. Absts. 9: 314). Because these Mabs were selected histologically, it was not certain that any in fact would regognize the secretory products of the neurons. We now report that two Mabs (2F5 and 6C5) appear to recognize two small cardioactive peptides (CAPs) that are present in the moth CNS.

These peptides accelerate the beat rate of isolated moth hearts, have MWs of approximately 1 and 1.5 KD and have been localized to 6 specific neurons in each abdominal ganglion (Tublitz and Truman, 1983, Neurosci. Absts. 9: 19). Of the 29 Mabs tested, only 2F5- and 6C5-immunoreactivity (as tested with ELISAs) co-elute with the two peaks of biological activity that result from gel filtration of abdominal ganglia. Furthermore, both Mabs specifically decrease the biological activity of either CAP (in partially purified forms) by as much as 90%. With immunocytochemistry, these two Mabs stain a similar set of neurons: the CAP-containing neurons stain strongly; as many as 20 of the 700 other neurons in each ganglion stain more weakly. Finally, CAP bioactivity and 2F5- and 6C5-immunoreactivity show very similar patterns of developmental modulation: both are detectable starting at approximately Day 4 of adult development. Together these lines of evidence suggest a specific recognition of the two CAPs by Mabs 2F5 and 6C5.

We are currently using these antibodies to further characterize the cellular distribution, physiological properties and molecular nature of the CAPs. In addition, we have made a cDNA library from Day 8 developing adult moth CNS poly A+ RNA (2 x 10^5 recombinants) in λgt10 and have converted it to the expression vector λgt11. This library is being screened with the various Mabs to try and isolate the message(s) that encode CAPs and as well those that encode the other peptidergic cell-specific antigens.

46.7 MORPHOLOGICAL ANALYSES OF IDENTIFIED SEROTONIN-PROCTOLIN CONTAINING NEURONS IN THE LOBSTER. B. Beltz and E.A. Kravitz. Neurobiol. Dept., Harvard Med. Sch., Boston, MA 02115.

Serotonin exerts a wide range of physiological actions on many different lobster tissues. Immunocytochemical studies of lobster ganglia have identified presumptive serotonergic neurons, their central and peripheral projections, and their terminal fields of arborization (Beltz and Kravitz, J. Neurosci. 3:585, 1983). More than 100 neurons have been found in the lobster nervous system that show serotonin-like immunoreactivity. We chose two pairs of these neurons (in the fifth thoracic [T5] and first abdominal [A1] ganglia) for extensive morphological and physiological studies because: (1) they appear to contribute varicosities to peripheral neurohormonal release sites and to have extensive projections within central ganglia; and (2) they stain immunocytochemically for the peptide proctolin (Siwicki and Kravitz, Soc. Neurosci. Abst. 9 (1):313, 1983). Physiological Identification. To identify these cells in living preparations, neurons in T5 and A1 were penetrated with microelectrodes. Lucifer yellow was injected into neurons that fulfilled the following criteria: (1) they were approximately the same size and in the general location of the immunocytochemically labeled cells; and (2) they could be backfired by extracellular stimulation of the ipsilateral connective two segments anterior to the cell body (stimulation site determined from immunocytochemistry of axonal projections). Following lucifer yellow injections, preparations were fixed and processed for serotonin immunocytochemistry using the PAP method. Cells that were correctly identified in the living preparation were therefore double-labeled with lucifer yellow and HrP. Using this method, we have found that the pairs of T5 and A1 neurons can be identified reliably. Cell Morphology. We then injected hexamine cobalt chloride or HrP into the cells to obtain detailed morphological pictures of individual cells. These studies showed that T5 and A1 paired neurons project anteriorly through at least five segmental ganglia. In each ganglion a distinctive, repeating pattern of branching of cell processes is seen. Branches from each cell project out the ipsilateral thoracic second roots of anterior ganglia to form varicosities of the peripheral plexus of endings; other branches form varicosities in neuropil regions of central ganglia.

Our current studies are focused on computer imaging of these neurons, and on their embryonic development, physiological properties, and relationship to behavior.
(Supported by NS-07848 to E. Kravitz).

46.8 PROCTOLIN COLOCALIZES WITH SEVERAL DIFFERENT TRANSMITTERS IN LOBSTER NEURONS. K.K. Siwicki* and E.A. Kravitz (SPON: S. Glusman). Neurobiology, Harvard Medical School, Boston, MA 02115

The peptide proctolin (Arg-Tyr-Leu-Pro-Thr) has been found in the nervous systems of many invertebrate species, including the lobster, Homarus americanus (Schwarz, et al, J.Neurosci. in press). Using two different antisera (one raised in our laboratory, the other kindly supplied by M. O'Shea), we completed a detailed immunocytochemical map of the distribution of proctolin-like material in the lobster nervous system, and found over 1000 stained cells. A few of these show evidence of colocalization of proctolin with other transmitters. Bilateral pairs of large cells in the fifth thoracic and first abdominal ganglia stain with antibodies to both proctolin and serotonin. These cells can be identified physiologically (Beltz & Kravitz, previous abstract) and dissected from ganglia. In addition, a single large cell in each circumesophogeal ganglion stains with antisera to both proctolin and tyrosine hydroxylase (kindly supplied by T. Joh). This cell, likely to be a homologue of the large dopaminergic cell in the circumesophogeal ganglion of other Crustacea (Cooke & Goldstone, J.Exp.Biol.53, 1970; Barker, et al, Br.Res.161, 1977), can be identified for dissection by both its size and position in a desheathed ganglion. Reverse phase HPLC analysis of extracts of these identified cells showed a peptide comigrating with authentic proctolin (average content per cell = 10 fmoles). We recently developed a procedure for HPLC analysis of both peptides and monoamines from single cells, and these analyses are now in progress. In addition to these large monoamine-containing cells, proctolin-like immunoreactivity was seen in both sensory and motor axons in the lobster respiratory system. In collaborative studies with V. Pasztor, we found that the sensory dendrites and two of the axons of the oval organ mechanoreceptor stain for proctolin, as do several axons in motor nerves innervating respiratory muscles. Proctolin-containing excitatory motoneurons have been identified previously in locust (Adams & O'Shea, Science 221, 1983) and crayfish (Bishop, et al., J.Neurosci. in press) neuromuscular preparations.

Thus proctolin or a closely related substance may colocalize with a number of other transmitter compounds. This may reflect a common peptide-activated mechanism useful in many different contexts. (Supported by NS-07848 to EAK)

46.9 SPONTANEOUS AND INDUCED RELEASE OF THE PEPTIDE ECLOSION HORMONE FROM IDENTIFIED NEURONS IN THE MOTH MANDUCA SEXTA. P.F.Copenhaver and J.W.Truman. Dept. of Zoology, Univ. of Washington, Seattle, WA 98195.

Eclosion hormone (EH) is an 8500 dalton peptide that triggers the stereotyped behavior of adult emergence in Lepidoptera. During adult development in the moth Manduca sexta, EH is synthesized in the animal's brain and transported out to the associated neurohemal organ (the corpora cardiaca-allata) for storage and secretion. The timing of release is strictly governed by two factors: the declining titers of the steroid 20-hydroxyecdysone specifies the day of release, while circadian input determines the time of day that release occurs (Truman JW, Am. Zool. 21, 655, '81). How these two controlling factors impinge on the individual neurosecretory cells to regulate their activity is unknown and requires the identification of the EH neurons.

To identify the EH cells in the moth brain, the nerves leading to the neurohemal organ were backfilled with cobalt, revealing three groups of cells in the protocerebrum and a fourth in the tritocerebrum. By cleanly dissecting each cell group and testing them with a sensitive behavioral bioassay, we localized the EH-containing cells to bilaterally paired clusters of 9 dorsolateral cells. Injection of cobalt into individual cells of this group revealed a distinctive morphology, with their dendritic arborization lying superficial to the major neuropilar regions of the brain. In addition, intracellular stimulation of individual lateral cells induced release of EH bioactivity into the bath, whereas stimulation of the medial cells did not.

The spontaneous activity of the EH cells around the time of adult emergence was monitored in a minimally dissected preparation which allowed both intracellular and extracellular recording from the neurosecretory cells and nerves. Continuous extracellular recording from these nerves around the time of expected adult emergence revealed a sharp increase in tonic firing of several units coincident with the release of EH into the surrounding bath. By combining this viable semi-intact preparation with intracellular recording techniques, it should be possible to examine directly the mechanisms controlling EH release at the level of the single neurosecretory cell.

46.10 Immunocytochemical Localization and cDNA Cloning of Small Cardioactive Peptides in Aplysia. A.C. Mahon*, P. Lloyd, K. Weiss, I. Kupfermann and R.H. Scheller. Dept. of Biol. Sci.,Stanford Univ.,Stanford, CA 94305 and Dept. of Neurobio. and Behav., Columbia Univ., New York, NY 10032

The amino acid sequence of the molluscan cardioactive peptide, SCP_B, has been determined (Morris,H.R. et al., Nature 300:643,1982). A role for this peptide in feeding behavior has been proposed based on physiological data (Lloyd, P.E. et al., PNAS, In press). We have determined the distribution of SCP_B immunoreactive neurons in cryostat sections of the CNS of adult Aplysia. In the buccal ganglion, identified neurons B1 and B2 and 3-5 smaller neurons in close proximity to these large (250 micron) cells react strongly with the antisera. A cluster of 20-40 small (5-10 micron) cells on the rostral margin of the dorsal surface also react strongly . Faint staining of 3-4 medium (100 micron) cells in the region of B3,B6,B9 and B10 is observed. All of the ring ganglia (pleural, pedal and cerebral) contain unidentified SCP_B immunoreactive cells. No reactive neurons have been detected in sections of the abdominal ganglion. All ganglia show a large amount of staining in the neuropil.

To determine the structure of the SCP_B precursor protein we have characterized a cloned cDNA segment which encodes SCP_B. Using a differential screening procedure putative SCP_B encoding cDNA clones were isolated from cDNA libraries to the buccal and ring ganglia but not to the abdominal ganglion. This mRNA distribution is consistent with the distribution of SCP_B immunoreactive neurons. The complete nucleotide sequence of one cloned buccal cDNA isolate predicts the sequence of a 136 amino acid precursor protein with a characteristic hydrophobic signal sequence. The sizes of the predicted pre-pro-precursor (14.8 kd) and of the pro-precursor (12.5 kd) are in agreement with in vivo and in vitro labeling studies. The precursor protein contains one copy of SCP_B and a related 11 amino acid peptide, SCP_A. The carboxy terminal end of both peptides is followed by gly arg, the signal for proteolytic cleavage followed by carboxy terminal amidation.

Hybridization of 32p-labeled buccal cDNA to size fractionated DNA from 8 individuals indicates that at least six genomic DNA segments contain SCP related sequences. This result suggests that more than one SCP gene may exist.

46.11 IMMUNOCYTOCHEMICAL STUDY OF THE DISTRIBUTION OF SMALL CARDIOACTIVE PEPTIDE (SCP_B) IN APLYSIA. I. Kupfermann, A. Mahon,* R. Scheller,* K.R. Weiss and P.E. Lloyd,*. Center Neurobiol. & Behav, NYPI; Depts of Physiol., Anat.& Cell Biol., and Psychiat., Columbia P&S, and Schl. Dent. & Oral Surg., New York, NY, and Dept. Biol., Stanford Univ. CA.

We have examined the distribution of small cardioactive peptide B in Aplysia using immunohistochemistry. Antibodies were raised in rabbits, using SCP_B conjugated to albumin. Staining was done on whole mounts of late juvenile stage animals, using a modification of the methodology of Goldstein et al. (Neurosci. No.2, 1984). Controls included preabsorption of antiserum with SCP_B or other peptides and replacement of primary or fluorescent second antibody with normal rabbit serum. In selected cases, SCP_B in given tissues or individual cells was independently measured by means of HPLC followed by bioassay (see Lloyd et al., Neurosci. Abs. 1984). SCP_B immunoreactive processes were observed in all central ganglia as well as gut, salivary glands, and muscles of the buccal mass. Relatively large numbers of strongly positive cells were observed only in the buccal ganglion, and included 1) B1 and B2 which innervate the gut, 2) a characteristic cluster of small neurons, and 3) many large neurons, which are located within a cluster of cells that includes identified cholingergic motor neurons involved in biting responses. The cerebral, pedal, and pleural ganglia each exhibited a small number of characteristic immunoreactive neurons as well as intense staining of the neuropil. The abdominal ganglion also showed intense staining of the neuropil, but only two lightly stained neurons were observed. Although SCP_B mimics the action of serotonin at several sites in the animal (Lloyd et al., PNAS, May 1984; Castellucci et al., Neurosci. Abs. 1984), unlike serotonin, SCP_B was not present in meshworks of fibers surrounding cell bodies. Our data suggest that although SCP_B appears to be extensively involved in a single behavioral function, i.e. feeding, its wide distribution indicates that it is likely to have multiple functions.

46.12 SEQUENCE AND NEURONAL LOCALIZATION OF A NEWLY CHARACTERIZED NEUROPEPTIDE IN APLYSIA. P.E. Lloyd,* I. Kupfermann, K.R. Weiss. Center Neurobiol. & Behav., Columbia Univ., NY 10032

The nervous tissue of Aplysia contains a number of neuropeptides that are active on periperal tissues. One class of neuropeptides that are particularly potent are termed the small cardioactive peptides (SCPs). There are two major SCPs in Aplysia, SCP_A, and SCP_B (Lloyd, Fed. Proc. 41:2948, 1982). The SCPs are broadly distributed in the CNS (Kupfermann et al, Neurosci. Abstr. 1984). The sequence of SCP_B has recently been reported (Morris et al, Nature:300, 1982). We have now purified SCP_A from acidic extracts of 2000 Aplysia guts (which contains high concentration of the SCPs) by the use of three sequential analytical procedures: high pressure (HP) ion-exchange chromatography and two modes of HP-reverse phase chromatography. Amino acid composition was: Ala_2, Arg_2, Gly, Leu, Met, Phe, Pro_2, Tyr. We have determined the sequence of the N-terminal 6 amino acids as Ala-Arg-Pro-Gly-Tyr-Leu-. From a combination of the composition and partial sequence determination, and assuming the C-terminal is homologous to SCP_B (see below), we hypothesized the following sequence for SCP_A:

SCP_A: Ala-Arg-Pro-Gly-Tyr-Leu-Ala-Phe-Pro-Arg-Met-NH_2
SCP_B: Met-Asn-Tyr-Leu-Ala-Phe-Pro-Arg-Met-NH_2

This sequence for SCP_A was confirmed by demonstrating precise co-elution of native (labelled in vivo with S^{35}-methionine) and synthetic SCP_A on each of the three analytical procedures used to originally purify the peptide. Furthermore, the synthetic peptide was equipotent with the native peptide on the isolated snail heart. Both SCP_A and SCP_B are present in equimolar concentrations in each of two giant neurons (B1 & B2) which innervate the gut and are located in the buccal ganglia. In addition, the two peptides are synthesized at identical rates in each of these neurons suggesting that the two peptides may be synthesized on a single precursor, a proposal consistent with the recent isolation of a cDNA clone that encodes both peptides (Mahon et al., Neurosci. Abstr. 1984).

46.13 TWO INHIBITORY MOTOR NEURONS IN THE LEECH USE GABA AS THEIR NEUROTRANSMITTER. H.P.Cline. Grad. Group in Neurobiology University of California, Berkeley, Ca. 94720.

In the leech Hirudo medicinalis, two pairs of inhibitory motor neurons, known as cells 1 and 2, are candidate GABAergic neurons by virtue of their ability to accumulate GABA by a high affinity uptake system. Cells 1 and 2 innervate the longitudinal muscles in the body wall, and within the ganglion, they monosynaptically inhibit the excitatory motor neurons to the same muscles.

I have recorded intracellularly from the postsynaptic excitatory motor neurons and found that focal application of exogenous GABA to the cell body or into the neuropil causes an increase in membrane conductance accompanied by a membrane hyperpolarization. Therefore, these cells have receptors for GABA which mediate changes in the membrane properties similar to those seen with stimulation of the inhibitory neurons. Bath application of bicuculline methiodide ($5X10^{-5}M$), a known GABA antagonist, reversibly blocks both the GABA-mediated change in membrane properties as well as the inhibitory synapse onto the excitatory motor neuron, without interfering with other inputs. The peripheral targets of cells 1 and 2 are also responsive to GABA. I recorded intracellularly from single muscle fibers. Spontaneous ipsps and epsps were recorded from the same muscle fiber, although different regions of the fiber were preferentially innervated by either inhibitory or excitiatory terminals. Focal application of GABA onto a site where ipsps are large and frequent results in an increase in membrane conductance. In contrast, application of GABA to a site where epsps predominate results in little or no increase in conductance.

I assayed the ability of cells 1 and 2 to synthesize GABA after I had isolated them from the ganglion and grown them in culture. The inhibitory motor neurons synthesize about 6 pmol of GABA in 3 hours, whereas cholinergic motor neurons synthesize less than 0.5 pmol (the limit of the assay).

In summary, GABA is very likely to be the neurotransmitter used by the inhibitory motor neurons, cells 1 and 2, in that it fulfills the following criteria: GABA is synthesized by these neurons. They accumulate GABA, thereby removing it from the synaptic cleft. Application of exogenous GABA onto the central and peripheral targets mimics the effect of stimulating the inhibitory motor neuron. Finally, the synapse is specifically blocked by bicuculline methiodide, a GABA antagonist.

This work was supported by NIH training grant GM 07045.

PEPTIDES: ANATOMICAL LOCALIZATION I

47.1 LOCALIZATION OF A TRANSFERRIN-LIKE PROTEIN IN THE ADULT RAT CNS. J.R. Connor and R.E. Fine*. V.A. Medical Center and Dept. of Physiology, George Washington Univ. Washington DC and Dept. of Biochemistry and Physiology, Boston Univ. School of Medicine Boston MA.

Recent studies have demonstrated that extracts of sciatic nerves have a myotrophic influence on muscles in culture. The myotrophic factor is an 80,000 MW glycoprotein given the name "sciatin". More recently, sciatin has been shown to be similar in structure and function to transferrin, the iron binding and transport protein in vertebrate blood. Further evidence suggests sciatin is manufactured in spinal neurons in vitro. The present study was designed to localize sciatin in the adult CNS. Four adult male rats (Sprague Dawley) were perfused with 4% paraformaldehyde. Sections were cut on a Vibratome at 50 µm thickness from the somatomotor cortex, pons, cervical and lumbar spinal cord regions. Following pretreatment, sections were incubated in antisciatin antisera (1:1000) or in pre-immune rabbit sera (controls) overnight and processed according to the method of Sternberger (1979). The most intensely immunoreactive cells in the CNS are the three classes of oligodendrocytes: perivascular, interfascicular, and perineuronal satellite cells. Immunoreactive oliogodendrocytes are visible throughout all CNS areas examined. Motor neurons in the ventral gray of the spinal cord and large pyramidal neurons in the somatomotor cortex are also immunoreactive. In the oligodendrocytes, the reaction product is frequently found as a cap on one end of the soma. Infrequently, processes of oligodendrocytes are immunoreactive. The reaction product in the neurons forms a thin rim in the cytoplasm surrounding the nucleus. Occasionally, the reaction product extends into the initial portion of dendrites and the axon. The reaction product in both the oligodendrocytes and neurons is granular, although some dispersed reaction product is also visible in oligodendrocytes. Further analysis at the ultrasctructural level is presently under consideration.

Supported by the Veterans Administration & NIH

47.2 **IMMUNOCYTOCHEMICAL LOCALIZATION OF NEUROPEPTIDES IN APLYSIA** Thane Kreiner* and Richard H. Scheller(SPON:A. DiBerardino) Dept. of Biological Sciences, Stanford University, Stanford, CA 94305

The Aplysia abdominal ganglion neurons R3-14 contain a 1.1 kb mRNA that is not expressed in other Aplysia neurons; this message encodes a 14 kd protein which is proteolytically processed to yield potentially biologically active peptides. Peptides corresponding to three regions of the precursor were synthesized and used to generate polyclonal antibodies. Immunocytochemical studies demonstrate that only the R3-14 neurons specifically react with the antisera to each of the three regions of the precursor protein.

Whole mount preparations of juvenile animals reveal numerous processes arising from R3-14 which terminate in the vascularized connective tissue sheath surrounding the ganglion. R3-14 also send prominent processes out the branchial nerve; these processes are varicose along their length, and terminate in the efferent vein from the gill, the anterior aorta, and the auricle. The distribution of varicosities suggests that these cells could exert effects both centrally and peripherally. Double-label experiments demonstrate that the same processes contain all three regions of the precursor; no processes containing one region and not another were observed (Kreiner,et.al.,in press,J. Neurosci.). The R3-14 neurons in Aplysia are thought to use glycine as a modulator of cardiovascular physiology (Price and McAdoo, 1981,Brain Res.). Electron microscopic studies should reveal if different regions of the precursor and/or glycine are packaged into the same vesicles.

The functional significance of the coexistence of multiple messengers in a single neuron is not clear; however, the relative simplicity of the Aplysia CNS affords opportunity to address these issues.

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47.3 COLOCALIZATION AND SEGREGATION OF PEPTIDES IN THE HYPOTHALAMIC ARCUATE NUCLEUS OF THE RAT.
B.M. Chronwall, R.M. Knight* and T.L. O'Donohue. Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20205.

The hypothalamic arcuate nucleus (arc) is the major source of opiomelanotropinergic (POMC) neurons in the CNS. ACTH, αMSH and β-endorphin are colocalized and are all derived from a common prohormone. In addition a number of other peptides have been localized in the arc. These include neuropeptide Y (NPY), somatostatin (SS), FMRFamide and enkephalin.

The question of possible colocalization of peptides was addressed employing double immunohistofluorescent staining. The primary antibodies were produced in different species and controls were performed to insure that the secondary antibodies did not bind to each other and result in false positives.

NPY and POMC are two distinct populations of neurons throughout the nucleus. In each frontal section the 60-80 NPY neurons were 5-8 μm wide, 8-10 μm long, with axes oriented towards the ventricle. The cells were clustered close around the ventricle and there was a dense fiber network occupying the entire area. The 15-20 POMC neurons were 10-15 μm in diameter, polygonal and situated in the lateral part of the nucleus. At mid levels the NPY perikarya numbered 140-160, the POMC:30-40. In caudal parts there were few perikarya. The NPY innervation was dense whereas that of POMC was sparse.

NPY and SS were present throughout the nucleus, in frontal and midparts SS perikarya were much fewer (10-20). Although the neurons had the same morphology and general distribution, colocalization was only found occasionally.

NPY and FMRFamide are colocalized in frontal and mid sections. In the caudal aspect, the dorsolateral part of the nucleus showed cells that contained only FMRFamide, in the ventral parts there was colocalization.

FMRFamide and enkephalin, SS and POMC, enkephalin and POMC: no colocalization was noted.

In conclusion, there appears to be coexistence as well as segregation between the peptides of the arc. It must be noted, however, that coexistence may be more widespread than our methodologies can detect as non-detectable concentrations of a particular peptide may coexist with another and that relative concentrations could be dramatically influenced by the physiological state (Kiss et al., Proc. Natl. Acad. Sci. USA 1984:84, 1854-1858).

47.4 GONADOTROPIN RELEASING HORMONE (GnRH) - PRODUCING NEURONS IN THE RAT PREOPTIC-DIAGONAL BAND AREA: AN ELECTRONMICROSCOPICAL STUDY. L. Jennes, W.E. Stumpf, M.E. Sheedy*. Dept. of Anatomy, Univ. of North Carolina, Chapel Hill, NC 27514.

With preembedding immunohistochemistry at the electronmicroscopical level two classes of GnRH positive neurons could be identified: A "smooth" GnRH neuron with even contours and a "spiny" GnRH neuron with numerous short protrusions at the perikaryon and at the cell processes. Both cell types contain a large round or ovoid nucleus which does not show invaginations, an extensive rough endoplasmic reticulum which occurs in the form of individual cisternae or multilayered stacks, and several well developed Golgi complexes. Under the conditions of the animals studied the lysosomal system is not extensive. GnRH cell bodies contain frequently nematosomes, kinocilia and lamellar whorls, as well as dense core vesicles with a diameter of 100nm and clear vesicles with a diameter of 30-40nm. Neurites of the "smooth" GnRH cell origniate as elongated, continuously thinning cones which contain all cell organelles but the nucleus. Neurites of the "spiny" GnRH cell are thin and show bifurcations, irregular contours and accumulate both clear and dense core vesicles in their spines. In areas distant to the perikaryon, a different type of neurite is found, which is characterized by a large number of immunoreactive dense core vesicles, as well as numerous unreactive clear vesicles. Some of these GnRH positive fibers are seen adjacent to immunonegative neurons, occasionally making contact via synaptic as well as non-synaptic terminals. In addition to giving rise to presynaptic elements, GnRH neurons form postsynaptic specializations when apposed by a presynaptic terminal. Synaptic contacts are seen rarely on "smooth" cells but occur frequently on "spiny" cells. The results indicate the presence of two types of GnRH neurons with different patterns of innervation, which suggests differing integrative capacities probably correlated to "tonic" and "cyclic" GnRH release.

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47.5 CAPSAICINE-INDUCED DEPLETION OF CORTICOTROPHIN RELEASING FACTOR (CRF) IMMUNOFLUORESCENCE FROM MEDULLA OBLONGATA AND SPINAL CORD OF THE RAT. J.Schipper*, H.M.W.Steinbusch, F.J.H.Tilders, (Spon: P.Bevan). *Dept. Pharmacology, Duphar B.V., 1380 AA Weesp; Dept. Pharmacology, Free University, 1081 BT Amsterdam, The Netherlands.

By use of antibodies raised against ovine CRF, immunoreactive cell bodies and nerve terminals have been observed in formaldehyde-fixed rat brain tissue. In addition to the presence of CRF immunoreactivity (CRF_i) in the hypothalamus, we reported also the presence of a dense plexus of CRF_i nerve terminals in the medulla oblongata and spinal cord (Schipper et al, Brain Res. 267:145). In order to unravel the function of this lower brain stem CRF_i neuronal system, we studied the effects of various experimental manipulations on these CRF_i fibers and compared them with the effects on the CRF_i in the hypothalamus and median eminence.

Hypothalamic lesions that induced a complete disappearance of CRF_i in the median eminence did not affect the lower brain stem CRF_i. Hypophysectomy, adrenalectomy and reserpine treatment that induced a reduction in the CRF_i of the median eminence, failed to affect the lower brain stem CRF_i. Mesencephalic lesions have indicated that the presence of CRF_i fibers in the lower brain stem are not caused by long descending efferents from hypothalamic neurones. Therefore, these CRF_i fibers are either intrinsic to the lower brain stem or they represent the afferents of primary sensory neurons.

Capsaicine, a drug known to deplete neuropeptides from primary afferents, induced a complete disappearance of the CRF_i, seven days after the last injection, in the substantia gelatinosa of the spinal cord, whereas the CRF_i in the median eminence was unaffected. In addition to the depletion of CRF_i there was also a marked reduction in the number of Substance P immunoreactive nerve terminals, but no effect could be observed on the number of serotonin immunoreactive nerve terminals in the substantia gelatinosa.

These results indicate that primary afferents contain a CRF-like peptide that can be stained with an antiserum raised against ovine CRF.

47.6 ORIGINS OF SOME DYNORPHIN-CONTAINING PATHWAYS IN THE SUBSTANTIA NIGRA-VENTRAL TEGMENTAL AREA OF THE RAT. J.H. Fallon, A. Mehta, J. Kirby, F. Leslie and R. Cone, Dept's of Anatomy and Pharmacology, University of California, Irvine, CA 92717.

Dynorphin (DYN)-containing fibers and terminal-like plexuses are distributed throughout all sectors of the substantia nigra-ventral tegmental area (SN-VTA). In this study we investigated some of the origins of these fibers with combined immunofluorescence and retrograde tracing techniques.

The location of DYN-containing fibers and cell bodies was determined, respectively, in uninjected and colchicine-injected adult female albino rats. In order to determine connections of DYN-containing cell bodies, animals were injected with .05-0.2ul of retrograde fluorescent tracer (Fast Blue, Nuclear Yellow, Propidium Iodide, SITS), then later injected with 0.1-2.0ul of colchicine. Animals were sacrificed and processed for combined FITC immunofluorescence and retrograde tracing. Adjacent sections were processed with primary antisera raised against either DYN B, DYN A 1-8, met-enkephalin-arg-gly-leu (MERGL) (Antisera kindly supplied by Dr. Eckard Weber), antisera preabsorbed with the appropriate blocking peptides, or in the absence of primary antisera.

The double-labeling experiments demonstrated the presence of the following pathways containing DYN B and DYN A 1-8 immunoreactivity: 1) Moderately dense projections from the medial caudate putamen to the pars reticulata of the SN, 2) Moderate projections from the amygdala to the dorsal VTA and the pars compacta and pars lateralis of the SN, 3) Light projections from the lateral and medial hypothalamus to the VTA, medial SN and brainstem, and 4) Light projections from the peripeduncular region and pars lateralis of the SN to the amygdala. Therefore, while most descending projections examined terminate in the SN-VTA, hypothalamic projections also continue en passage to the brainstem.

The present studies provide neuroanatomical evidence for heterogeneous, topographically organized DYN-containing pathways in the SN-VTA. Similar pathways were visualized with MERGL antisera, except that the striatonigral projections were less pronounced. Thus pro-dynorphin (DYN B, DYN A 1-8) and pro-enkephalin products are present in parallel pathways and, perhaps, in some cases, in the same neuron.

(Supported by NIH Grants NS 16017 and NS 18843).

47.7 OXYTOCIN PROJECTIONS TO THE RAT CEREBELLUM. G. Nilaver, M.J. Perlow, G. Valiquette*, J. Haldar, G. Abrams, and E.A. Zimmerman. Dept. Neurol., Columbia Univ., Coll. of Phy. & Surg., New York NY 10032; & Univ. of Illinois, Chicago, IL 60612.

Oxytocin (OT), vasopressin (VP) and their carrier proteins, the neurophysins (NPs) have been demonstrated in several extrahypothalamic sites by radioimmunoassay (RIA) and immunocytochemistry (ICC). There is however, little information about the cerebellar content of these peptides. A recent study demonstrated the presence of direct hypothalamo-cerebellar projections as evidenced by retrograde labeling of hypothalamic neurons following injection of HRP tracer into the lateral cerebellar cortex (Dietrichs, Science: 223, 1984). Neurons labeled included those in the periventricular and supraoptic nuclei. Since these nuclei contain OT, VP and NP, we studied the possible innervation of the cerebellum by these peptides employing ICC. Long-Evans rats were perfusion fixed with 10% buffered formalin. Brainstem and cerebellar sections (100um; coronal & sagittal) were immunoreacted with specific rabbit antisera to OT, VP and a rabbit antiserum that recognizes both NPs, employing biotinylated protein A with avidin-biotin peroxidase in the preembedding staining technique. These studies demonstrated a significant OT fiber projection to both the folial and deep nuclear regions of the cerebellum. The fibers appear to enter the cerebellum via the superior cerebellar peduncle. This finding, and the absence of immunoreactivity in cerebellar perikarya even upon colchicine pretreatment suggests an extracerebellar origin of these fibers. Dense NP fiber staining was also seen in the pia-arachnoid overlying the cerebellar folia. Very few VP fibers were found in the cerebellum despite comparable staining in the hypothalamus. This anatomical observation was confirmed with regional RIAs which also demonstrated OT predominance in this brain region. A similar pattern of OT and NP immunoreactivity was also seen in cerebellar primordial tissue allografted into various regions of adult rat brain. Our demonstration of OT projections to the rat cerebellum is consistent with the hypothalamocerebellar projection reported by Dietrichs. The precise hypothalamic origin of these OT fibers, and their pattern of cerebellar arborization however, remain to be determined. Since a cerebello-hypothalamic projection has been described from the fastigial nucleus (Harper & Heath, Exp. Neurol.:285,1983) these results imply reciprocal interactions between these two brain regions, and a role for OT in the hypothalamic modulation of cerebellar autonomic activity. (Supported by NIH grants HD 13147, NS 18324, and the Parkinsons' Disease Foundation).

47.8 NEUROPEPTIDE Y INNERVATION OF THE AMYGDALOID COMPLEX IN THE RAT: AN IMMUNOHISTOCHEMICAL ANALYSIS. E.L. Gustafson*, J.P. Card and R.Y. Moore. Dept. of Psychology and Depts. of Neurology and Neurobiology and Behavior ,SUNY, Stony Brook, NY 11794.

Neuropeptide Y is a 36 amino acid polypeptide extracted and characterized from brain (Tatemoto,1982). NPY has 20 amino acid homologies with avian pancreatic polypeptide(APP), and antisera to NPY (Emson,1984), APP (Kimmel et al,1976), and the molluscan cardioexcitatory peptide (FMRF; Weber et al,1981) demonstrate identical patterns of immunoreactivity in the rat lateral geniculate-suprachiasmatic nucleus projection Moore et al,1984). Previous studies have reported NPY-, APP- and FMRF-like immunoreactivity in the rat amygdaloid complex but no detailed description has been made nor has the issue of cross-reactivity been addressed. The present study is directed to the following: 1) providing a detailed description of NPY-, APP- and FMRF-like immunoreactivity in the amygdala, 2) determining whether immunoreactive cell bodies in the amygdala provide a component of the amygdala innervation of the hypothalamus and 3) determining whether the three antisera demonstrate the same pattern of immunoreactivity. All tissue was processed according to the PAP method of Sternberger (1979) and the material was analyzed by light microscopy.

The distribution of immunoreactive cell bodies and fibers in the amygdaloid complex is identical with antisera generated against NPY, APP and FMRF. Blocking studies further suggest that NPY is the endogenous peptide. Most of the immunoreactive perikarya are found at intermediate and caudal levels of the complex with many cells located in the medial and cortical amygdaloid nuclei and scattered cells in the base of the stria terminalis and along the course of the stria. Scattered immunoreactive perikarya are also found throughout the remaining amygdaloid subnuclei. Immunoreactive fibers in the amygdala are generally sparse but a moderately dense plexus is present in the medial one-third of the central nucleus and a moderate to dense plexus in the caudal medial nucleus adjacent to the optic tract. Lesions of the stria terminalis and medial nucleus do not affect the the NPY innervation of hypothalamus. These observations suggest that NPY immunoreactive neurons in the amygdaloid complex project within the amygdala and do not contribute to the amygdala innervation of the hypothalamus.
Supported by NSPHS Grant NS-16304

47.9 OXYTOCIN AND VASOPRESSIN IN RAT BRAIN AND SPINAL CORD. G.Valiquette*, J.Haldar, G.M.Abrams, G.Nilaver and E.A.Zimmerman. Dept. of Neurology, Columbia Univ. P & S, New York, NY 10032 and Dept. of Biological Sciences, St. John's Univ., New York, NY 11439

We have previously reported the distribution of vasopressin (AVP) in rat brain by radioimmunoassay. We are now reporting the study of the same extracts by oxytocin (OT) radioimmunoassay. Brains and spinal cords from normal male Long Evans rats were dissected and extracted with boiling 2M acetic acid. The extracts were lyophilized, reconstituted in buffer and assayed. Results are expressed in ng/g wet weight (mean ± SEM).

Area	AVP	OT	OT/AVP
P. Pit.	215±50.9(ug)	219±45.3(ug)	1.02
A.Pit.	51.8±15.4	33.0±10.1	0.64
Hypoth.	648±38.0	1171±175	1.81
Amyg.	25.5±6.03	1.67±0.131	0.07
Fr.Cx.	0.299±0.136	0.968±0.284	3.24
Hippo.	0.359±0.0602	0.934±0.183	2.60
Mid.Br.	1.20±0.276	2.05±0.361	1.71
Pons	0.808±0.174	4.42±1.01	5.47
Medulla	4.79±2.29	10.2±1.95	2.14
Cerebl.	0.208±0.0181	0.670±0.0760	3.22
C.Cord	1.76±0.273	3.59±0.199	2.04
T.Cord	2.35±0.429	6.46±1.55	2.75
LS.Cord	5.24±0.608	9.85±0.294	1.88

The predominance of OT over AVP throughout the extrahypothalamic CNS is remarkable. The amygdala is the only site where AVP is more abundant than OT. In the spinal cord, both peptides increase in concentration along a rostral to caudal gradient while their ratio remains essentially unchanged; this roughly parallels the relative importance of the intermediate horn in the cord. AVP has been implicated in a number of homeostatic mechanisms, including blood pressure regulation, and its presence in areas such as midbrain and pons is consistent with this function. The high concentrations of AVP in the amygdala and its striking predominance over OT, limited to this area, suggests that this peptide may play a yet unidentified physiological role in these nuclei, possibly relating to its reported effects on memory. The function of OT in extrahypothalamic CNS is unknown and merits further attention. (Supported by NIH grant HD13147 and the Parkinson's Disease Foundation)

47.10 HYPOTHALAMIC IMMUNOREACTIVE PROLACTIN NEURONS ARE TARGETS FOR ESTROGENIC ACTION. B.D. Shivers*, R.E. Harlan*, and D.W. Pfaff. The Rockefeller University, New York, NY 10021.

An estimated two-three thousand neurons in the mediobasal hypothalamus of the rat contain immunoreactive (ir)-prolactin, which is not of pituitary origin (Shivers, et al., Abstracts Soc. Neurosci. 9: 1018, 1983). These neurons send a strong projection to the dorsal midbrain, where microinfusions of rat prolactin facilitate the estrogen-dependent reproductive behavior, lordosis (Harlan et al., Science 219: 1451, 1983). Many hypothalamic cells are targets for estrogenic action i.e., they concentrate estradiol in their nuclei (Pfaff and Keiner, J. Comp. Neur. 151: 121, 1973). The purpose of the present study was to determine whether any of these estradiol-concentrating neurons contain ir-prolactin, by combining on the same tissue section steroid autoradiographic and immunocytochemical methods.

Results were obtained from analysis of paraformaldehyde-fixed, 6 µm cryostat sections of brains derived from three colchicine-treated, ovariectomized rats receiving tritiated estradiol (S.A. 134 Ci/mmol, 0.25-.29 µg/100g rat). Autoradiograms were exposed at -15C for 144 to 186 days. Prolactin-like immunoreactivity was localized using rabbit anti-rat prolactin antiserum (1:1000; National Hormone and Pituitary Program) and the avidin-biotinylated peroxidase complex (ABC) method. Sections were counterstained with cresyl violet. Only ir-prolactin cell bodies with visible nuclei were included in the analysis. Nuclei having reduced silver grains at least three times background were designated estrogen-concentrating cells.

Ir-prolactin neurons were observed in a continuum from the arcuate nuclei, ventral and lateral to the ventromedial nuclei. Ir-prolactin neurons were localized from the retrochiasmatic area caudally to premamillary nuclei levels. The distributions of estrogen-concentrating cells and of ir-prolactin neurons appeared similar to those observed previously. Of the 1693 ir-prolactin neurons examined to date, 518 (or about 30%) concentrated estradiol. In a preliminary analysis, a higher percentage of the more caudally-located ir-prolactin neurons were estrogen-concentrating.

Many ir-prolactin neurons concentrate estradiol and presumably are primary targets for regulation of gene expression by estrogen. The results support the proposal that ir-prolactin neurons are of central importance in the regulation by estrogen of behavioral and autonomic phenomena.

48.1 DEFICITS IN MANIPULATIVE BEHAVIORS INDUCED BY LOCAL INJECTION OF MUSCIMOL IN DIFFERENT AREAS OF THE HAND REGION OF CONSCIOUS MONKEY SOMATOSENSORY CORTEX (SI). Y.Iwamura, O.Hikosaka, M.Tanaka* and M.Sakamoto*. Dept. of Physiol. Toho Univ. Sch. of Med., Otaku, Tokyo, Japan 143

A small amount of muscimol, a potent agonist of GABA, was injected at various sites in the hand region of the conscious monkey SI to investigate deficits in the hand manipulative behaviors caused by the reversible and localized dysfunction of the cortex. We first recorded single cell activities using a glass-coated platinum-iridium microelectrode and determined their receptive field characteristics. Then we inserted a pipette for muscimol injection through the same hole on the dura resulted from the preceding electrode penetration. Changes in monkey's behavior following the injection were examined using various tasks that required the monkey to manipulate her hand and fingers in precise or complex ways to pick up small pieces of food pellets from various containers. These tests were done also without monkey's vision. Eighteen injections of muscimol were made and changes in tactile behavior were detected following 15 injections. The behavioral changes varied depending on the injection site and time after the injection. Injections in area 2 disrupted behaviors that required organized informations from multiple fingers. For example, in the antero-medial part of area 2 a cluster of neurons was recorded along a penetration perpendicular to the cortical surface. Their receptive fields were on the 2nd - 5th fingers. They responded to either the passive nail stimulation or the finger joint manipulation, or to both. However they were activated most vigorously when the animal actively picked up a small piece of apple from a small hole (20 mm in diameter) on the wooden block. The subsequent injection of muscimol to the same site disrupted this behavior. The 3rd - 5th fingers lost stability and repeated clumsy flexion and extension while the thumb and the index finger attemted to dig a piece of apple out of the hole. Injections in area 3b interfered with the same behaviors, but in different ways, possiblly by the disruption of somatotopically discrete information. The results support our hypothesis that area 2 of the SI hand region is composed of various neuronal clusters each of which is related to certain types of manipulative behaviors by integrating and sending essential feedback signals to the motor cortex.

48.2 REGIONAL (^{14}C) 2-DEOXYGLUCOSE UPTAKE DURING VOLUNTARY FORELIMB MOVEMENTS IN THE RAT. M.F. Gonzalez*, R. F. Gariano*, and F.R. Sharp (SPON: D.A. Trauner). Dept. Neurology, SFVAH, San Francisco, CA 94121 and Dept. Neurosciences, UCSD, La Jolla, CA 92093

The rat brain regions activated during repetitive left forelimb movements induced by right motor cortex stimulation have been mapped using the (^{14}C) 2-deoxyglucose (2DG) technique (Sharp, JCN 224:259, Sharp and Ryan, JCN 224:268). The present study describes the pattern of (^{14}C) 2DG uptake in trained rats.

Sprague-Dawley rats were trained to grasp and rapidly press a lever on fixed-ratio schedules of 16 or 32 presses per reinforcement using standard operant conditioning techniques. Then they were taught to use only one forelimb by punishing undesired motor behaviors with electric shocks and habituated to tail vein injections of saline. When the training was complete, (^{14}C) 2DG was injected instead of saline. After a 45 min. lever-pressing session, the subjects' brains were removed, sectioned, and autoradiographed as previously described. Controls received the same schedule of food pellet reinforcements, but had no forelimb movements.

Lever-pressing rats exhibited clear increases of 2DG uptake in the contralateral primary motor cortex (MI), and in both the first (SI) and second (SII) somatosensory cortices. The pattern in SI was complex. Two laminae of 2DG uptake were identifiable in activated regions of MI and SI. A very distinct, small region of lateral prefrontal cortex was also activated--primarily in an intermediate lamina. 2DG uptake also increased in contralateral thalamus. The ventrolateral (VL)--ventralanterior (VA), ventrobasal (VB), paracentral and centrolateral intralaminar, reticular, and parafasicular nuclei were activated. Only portions of each thalamic nucleus were active. Discrete areas of globus pallidus, caudate-putamen, subthalamic nucleus, substantia nigra, cerebellar granule cell layers, and many brainstem regions also increased 2DG uptake.

Most of the above changes in the trained animal were also seen during forelimb movements elicited by motor cortex stimulation. However, the increased 2DG uptake in the lateral prefrontal cortex of trained animals did not occur during motor cortex stimulation (Sharp, JCN 224:259). This result could be related to the fact that the limb movements in the trained animal were not electrically induced but initiated on the rat's own volition.

47.3 THE EFFECTS OF PARIETAL AND FRONTAL LESIONS ON A SUSTAINED ATTENTION PARADIGM G.Gücer, L.Viernstein*, R.Syzmanski*. Department of Neurosurgery, Baltimore City Hospitals and Johns Hopkins Hospitals Balt. Md. 21224

The neural mechanisms involved in attention are being studied by various methods. The local rate of cerebral blood flow has been used as an index of local brain activity. Such measurements have been made in humans who were required to shift their attention from one modality to another in performing a task. The measurement showed increased cerebral blood flow in the superior lateral part of the pre-frontal cortex. We have developed a similar paradigm for trained monkeys who for rewards must discriminate between two separate frequencies (10Hz vs 100Hz) of threshold vibration applied to the glabrous skin of the hand. The task is repeated in a different sensory modality requiring the discrimination between two frequencies (10Hz vs 100Hz) of auditory clicks. The threshold amplitude of the stimuli used in the two tasks is such that the monkeys must sustain their attention to the task at hand to maximize their performance. The threshold amplitude and random time of occurrence of the stimuli used in the two tasks require that the monkeys give sustained attention to the task. Four well trained monkeys were used in the study. In two monkeys the peri-arcuate cortex was bilaterally removed and in two monkeys the parietal cortex contralateral to the hand receiving the stimulation was removed. Monkeys with bilateral arcuate lesions were unable to perform either task for two months after lesions even though they had little motor deficits in feeding or grooming. Their reaction times were generally delayed. In contrast monkeys with parietal lesions were able to perform these tasks within two to three days after lesions were made. We interpet the inability of arcuate lesioned monkeys to perform the tasks to be caused by their inability to attend to the task at hand and/or initiate a go-command to carry out the motor task. Therefore further studies of the peri-arcuate cortex in sustained attention paradigms are indicated.

47.4 THE INTER-STIMULUS SPONTANEOUS ACTIVITY OF PERI-ARCUATE NEURONS DURING A SUSTAINED ATTENTION PARADIGM L.Viernstein*, G.Gücer, R.Syzmanski*, (Spon:M.Greenberg) Department of Neurosurgery Baltimore City Hospitals and The Johns Hopkins Hospitals Balt. Md 21224

Single unit studies of the peri-arcuate cortex have demonstrated discharge patterns selective for auditory, visual and tactile stimuli. In this same general area of the monkey brain. lesions made in the arcuate gyrus disrupt the ability to perform tasks in alternating attention paradigms. Studies of the posterior bank of the arcuate gyrus have shown the presence of cells whose discharge patterns are enhanced when there is a rewarding saccade to a target but not for hand movement to the target.Hence, in monkeys trained in paradigms of alternating attention the neural pool in areas 4,6,and 8 appear to be important in coordinating the information from different sensory modalities and in sequencing of the appropriate motor response. We have performed an initial survey of these neurons in these areas to classify them in terms of their activity while the monkey is engaged in an attention paradigm. In four monkeys, the discharge patterns of 160 neurons near the arcuate sulcus was studied in both hemispheres of the brain. This was done while the monkeys were engaged in a task requiring sustained attentiveness to somatosensory stimuli and also for task requiring sustained attentiveness to auditory stimuli. The mean impulse rate of the inter-stimulus spontaneous activity of 37 of the neurons differed as a function of the sensory channel to which the monkey was giving sustained attention. The nature of the neural activity of all of the neurons could be classified into five categories based on peristimulus histograms. The general results seem to indicate that the average level of activity of certain classes of neurons is associated with sustained attention to a particular sensory channel.

48.5 DENDRITES OF DEEP LAYER, SOMATOSENSORY SUPERIOR COLLICULAR NEURONS EXTEND INTO THE SUPERFICIAL LAMINAE. R.D. Mooney, B.G. Klein, M.F. Jacquin and R.W. Rhoades. Dept. of Anatomy, Univ. of Medicine and Dentistry of New Jersey, School of Osteopathic Medicine and Rutgers Medical School, Piscataway, NJ 08854.

There has been considerable debate (see Edwards, S.B., in The Reticular Formation Revisited, J.A. Hobson and M.A.B. Brazier eds. New York, Raven Press, 1980, for a review) regarding the extent to which the superficial and deep layers of the mammalian superior colliculus (SC) are interconnected and whether the sensory processing in the superficial laminae affects the responses of deep layer neurons. We have used intracellular recording and horseradish peroxidase (HRP) injection techniques to demonstrate that some deep layer SC cells in hamsters extend dendrites into the superficial, retinorecipient laminae, and that these same neurons possess purely somatosensory receptive fields.

Conventional methods were used to physiologically characterize, impale and inject HRP into 44 SC neurons in 25 normal adult hamsters. Of these, 34 were recovered in the deep laminae (the layers ventral to the stratum opticum - SO). Two of these cells had visual receptive fields, 6 were unresponsive and the rest (N=26) could be discharged only by somatosensory stimuli. Ten deep layer cells extended dendrites into the superficial laminae. In 3 instances these processes reached the SO and in the rest they were visible in the stratum griseum superficiale. Three of the latter had dendrites which extended to the collicular surface. All ten of these cells had exclusively somatosensory receptive fields, and they could not be differentiated physiologically from somatosensory neurons whose processes were restricted to the deep laminae. None of the deep layer neurons we have recovered so far possessed axon collaterals which ascended into the superficial laminae.

Ascending dendrites of deep layer cells may not be the only anatomical substrate for communication between the superficial and deep laminae. Six of the superficial layer neurons we have recovered so far gave off axon collaterals which descended at least as far as the stratum griseum intermediale. Four of these cells were exclusively visual, one was somatosensory and one was unresponsive.

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48.6 ALTERATIONS IN RHYTHMICAL MASTICATORY MOVEMENTS CAUSED BY AN OBJECT BETWEEN THE TEETH. G. Lavigne*, C. Griffiths*, J.S. Kim*, C. Valiquette* and J.P. Lund. Cent. de Rech. Sci. Neurol., Université de Montréal, Canada.

Peripheral inputs are important in the control of rhythmical mastication. Tactile stimulation of the oral cavity activates the central pattern generator (C.P.G.) and the final output is under sensory control. As in other systems, feedback from muscle spindles is important; its removal by paralysis probably contributes to the slowing of the masticatory rhythm and the fall in the output of jaw closing motoneurons. Paralysis also stops feedback from periodontal pressoreceptors, which, as well as their direct effects on motoneurons, may act at the level of the C.P.G. In this study, we have investigated the effect on the pattern of mastication, of introducing a hard object between the teeth of rabbits anesthetized with urethan. Jaw movements were recorded by a photodiode transducer system. These signals and electromyograms (EMGs) from the major jaw muscles, were recorded on magnetic tape and later analysed with computer assistance. Stereotyped masticatory movements with a large swing to left during closure were evoked by stimulation of the right motor sensory cortex (6 - 25V, 50 Hz, 15S). After a control period, a small ball was pushed between the left upper and lower anterior molars by a pneumatic piston. When the teeth made contact with the ball, there was a large, but unequal increase in EMG activity of all closing muscles, one result of which was a rapid swing of the mandible back to the midline. The closing movement was not complete and all the other phases of the movement were reprogrammed. In order to see if the changes in the pattern of movement were primarily dependent on information coming from the periodontal receptors, the areas around the teeth or the afferent nerves, were infiltrated with a local anaesthetic (prilocarpine HCl). This reduced or abolished the changes in the masticatory pattern caused by the perturbating stimulus for about 60 minutes. If the superior and inferior dental nerves were cut, the effect was permanent. These results suggest that changes in periodontal pressoreceptor input, caused by differences in the texture and hardness of food or by alterations in the form of the teeth, cause adaptive modifications of the masticatory pattern.

(Supported by Canadian Medical Research Council)

48.7 EFFECTS OF TOTAL BODY ROTATION ON MASSETERIC MOTONEURON EXCITABILITY. T. M. Moriarty*, R. S. Hickenbottom*, and B. Bishop. Depts. of Physiology and Physical Therapy, SUNY at Buffalo, Buffalo, NY 14214

Vestibular stimulation is a popular clinical treatment for certain motor dysfunctions, yet the neurophysiological mechanisms by which vestibular input contributes to brain-stem motor control are not known. The purpose of this study was to determine whether full body rotation modified the excitability of the masseteric motoneuron pool. Using the monosynaptic test, masseteric compound action potentials (CAPs) evoked by standard chin taps served as our measure of motoneuron excitability.

Twenty experiments were performed on 10 healthy adults with no oro-facial or otological disorders. Each subject (S) sat in a modified dental chair with head and body stabilized by a halo head piece and seat harness. The force and frequency (3/sec) of the chin taps were maintained constant in each trial by micro-computer control. Responses of the right masseter muscle were recorded by surface electrodes. After each tap, a 16 ms sample of EMG was analysed for amplitude and latency of the response and stored for subsequent visual inspection. Only CAPs meeting rigorous criteria in terms of latency, duration and waveform were considered as responses. The chair was rotated at 30 rev/min. Data from each experiment were divided into 4 phases: 1) "control" before rotation (30 representative trials), 2) during rotation (30 representative trials), 3) during deceleration (all 60 to 80 trials) and 4) post-rotation (30 trials). The frequency of occurrence and the means ± SD of the amplitudes were compared within and across subjects. Three Ss had no responses during phases 1, 2 or 3, but at cessation of rotation, when ampullary hair cells reverse direction, a burst of several successive responses occurred. The other 7 Ss had responses in every phase, but the frequencies of occurrence were significantly greater during phases 2 and 3 than during control. During phase 4, the increases in frequency of occurrence and in response amplitude were further enhanced. This post-rotational facilitation decayed slowly over one minute. In two Ss the facilitation persisted for at least 5 minutes. When the experiment was repeated one minute later, the changes in response parameters during phases 3 and 4 were significantly less than for the first experiment, suggesting habituation. These results provide quantitative evidence that the dynamic input from vestibular ampullary receptors is facilitatory to masseteric motoneuron output.

48.8 NEUROMUSCULAR CONTROL MECHANISMS IN OROMOTOR BEHAVIOR. K.V. Anderson, N.F. Capra and M. Bailey*. Dept. of Anatomy, Univ. of Miss. Med. Ctr., Jackson, MS 39216.

Recent experimental studies in cats and monkeys have suggested that the basic neurological control mechanisms that underlie spinal motor behavior might function differently than those that underlie oromotor behavior. The present studies addressed this issue by analyzing the activity patterns of muscles that cause jaw opening and closing movements and comparing these results with those from studies of muscles that serve opposing functions around limb joints. To accomplish our goals, jaw movements were studied in 13 cats, which had stimulating electrodes implanted into the pulp chamber or periodontal ligament receptors of right maxillary canine teeth. Electrical stimulation through these electrodes produced an active opening of the jaw, as has been reported previously. Electromyographic (EMG) activity was recorded from indwelling electrodes implanted into jaw opening (JO; digastric) and jaw closing (JC; masseter) muscles. In addition, the posterior fossa was opened to permit surgical access to the medullary and pontine regions of the lower brain stem so that known sensory-motor pathways involved in oromotor behavior could be surgically disrupted to help determine the neurological basis for the activity recorded from JO and JC muscles.

The results showed that both JO and JC muscles were simultaneously active during movements of the jaw. While the digastric muscle was dramatically active during JO, the masseter muscle was also activated, but to a lesser degree. Conversely, JC was accompanied by substantial EMG activity from the masseter, with somewhat less, but nevertheless substantial, activity present in the digastric muscle. Analyses of the quantitative and temporal features of JO and JC muscles during oromotor responses indicate that antagonistic muscles that operate around the temporomandibular joint (TMJ) behave synergistically by co-contracting to produce movements of the jaw. These results are in agreement with some recent observations in antagonistic muscles that function around a limb joint, but stand in sharp contrast to the common finding of reciprocal inhibition as the major pattern of activation of antagonistic muscles that act about a particular limb joint. While the functional significance of simultaneous co-contraction of opposing muscle groups operating around the TMJ is not yet known, such actions could help to control the rate and extent of JO and JC movements during various important oromotor activities, such as mastication, deglutition, phonation and, even, breathing.

48.9 GIANT VISUAL INTERNEURONS IN THE BLOWFLY SEGREGATE TO THE LEG AND NECK MOTOR. N.J.Strausfeld,H.S.Seyan and U.K.Bassemir.EMBL,D-6900 Heidelberg,F.R.G.

The fly, Calliphora erythrocephala,has eleven vertical (VS) and three horizontal (HS) motion-sensitive neurons arising in the lobula plate of each optic lobe and ending in the ipsilateral mid-brain (Hengstenberg,R.,et.al.,J.Comp.Physiol.149: 163,1982). Behavioural and physiological studies propose their cardinal role in fast "optomotor" visual control of flight (Hausen,K., Verh.Dtsch. Zool.Ges. 1981: 49,1981).

We transsynaptically filled specific subsets of VS and HS cells with cobalt by backfilling selected axons from the brain within, and branching from, the cervical connective. As demonstrated earlier, cobalt ions cross such coupled systems at areas of gap junctions (Strausfeld.N.J. & Bassemir,U.K., J. Neurocytol. 12:971.1983).We found that the downward looking HSS neurons in both lobes, and the frontally viewing VS2,3 neurons of the ipsilateral optic lobe are coupled to motor neurons leading to the ipsilateral neck muscles. This result lends strong support to the proposal that motion-sensitive pathways control saccadic head movement (Land,M.S.In: The compound eye and vision in insects.Ed.G.A.Horridge, O.U.P:469,1975). We also showed by light and electron microscopy that only VS4-9 are coupled to a uniquely identifiable descending neuron (DNOVS) which receives also many chemical presynaptic endings from ocellar interneurons. The remaining giant visual cells (VS1,VS10,VS11;HSE,HSN) contribute but a fraction of the total input to a tightly packed cluster of descending neuron dendritic trees whose main inputs are many hundreds of small-field visual interneurons and mechanosensory afferents that are mostly derived from the antennae.

We conclude that VS and HS neurons comprise a heterogenous assembly of lobula plate outputs of which only two subsets lead directly to motor neurons for head movement. The subset VS4-9 leads to leg motor neurons via DNOVS. The remainder contribute to a complex multimodal integration region whose descending axons project to numerous centers of the thoracic ganglia. We have not yet found compelling anatomical evidence for direct VS and HS relays to flight motorneurons.

48.10 CENTRAL PROCESSING OF EXTEROCEPTIVE INFORMATION IN THE LOCUST FLIGHT CONTROL SYSTEM. H. Reichert and C. H. F. Rowell*. Dept. of Zoology, Univ. of Basel, Basel, Switzerland.

Interneuronal circuits process sensory information about course deviations in flying locusts and evoke corrective steering reactions by influencing the activity of flight motoneurons. We study the cellular basis of these interactions by recording intracellularly with lucifer-filled electrodes from thoracic neurons in a preparation which exhibits flight motor activity. An electronically controlled artificial horizon delivers spatially coordinated stimulation to the compound eyes, ocelli and head windhairs in a way which simulates angular deviation of the animal in 3 rotational axes.

We find that course deviations are encoded in the activity of numerous descending interneurons, many of which are multimodal. The different modalities carry spatially compatible sensory information. The descending interneurons make connections with numerous thoracic interneurons. Simulated course deviations evoke suprathreshold excitation in many of these. These interneurons are structurally and functionally similar to cells which we have previously described carrying ocellar information. Many are modulated at flight frequency by the central rhythm generator for flight in a way which effects a phasic gating of descending sensory information flow. It is anticipated that most of these thoracic interneurons, like the corresponding ocellar driven neurons, are presynaptic to specific flight motoneurons and are an integral and important part of the flight steering system. Direct recordings indicate that the effects of the interneuronal steering circuitry on flight motoneurons vary from weak and relatively non-specific input to some motoneurons to highly selective and spatially well described suprathreshold input to other motoneurons.

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48.11 MICROADJUSTMENTS IN BASELINE FORCE MAINTENANCE PRIOR TO THE ISOMETRIC BUTTON-PRESS RESPONSE. P. B. Vrtunski and M. B. Patterson*. Cleveland VA Medical Center, Brecksville, OH 44141.

Recent studies have demonstrated numerous effects of sensory and kinesthetic inputs on movement trajectories. Preparatory adjustments prior to the response, however, frequently remain unreported. In a recent study with four choice reaction time tasks, baseline force levels preceding the movement did not vary appreciably (Vrtunski, et al., Brain,106:929, 1983), possibly because the tasks were too similar. The present study investigates the baseline force maintenance preceding an isometric button-press response in two, more different tasks. Performance of 20 adult, right-handed subjects with simple and choice reaction time (RT) task was compared. Stimuli were tachistoscopically presented slides of line drawings. In simple RT, the task was to respond to each stimulus, while in choice RT, the response was to be made to one class of stimuli and omitted to another. The design was counterbalanced for stimuli and hand. The response consisted of the index fingers' pressure upon a button. Response forces were recorded with two transducers fitted in the armrests of the testing chair, amplifiers and an on-line computer. Baseline was defined as the amount of force measured at the transducer between the stimulus onset and the point where the response was initiated (the interval called premotor, or decision time).

There were numerous differences between simple and choice RT responses. The principal finding was that in the simple RT task, baseline force maintained was significantly higher than in the choice RT, 72.37 vs 48.67 cN, respectively (1 cN of force corresponds to 1.02 gram of mass). Because the choice task is more difficult and requires greater attention, we conclude that baseline maintenance represents a specific, feedback-loop regulated function. This function, presumably mediated by proprioceptors, is in a trade-off relationship with attention (a centrally mediated function), which in turn, is represented by the nature of the task.

48.12 THE EFFECT OF "XING" ON COMPETITIVE MOTOR PERFORMANCE. P.R. Burgess and J.Y. Wei*. Dept. of Physiology, Univ. of Utah Sch. of Med., Salt Lake City, UT 84108.

If a person who is able to successfully resist being displaced in a contest of strength is "Xed" by his opponent, he appears to become more displaceable; i.e., he loses the contest. The contestants stand opposite each other with their right arms half extended at the elbow such that the palm of one contestant faces that of the other. At an agreed upon signal, the subject is instructed to make a vigorous but not violent effort to push the experimenter's hand to the subject's left. We call this the "cross body press." Unless the subject is defeated (displaced 10 cm), the contest lasts for 3 sec. Between the palms of the contestants is a transducer assembly containing a strain gauge oriented toward the subject such that the measured force is the force that comes against the subject's palm. The transducer is suspended from a potentiometer so that the position of the transducer can be measured. The force and position data are digitized on line by a computor for later analysis. In some trials the subject is "Xed". Xing is done by drawing the hand rapidly across the body axis twice at close range but without touching so as to form an X centered over the lower end of the sternum. Twelve subjects participated in a total of 150 control (no Xing) and 15 experimental (Xing) trials. No defeats occurred in the control trials, but the subjects were invariably defeated after Xing. Measurements of force and position show that subjects were displaced after Xing at lower forces than they could resist during control trials even though the force did not start earlier or rise more rapidly after Xing. Thus, the data suggest that the subject is changed by the Xing so that he/she can be more easily displaced by the Xer. The alternative is that the changes are confined to the Xer. However, no consistent differences have been found in the way the force is applied to the subject's hand in the experimental as compared with control trials. If the subject is changed by the Xing, then the mechanism is obscure since Xing effects appear to be slightly stronger when the subject does not know she/he has been Xed.

49.1 SPACE MOTION SICKNESS ON SPACELAB MISSION ONE C.M. Oman, B.K. Lichtenberg*, K.E. Money* and R.K. McCoy*. Man Vehicle Laboratory, Mass. Inst. of Technology, Cambridge, MA 02139.

Symptoms of space sickness were documented in 4 specially trained crewmen. Two wore head mounted accelerometers. 3 of 4 reported persistent symptoms, which modulated with activity level, and vomited repeatedly on days 1 or 2. Symptoms diminished by day 3, but could be elicited with vigorous head movements through days 4-5. One subject who explored different types of head movement found pitching and rolling head movements particularly provocative on day 1, but pitch was less disturbing by day 4. On day 9, this subject was asymptomatic after performing 5 minutes of vigorous head to knee movements. The relationship between symptoms and the objectively recorded head activity patterns on different axes is discussed.

Among symptomatic subjects, tactile and proprioceptive contact cues provided by "wedging" the body into a corner of the cabin or into a bunk cubicle were palliative, as was closing the eyes, provided that these contact cues were simultaneously present. When they travelled through the Spacelab tunnel; assumed an unusual orientation with respect to the cabin floor, or viewed another crewman in such an orientation, the associated ambiguous visual cues could trigger "reorientation" illusions which were provocative early in the flight.

Symptom pattern was generally similar to that seen in the same individuals preflight, except that facial pallor and cold sweating were usually not seen, and for 2 subjects, nausea was brief or absent prior to vomiting. Sudden vomiting is characteristic of long duration motion sickness, and also of the responses of relatively resistant subjects. We tentatively attribute findings on pallor and sweating to the effects of fluid shift on capillary circulation, and to the cool, dry environment of Spacelab, respectively. One subject experienced a persistent, uncomfortable feeling of "stomach elevation", sore abdomen and difficulty burping. Drugs (0.5 mg scopolamine/2.5 mg dexedrine or 25 mg promethazine/25 mg ephedrine) were eventually taken by all, and judged helpful, with minimal side effects. Only 2 of 12 vomiting episodes occured during presumed intervals of maximal drug effectiveness. Although all subjects reported persistent head fullness and congestion, and "fluid shift" appearance throughout the mission, they denied difficulty hearing or clearing the ears. Our results support the view that space sickness is a form of motion sickness. (Supported by NASA Contract NAS9-15343; RKM is 1st Lt,USAF).

49.2 POSTURAL RE-ADAPTATION FOLLOWING EXPOSURE TO WEIGHTLESSNESS (SPON:H.T. Hermann) R.V. Kenyon* and L.R. Young. Man-Vehicle Laboratory, Mass. Inst. of Technology, Cambridge, MA 02139.

Postural instabilities have been observed on space crews after exposure to weightlessness. The general observation is one of wide stance and difficulty maintaining posture with eyes closed (Homick and Reschke, Acta Otolarynolgol. 83: 455, 1977). We hypothesize that postural stability with eyes closed is based on otolith signals, particularly cues from the utricular maculae. Weightlessness might alter interpertation of otolith signals causing changes in timing or magnitude of postural responses.

Ss stood on a pneumaticly driven posture platform which pitched rapidly (30ms) and unexpectedly causing dorsi- or plantar- flexion. Platform torque, body sway, and EMG activity from the tibialis anterior and the gastrocnemius muscles were measured during eyes open and eyes closed trials.

Four hrs post-flight Ss avoided rapid head movements or bending, assumed a wide stance, had slightly crouched posture and lost balance on an eyes closed tilt-up trial, which did not occur pre-flight or on subsequent days. Their EMG responses showed no changes in latency or amplitude in the first peak of the response (250ms epoch). EMG activity after this period was highly elevated and oscillatory when compared to pre-flight. The epochs of the oscillations were 3 secs for eyes open and 6 secs for eyes closed; pre-flight EMG oscillations rarely exceded 1 sec eyes open and 3 secs eyes closed. Thus subjects were more unstable with eyes closed post-flight but the initial response was unchanged in each case. The functional stretch reflex does not appear to have changed yet the long latency response perhaps indicative of interpertation of otolith signals showed elevated and oscillatory activity.

Re-adaptation was rapid; by 3 days post-flight Ss reported no noticeable instabilities. However, EMG activity following the initial response was above pre-flight levels in all Ss until 5 days post-flight.

These results will be discussed in terms of re-interpertation of otolith signals which emphasize lateral acceleration rather than tilt. (Supported by NASA Contract NAS9-15343).

49.3 VISUAL-VESTIBULAR INTERACTION IN WEIGHTLESSNESS: CIRCULAR-VECTION DURING SPACELAB-1. L.R. Young, B.K. Lichtenberg*,M. Shelhamer* and R. Renshaw*. Man-Vehicle Lab.Dept. of Aero. and Astro., Mass. Inst. of Tech., Cambridge, MA 02139.

When a wide field visual stimulus is rotated about a sagittal (roll) axis horizontal, a subject who is sitting or standing on earth will normally perceive the paradoxical sensation of visually induced self rotation and tilt (Dichgans et al, Science 178,1217, 1972). A limitation on visually induced tilt has been attributed to graviceptor signals, particularly those from the otolith organs, which do not confirm the visual input suggesting continuous roll rate (Young et al Aviat. Space Environ. Med. 46,264, 1975;Dichgans et al Acta Oto-laryngol. 78,391, 1974). During weightlessness, the absence of any inhibiting otolith signals might be expected to produce stronger and more compelling visually induced roll. The "Rotating Dome" experiment exposed Ss to a cylindrical display which rotated in roll. Self-motion was indicated by magnitude estimation. For alternate 6 min sessions localized tactile cues were provided by pressure on the feet produced by standing against stretched elastic cords which created an upward force roughly equal to body weight. Despite considerable inter-subject variability, there was enhancement of vection in weightlessness relative to ground erect or supine tests for all subjects. Two of the S's experienced full saturated vection, perceiving that they and the entire Spacelab were rolling around a space-fixed dome. Another S felt continuous unsaturated vection with shorter onset latencies and fewer "drop outs" than on the ground. The fourth experienced similar incomplete vection to that on the ground. Latency to onset of vection and average intensity of the self-motion indication confirms the subjects' reports of stronger visual effects in weightlessness.

Localized tactile cues to the feet in the first 3 days produced some inhibition of visually induced tilt in all subjects. However, all Ss reported that the sensation of visually induced tilt was the same with or without tactile cues by the end of the mission. In the absence of inhibitory signals from the otolith system in weightlessness, visual cues concerning orientation apparantly take on an increasing role. Furthermore, the localizable tactile cues, which act as a partial otolith substitute,are disregarded after full vestibular adaptation. (A preliminary report is in Science, July, 1984, and the full paper is submitted to Exp. Brain Res.) Supported by NASA (NAS9-15343).

49.4 EYE MOVEMENTS DURING VERTICAL AXIS AND 'BARBEQUE SPIT' ROTATIONS ARE RELATED. C. Wall III. Dept. of Otolaryngol., Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15213.

Rotation in the dark about an earth horizontal axis (like a barbeque spit) is known to cause a continuous horizontal nystagmus when the stimulus is constant velocity. The nystagmoid slow phase velocity (SPV) response in humans has been previously shown to have two components. One of these is a so called "bias" component: the nystagmus SPV has a non-zero mean value. The other is a so called "modulation" component, a nystagmus whose SPV changes in direction at a frequency related to the rate of angular rotation. These response components are not present during earth vertical axis rotation which stimulate the semicircular canals but not the saccule and utricle. Rostral-caudal (yaw) axis rotations were given to 6 normal human subjects in two different orientations: earth vertical axis (EVA) and earth horizontal axis (EHA) to test the hypothesis that responses induced during EHA alone are in fact related to responses induced during EVA alone in the same subjects.

During the EHA 60 degree/second velocity trapezoid protocol, the linear gravitational force vector is rotating with respect to the subject's head at the rate of 1 revolution in 6 seconds or 0.16 Hz producing a 0.16 Hz SPV modulation component. When the EVA VOR is tested using wide band white noise, there is also gain and phase data over the noise bandwidth, in this case between 0.02 and 1.67 Hz. This permits comparisons of EHA and EVA responses at the same frequency of 0.16 Hz.

Subjects having EVA phase values far away from zero (ref. velocity) tended to have large magnitude EHA modulation components. In contrast, subjects with phase values near zero tended to have small modulation components. The null hypothesis that these two variables are independent was rejected at the $p = 0.008$ level.

All the gain and phase points of the same EVA VORs were fit using linear system parameters to estimate the "long VOR time constant". This quantity has been associated with the dynamics of peripheral horizontal semicircular inputs combined centrally by the "velocity storage mechanism". The individual VOR time constants for EVA testing were compared once again with the magnitude of the dynamic otolith modulation component from EHA experiments on the same subjects. It was possible to reject the null hypothesis that the long time constant and the modulation component were uncorrelated at the $p = 0.028$ level. Thus, the shorter the EVA VOR time constant, the greater the apparent eye movement in response to dynamic otolith stimulation.

49.5 OPTOKINETICALLY INDUCED RABBIT HEAD MOVEMENTS: James H. Fuller Dept. of Oral Anatomy, Univ. of Ill. Med. Ctr. Chicago IL 60612

The vestibulo-collic reflex (VCR) has been studied in rabbits facing a Ganzfeld and found to have a gain of 0.1-0.5 (head/platform rotation). However, when viewing a textured background the gain was typically much higher (0.6-0.95). This raised the possibility that a visual-neck or opto-collic reflex assisted the VCR to improve head stability in space. The same rabbits (adult Dutch belted) were thus exposed to optokinetic stimulation with the head free to rotate in the horizontal plane as before, but with the platform stationary. Triangular, continuous velocity and sinusoidal stimulation was employed. With the first two forms of stimuli, optokinetic drum (OKD) velocities of 1-5°/sec rarely evoked head movements (neck angular deviation, or NAD), and there was little or no retinal image motion (RS, the sum of eye and NAD (gaze) and the inverted OKD signal). At velocities of 10-20°/sec there was substantial undercompensation in gaze velocity, and head movements were evoked with a rather low gain (NAD velocity/OKD velocity) of 0.1-0.3. During the slower velocities, there were occasional brief (2-6 sec) periods in which very small, slow head movements were evoked--usually with sudden acceleration or deceleration of OKD--during which gaze moved ahead of the drum (overcompensation). During sinusoidal stimulation of < 1°/sec^2 at 0.01-0.02 Hz RS was generally flat (gaze velocity=OKD velocity), but during very slow (self-generated) head movements, there was overcompensation, presumably due to the lack of a vestibulo-ocular reflex, which would subtract from head velocity, at these low frequencies. The gain of NAD/OKD was greatest (0.2-0.5) with peak sine acceleration of 1.2-2.3°/sec^2, while above and below this acceleration gain was 0.2-0.3. While it is concluded that substantial RS is required for head movements and that this is not responsible for the higher VCR gains with vision, a subsidiary issue arose from the observation during maximal NAD/OKD gains with sines: there were intervals of continuous overcompensation lasting for up to 20 sec. This does not fit with current models of the optokinetic system; no simple explanation is currently at hand for this observation. It differs, however, from previous studies of visual-vestibular interactions in that head movements are provided by the subject, rather than passively imposed.

49.6 THE INFLUENCE OF GRAVITY ON HORIZONTAL AND VERTICAL VESTIBULOOCULAR AND OPTOKINETIC REFLEXES IN THE RABBIT N. H. Barmack. Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, OR 97210

When a rabbit is placed in normal prone orientation, angular deviations of the head about the longitudinal axis evoke a vertical vestibuloocular reflex (VVOR) which is a function of both angular acceleration encoded by the vertical semicircular canals, and linear acceleration encoded by the utricular otoliths. In addition there is a steady state signal originating from the sagittally oriented saccular otoliths, which is relatively unmodulated by small angular deviations about the prone orientation. If a rabbit is placed in a supine orientation, then a phase reversal is introduced for the modulated utricular otolith and vertical semicircular canal signals. Furthermore there is a steady state signal reversal of saccular origin. The present experiment was designed to investigate the influence of these stimulus-modulated (utricular) and unmodulated (saccular) phase reversed signals on vertical and horizontal vestibuloocular as well as optokinetic reflexes in the rabbit.

Rabbits were mounted in a biaxial rate table in front of a rear projection tangent screen. Eye movements were measured with an infrared light projection technique. Horizontal and vertical vestibuloocular reflexes (HVOR, VVOR) were measured with rabbits in both prone and supine orientations ($\pm 10^{o}$, 0.005Hz-0.800Hz). Horizontal and vertical optokinetic reflexes (HOKR,VOKR) were measured in both prone and supine orientations.

The gain of the HVOR for supine orientation was reduced at frequencies above 0.02Hz by at least 40%. Similarly there was a 20-40% reduction in gain of the monocular HOKR evoked by posterior-anterior stimulation. By contrast, the VVOR gain in the supine orientation was enhanced over a lower range of frequencies of (0.02-0.06Hz) and reduced at higher frequencies (0.08-0.80Hz). The gain of the VOKR was not reduced for ventral-dorsal monocular stimulation in the supine orientation. These data demonstrate gravitationally induced decreases in the gains of both the HVOR and VVOR over a frequency range which is encoded by the semicircular canals. The data imply that there is a significant gravitational influence, probably encoded by the saccular otoliths, on the gains of the VVOR, HVOR and HOKR. (Supported by NIH grant EY04167 and the Oregon Lions Sight and Hearing Foundation.)

49.7 TRANSFORMATION IN THREE DIMENSIONS BETWEEN PRIMARY AND SECONDARY VESTIBULAR NEURONAL SIGNALS IN THE RHESUS MONKEY. H. Reisine and V. Henn. Neurology Dept., University Hospital, 8091 Zürich, Switzerland.

Single unit recording in the vestibular nerve and nuclei was performed in alert Rhesus monkeys chronically prepared for EOG monitoring of eye position. One normal and three animals with different combinations of plugged semicircular canals (SCCs) were employed (plugged canals: 2 lateral SCCs, 2 lateral and a posterior and anterior SCC, and a posterior and an anterior SCC).

Functional canal planes for primary vestibular afferents were determined. When animals were positioned so that functional canal planes coincided with the horizontal rotational plane, the decay of unit activity following the end of an acceleration pulse provided a measure of the dominant time constant, which for anterior and posterior SCCs was similar to those reported for lateral canal afferents, i.e. 3 to 6 s. Functional canal planes closely coincided with canal orientations reported in anatomical studies.

A similar paradigm was employed in recording activity from central vestibular neurons (CVNs). Most CVNs received input from only one canal or canal coplane. Thus, directional sensitivities for responsive neurons were similar in normal monkeys and animals with canals plugged. Time constants of the decay of unit activity after velocity trapezoids were greater than 8 s and were similar to the time course of the decay of the slow phase eye velocity of the induced vestibular nystagmus. Little difference was noted between vestibular-only and vestibular-pause unit activity.

Conclusion: CVNs have similar direction coordinates as those of primary afferents, yet have activity with longer time constants indicating an integration process; hence, the integration process itself for a given CVN appears to be directly related to the incoming peripheral signal. Supported by: Swiss National Foundation for Scientific Research, 3.718.80.

49.8 MORPHOLOGY OF VERTICAL SECOND ORDER VESTIBULAR NEURONS IN THE CAT. Werner Graf and Kazuhisa Ezure*. The Rockefeller University, New York, N.Y. 10021

The most direct vestibulo-ocular reflex pathway consists of three-neuron-arcs connecting the semicircular canals to particular sets of eye muscles. Vertical canal neurons were studied using intracellular horseradish peroxidase. Neurons were identified with respect to canal-specific monosynaptic activation after bipolar electrode implantation into individual canal ampullae. The anterior canal neurons studied projected contralaterally and ipsilaterally to the oculomotor complex. The contralaterally projecting neuron (presumably excitatory) crossed the midline at the level of the abducens nucleus and bifurcated into ascending and descending branches which travelled in the medial longitudinal fasciculus (MLF). The descending branch, heading towards the spinal cord gave rise to collaterals which terminated in the vestibular nuclear complex, the perihypoglossal nuclei, the facial nucleus and the medullary reticular formation. The ascending collateral had its major terminations among superior rectus and inferior oblique motoneurons. Smaller collaterals branched into the pontine reticular formation, the trochlear nucleus, and the interstitial nucleus of Cajal. As previously described for posterior canal neurons, major collaterals also recrossed the midline within the oculomotor nucleus to terminate in comparable areas on the ipsilateral side (superior rectus subdivision). The ipsilaterally ascending anterior canal neurons (presumably inhibitory) had their major termination sites in the trochlear nucleus and in the inferior rectus subdivision of the oculomotor nucleus. Smaller collaterals reached areas in between the fiber bundles of the MLF, in an area which contains medial rectus motoneurons, and also crossed the midline within the oculomotor complex to terminate in the inferior rectus area on the contralateral side. Ipsilaterally ascending posterior canal neurons (presumably inhibitory) terminated mainly in superior rectus and inferior oblique motoneuron areas. Cell somata were found in an area which included portions of the medial, superior and descending vestibular nuclei. The oculomotor termination sites are in agreement with calculated reflex connectivity based on the geometry of the sensory and motor periphery (Ezure & Graf, Neuroscience 11, 1984ab). The described axonal trajectories reflect the organization of intrinsic coordinate systems and provide a matrix for the transformation of vestibular into oculomotor reference frames.

Supported by NIH grants EY04613 and NS02619.

49.9 KINEMATIC ORGANIZATION OF CAT VESTIBULO-OCULAR REFLEX (VOR) B. Peterson, J. Baker, J. Goldberg, & C. Wickland*. Dept. Physiol., Northwestern U. Med. Sch., Chicago, IL 60611.

We are studying the 3-dimensional organization of the VOR by recording extraocular muscle electromyographic (EMG) responses in thalamic level decerebrate cats to 0.5-1.6 Hz whole body rotations in many horizontal and vertical planes. Our questions and preliminary answers follow, based upon 7 horizontal rectus, 4 oblique and 2 vertical rectus muscles.

1. Does each muscle have clear optimal and null responses in planes at right angles to each other? Yes, in 13/13 cases.

2. Are optimal planes of antagonistic muscle pairs sufficiently similar to consider the pair a single kinematic element? Preliminarily yes, validating the use of simple 3 x 3 input-output models of VOR kinematics (Robinson, Biol. Cybern. 46:53; Ezure and Graf, Neuroscience, in press).

3. What are the relative contributions of the three pairs of semicircular canals to excitation of each of the three pairs of eye muscles? Multiplying our data matrix by the inverse of the canal response matrix (C) gives the brainstem connections (B) necessary to produce our muscle responses. (Coordinate frame is rotated 21.4° to place horizontal canals in yaw plane.) The brainstem matrix, below right, shows nearly pure horizontal canal input (rH1H) to horizontal recti (row 3), and little horizontal canal input to vertical recti (row 2) and obliques (row 1). Vertical recti receive strong input from both vertical canal pairs (rA1P, rP1A), obliques mainly from the ipsilateral posterior, contralateral anterior pair.

4. What directions must the eye muscles pull to produce a compensatory VOR? The inverse of our data matrix, below left, corresponds to Robinson's muscle matrix (M). It indicates the expected major pulling actions, and shows little roll (row 1) and almost no pitch (row 2) pull by horizontal recti (rLRMR), and almost no yaw (row 3) pull by vertical recti (rSRIR) and obliques (rSOIO). Preliminary recordings from alert cat VI nucleus support these findings. Supported by EY 04058.

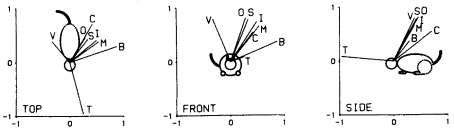
	[M]				[B]		
	rSOIO	rSRIR	rLRMR	rP1A	rA1P	rH1H
VOR=	-.927	-.564	.116	.977	.178	.004
	.424	-.843	.013	-.364	.945	-.014
	.032	.002	-1.003	.031	.007	-.997

* [C] =-[I]

49.10 SPATIAL AND TEMPORAL PROPERTIES OF VESTIBULO-NECK REFLEX EMG J. Baker, J. Goldberg, C. Wickland*, & B. Peterson. Physiol. Dept., Northwestern Univ. Medical School, Chicago, IL 60611.

Vestibulocollic reflex (VCR) electromyographic (EMG) responses of 7 neck muscles in decerebrate cats were studied during 0.07 - 1.6 Hz rotations of the whole body in many vertical and horizontal planes.

Responses at frequencies >0.5 Hz revealed the spatial pattern of VCR motor output, and were consistent with muscle activation from semicircular canals via brainstem circuitry that modifies canal dynamics. EMG response gains were sinusoidal functions of stimulus plane orientation, and responses advanced from velocity phase toward acceleration phase as frequency was increased. Responses at high frequencies were used to calculate axes of maximal response of the muscles in three dimensional space, and the figure below shows normalized response axis vectors for right side muscles, averaged over 6 experiments. Use the right hand rule to find planes of maximal response. Biventer cervicis (B) was activated predominantly by pitch rotations, triceps brachii (forelimb muscle, T) predominantly by roll, and longus capitis (V), obliquus capitis inferior (O), and splenius (S) most strongly by yaw. Complexus (C), occipitoscapularis (I), and rectus capitis major (M) were significantly excited by rotations in all three coordinate planes. Measurement of muscle origins and insertions showed that muscles do not necessarily pull in their plane of best VCR activation, as expected for a non-orthogonal set of muscles.



At lower frequencies, neck muscle vestibular responses were more complex than eye or forelimb responses. At the lowest frequencies tested, responses approached position phase suggesting an otolith influence on VCR EMGs. At 0.2 to 0.4 Hz, neck muscle response phase sometimes varied depending on the vertical plane in which the cat was rotated, and the optimal response plane was poorly defined and varied with frequency. Such complex responses, also found in secondary vestibular neurons, may result from summation of inputs with differing spatial orientation and dynamics, probably otolith and canal inputs. Supported by EY04058.

49.11 OPTIMAL GEOMETRY OF EXTRAOCULAR MUSCLES. J. Goldberg
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The planes in which individual eye muscles rotate the eye tend to be similar to the planes of the semicircular canals in various mammals. Each of the semicircular canals affects the extraocular muscle to which it is most nearly parallel via a "primary" vestibuloocular reflex (VOR) connection. "Secondary" VOR connections exist between the same muscle and canals that are not parallel but closer to being perpendicular to the muscle. Even if muscle planes were perfectly aligned with canal planes, secondary connections would still be required in order to correct for any non-orthogonality of the canal planes. A different geometrical arrangement of extraocular muscles could possibly make the secondary connections unnecessary. This paper examines the questions of what muscle geometry is required to compensate for canal non-orthogonality thus minimizing secondary VOR connections and how closely the actual eye muscles match the optimal geometry relative to their match of the canals.

The solution to the first question is very simple for a VOR with a unity gain in all planes. If each plane of an agonist-antagonist muscle pair is made perfectly perpendicular to two canal pair planes, the connections from these canal pairs to the muscle pair will necessarily be zero (see the LRMR muscle pair in the abstract by Peterson et al, this volume). Algebraically, if the canal vectors are given by rows of a 3 x 3 matrix [C], then the optimal muscle vectors will be given by the columns of its inverse $[C^{-1}]$. By the definition of the inverse, the two sets of vectors are orthogonal.

To answer the second question, measures N_C and N_O of how closely actual muscle vectors match canal vectors and their orthogonals, respectively, were constructed. N_C and N_O were chosen to be Euclidian matrix norms (distances) as follows:

$$N_C = \| M^T * C^T - C * C^T \| \qquad N_O = \| M^T * C^{-1} - C * C^{-1} \|$$

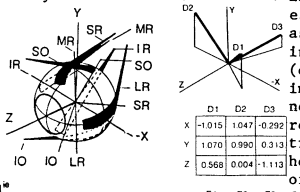
Rows of M^T (3 x 3) give the muscle pair vectors and subscript T signifies transpose. The two measures, computed using several published data sets for the cat and rabbit, did not differ by more than 3% for any data set. Thus the extraocular muscle planes appear to match the canal-orthogonal planes at least as well as canal planes in these species. Other species, whose canals deviate more from orthogonality will be considered.

Supported by the Clayton Foundation for Research

49.12 TENSORIAL COMPUTER MOVIE DISPLAY OF THE METAORGANIZATION OF OCULOMOTOR METRIC NETWORK. G. Ostriker[1], A. Pellionisz & R. Llinás. Depts. Physiol. & Biophys. and Ophthalmol.[1], New York Univ. Med. Ctr., 550 First Ave, New York 10016.

Sensorimotor transformations can be interpreted tensorially, where a covariant embedding procedure yields motor intention vectors. These are transformed into physically executable contravariants by a network matrix which can be calculated as the Moore-Penrose generalized inverse of the covariant metric tensor (Pellionisz 1983).

The principle of metaorganization (Pellionisz & Llinás 1984), which is capable of demonstrating the genesis of the required metric-type networks, is applied here to the extraocular motor system. The process is based on the notion that motoneuron innervation is contravariant and tendon-organ proprioception is covariant. A re-entry of proprioception resulting in motor execution yields eye-oscillations, reaching a steady state in the eigenvectors of the motor frame. This explains the necessity of muscle proprioception even in systems lacking myotatic reflex organization. A computer movie-display of the oscillatory behavior, to be presented, provides direct comparison of theory & clinical observations. Eigenvectors & eigenvalues, establishable physically as shown (E,L) can be used in spectral representation (eigendyadic expansion) to imprint g^{ie}, a neuronal network that serves as the required co- contravariant transformer. As displayed here and on film, the axes of eye oscillations differ from horizontal & vertical directions, thus a separation along these axes may be improper -- only to be applied along principal rotation directions D1-D3.



	D1	D2	D3
X	-1.015	1.047	-0.292
Y	1.070	0.990	0.313
Z	0.568	0.004	-1.113

g^{ie}

0.214	-0.214	-0.075	-0.057	-0.030	0.044	LR
-0.214	0.214	0.083	0.048	0.032	-0.045	MR
-0.075	0.083	0.321	-0.268	0.081	-0.063	SR
-0.057	0.048	-0.268	0.296	-0.060	0.035	IR
-0.030	0.032	0.081	-0.060	0.273	-0.268	SO
0.044	-0.045	-0.063	0.035	-0.268	0.266	IO

	E1	E2	E3
LR	-0.427	-0.484	-0.199
MR	0.436	0.470	0.212
SR	0.367	-0.298	0.558
IR	-0.119	0.585	-0.424
SO	-0.488	0.205	0.473
IO	0.475	-0.269	-0.448
L	2.497	2.077	1.423

As shown in the general sensorimotor tensorial scheme from vestibular sensory receptors to eye muscle motor executors (Pellionisz 1984), such co-contravariant transformer plays the role of a final converter of the premotor vector into motoneuron signals that generate movements of the eye. -- Supported by USPHS grant NS13742 --

50.1 NEUROFILAMENT (NF) SUBUNITS IN BOVINE CEREBELLAR NEURONS. J.Q. Trojanowski, M.A. Obrocka* and V.M.-Y. Lee*. Division of Neuropathology, Univ. of Penn. Sch. of Med., Phila. PA

The distribution of individual NF triplet proteins (68kD, 150kD, 200kD) in bovine cerebellar neurons was examined. NF subunit specific monoclonal antibodies (MAs) were generated with bovine immunogens as described (P.N.A.S. 79: 6089, 1982; J. Neurochem. 42:25, 1984). For immunohistochemistry fresh bovine cerebellum was fixed in 1 of 4 different fixatives (4% mercuric chloride and 8% formaldehyde, 10% formalin, Bouin's or Karnovsky's fixatives) and embedded in paraplast. NF subunit distribution was determined in cerebellar sections with the anti-NF MAs and a previously published peroxidase anti-peroxidase procedure (J. Histochem. Cytochem. 31:1217, 1983). The MAs used here were grouped according to their specificities for NF subunits (determined by immunoblot) as follows: 68kD group (8 MAs); 150kD group (5 MAs); 200kD group (9 MAs); 150+200kD group (30 MAs). These 52 MAs were selected from among 140 MAs specific for NF subunits which were found to recognize NF antigens in immunofluorescence and/or immunoperoxidase procedures.

Some, but not all MAs from each group, stained neuronal perikarya (stellate, Purkinje, basket, Golgi and granule cells) and dendrites in cortex as well as axons in white matter regardless of fixative. Purkinje cells, with their more abundant cytoplasm, were the most common neuronal cell type in cerebellum observed to contain immunoreactive NF antigens in the perikaryon. Not all neurons of a given type stained with the same anti-NF MA in tissue fixed with the same fixative; similar variability in axonal staining was not detected. Diffuse staining of the neuropil of the molecular and granular layers was observed only with some MAs from each group. Finally, the same MA did not produce the same pattern of NF staining in tissue fixed with different fixatives; the demonstration of NF proteins in perikarya and the diffuse neuropil staining were the most variable in different fixatives.

We conclude that anti-NF MAs permit the detection of each of the three NF subunits in all portions (perikarya, dendrites and axons) of cerebellar neurons. The variable detection of NF subunits in different portions of cerebellar neurons using MAs with the same NF subunit specificity may reflect "neurotypy" or fixation dependent modifications of NF subunits.

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50.2 TRANSCELLULAR FILAMENTS AT THE NODE OF RANVIER. Thomas J. Deerinck* and Mark H. Ellisman. Lab. for Neurocytology, Dept. Neuro., UCSD, La Jolla, CA 92093. (Spon. A. Miller)

We recently observed a highly developed system of fibrils, forming a transcellular filament network (TCFN) in eel Electrophorus electricus electric organ with the aid of high voltage EM (HVEM). The distribution of the TCFN has now also been examined in peripheral nerve and specializations associated with the nodes of Ranvier noted. Techniques used to visualize these structures have included 3-D imaging by HVEM of thick sections, contrast enhancement by immunolabeling, deep-etch rotary shadowing and thiocarbohydrazide or tannic acid mordanting techniques. In the nodal zone, where microvilli from the Schwann cell approach the extracellular surface of the axolemma, fibers within the villus give rise to filaments that issue from the tips and appear to proceed through plasma membranes, in direct continuity with filaments coursing across the axoplasm. These axonal filaments are oriented orthogonal to the longitudinal axis of the axon and are formed by two or more subunits of 9-10nm intermediate filaments twisted in the form of a multiply-coiled helix. In addition to examples of direct cytoskeletal continuity between Schwann cells and axons, extracellularly projecting fibrils associate with the extracellular matrix. In the paranodal region, where myelin terminal loops are closely associated with the axolemma forming the glial-axonal junction (GAJ), filaments approach and traverse both junctional membranes. Here, the TCFN fibers appear interwoven linking the cytoskeletons of both cellular participants. Several "integral membrane particles" of the GAJ and nodal axolemma seen by freeze-fracture now appear to represent molecular residue of filaments traversing plasma membranes. Why the distribution and nature of this pervasive and clearly important TCFN had not been observed previously appears to relate to the physical properties of the network's molecular constituents and the principles by which structures are generally imaged in electron microscopy. These structures are not positively contrasted by conventional electron dense stains rendering them refractory to direct electron microscopic visualization. Further, in concordance with the identification of intermediate filament (IF) constituents in the TCFN, the electron scattering of these structures, embedded in conventional epoxy, is lower than the surrounding resin. These factors combined yield a network delineated in micrographs by its lack of electron contrast or negative image thus difficult to recognize except when viewed in 3-D.

50.3 DEPHOSPHORYLATION ENHANCES THE DETECTABILITY OF AXONAL MICROTUBULE-ASSOCIATED PROTEIN 2 (MAP2) BY A MONOCLONAL ANTIBODY. S. Ch. Papasozomenos and L. I. Binder*. Division of Neuropathology and Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22908.

We have studied the effects of dephosphorylation on the detectability of MAP2 in nervous tissues of rats weighing 250-500 g. Three monoclonal antibodies, designated AP7, AP9 and AP13, which are directed against different epitopes on MAP2, and the peroxidase-antiperoxidase technique for light and electron microscopic immunohistochemistry were used. Vibratome sections were taken from the lumbar and cervical segments of spinal cord, ventral and dorsal roots, dorsal root ganglia, brainstem, cerebellum, cerebrum and optic nerves. Dephosphorylation was carried out by incubating the sections with 150 µg/mL of alkaline phosphatase (Sigma VII-L, from bovine intestinal mucosa) in 0.1M Tris-HCl, pH 8, for 2.5 h at 32°C. To inhibit proteolysis, 2mM phenylmethylsulfonyl fluoride, 10µg/mL leupeptin and 20µg/mL pepstatin A were also included in the incubation solution. Both AP9 and AP13 stained intensely microtubules in neuronal cell bodies and dendrites, but AP7 did not. In non-dephosphorylated sections, AP9 and AP13 stained with varying intensities certain types of axons, i.e. axons of upper and lower motor neurons, deep cerebellar nuclei, fornix, facial nerve, the peripheral process of dorsal root ganglion cells and various other axons. While dephosphorylation had no effect on the staining intensity of axons with AP9, it enhanced the staining of axons with AP13. The enhancement of staining was more pronounced in older animals. When 0.1M pyrophosphate was included in the alkaline phosphatase solution, no increase in the intensity of staining was observed. These findings suggest that AP13 is directed against a dephosphorylated epitope on the MAP2 molecule, which in the axons becomes progressively phosphorylated with age and thus inaccessible for immunostaining.

Supported by NIH grant NS19351.

50.4 MOBILIZATION OF CYTOSKELETON-ELEMENTS DURING NGF INDUCED NEURITE OUTGROWTH IN PC12 CELLS. S. Feinstein* (1), D. Drubin* (2), R. Sherman-Gold* (1), M. Kirschner* (2) and E.M. Shooter (1). (1) Dept. of Neurobiology, Stanford Univ. Sch. Med., Stanford, CA 94305 and (2) Dept. of Biochemistry and Biophysics, Univ. of California, San Francisco, CA 94143.

Differentiation of neuronal cells involves a major reorganization of the cytoskeleton. We have studied this reorganization using NGF induced neurite outgrowth in PC12 cells. Measurement of the tubulin monomer and polymer pools demonstrates a major shift favoring polymer formation in NGF treated cells. Protein blotting experiments show that tau proteins are increased greater than 20 fold, as are MAP 1 levels. Both are factors which promote in vitro assembly of microtubules from tubulin monomers. In contrast, tubulin proteins are induced merely two fold. The tubulin polymerization shift, tau induction, MAP 1 induction and neurite outgrowth all precisely correlate kinetically, being preceded slightly by the tubulin induction.

In attempting to determine the mechanism of the tau and tubulin inductions, we performed RNA blotting analysis using tau and tubulin cDNA probes. Tubulin mRNA levels increase two fold, commensurate with the protein induction. In marked contrast, tau mRNA levels increase, but to a much lesser extent than the corresponding tau protein rise.

Our data demonstrate that NGF induced neurite outgrowth is accompanied by a massive shift in the percentage of tubulin assembled into microtubules. This appears to be accomplished by increasing dramatically the amounts of tau and MAP 1 proteins, regulatory factors for the assembly of tubulin into microtubules. We have shown that there appear to be two classes of NGF induced cytoskeleton protein inductions, distinguished from each other by the quantitative and kinetic nature of the inductions. Structural elements are modestly induced relatively rapidly after NGF administration, whereas microtubule assembly regulatory factors are dramatically increased, but somewhat later than the tubulin increases. Further, we have shown that the tubulin protein increases are likely a direct result of increased tubulin mRNA levels. Importantly, tau mRNA levels are induced by NGF, but the magnitude of the induction is insufficient to fully account for the size of the tau protein increase. This implies that some additional means of regulation is likely involved.

50.5 EFFECTS OF SUBCHRONIC MORPHINE TREATMENT ON THE FINE STRUCTURE OF THE RAT SUPERIOR CERVICAL GANGLION . J. E. Johnson, Jr.[1], J. White, Jr.[1]*, A. Hervonen[2]*, R. C. Walovitch[3], and E. D. London[3]. Section on Exper. Morphology[1] and Lab. of Neurosci.[2], NIA Gerontology Res. Center, and Neurochem. Section, NIDA Addiction Research Center[3], Baltimore, MD 21224.

Opiate abuse is an ever-increasing and pervasive worldwide problem. Although a great deal is known about the subjective and physiological effects of opiates, comparatively little is known regarding morphological effects of this class of drugs. This study therefore has been initiated as part of a collaborative program to gather electron microscopic information and to correlate structural and functional features of opioid addiction.

Morphine (M) was administered to male Fischer-344 rats (4 - 6 mo old) by implantation of subcutaneous pellets. Rats received one M pellet (75 mg M, free base + 75 mg microcrystalline cellulose) on the first day of treatment, and 2 additional M pellets on day 4. Control rats received placebo pellets (150 mg cellulose only) at corresponding times. In rats subjected to the same pellet implantation procedure, but not used for morphological studies, naloxone (1 mg/kg, i.p.) was injected on day 8 to demonstrate M addiction by the precipitation of abstinence behaviors. Rats were sacrificed on day 8, by perfusion with fixative containing 1% paraformaldehyde and 1.5% glutaraldehyde in 0.12 M cacodylate buffer (pH 7.4, 18° C). Superior cervical ganglia were removed and processed for electron microscopy as previously described (Johnson, J.E., Jr., Current Trends in Morphological Techniques, Vol. I, CRC Press, 1981).

Morphometric analysis indicates that the relative frequency of secondary lysosomes, compared to primary lysosomes, increased in neuronal perikarya in ganglia from M-treated rats. In some neurons, mitochondria were enlarged twofold or more, and cristae were irregular, giving many of these mitochondria an empty appearance. Mitochondria appeared relatively normal in some neurons adjacent to those containing the apparently empty mitochondria, suggesting a differential drug effect among neurons.

These effects of M on lysosomes and mitochondria, respectively, suggest an increased catabolic activity and disruption of oxidative metabolism in the superior cervical ganglion, and may relate to the known physiological effects of M on autonomic function.

50.6 ELECTRON MICROSCOPIC ANALYSIS OF THEOPHYLLINE EFFECTS ON CALCIUM LOCALIZATION IN SYMPATHETIC GANGLIA OF BULLFROG. Shao Zuo-hua* and F. F. Weight (SPON: G. C. Salmoiraghi). Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

Electrophysiological and biochemical investigations indicate that theophylline can release calcium ions (Ca^{2+}) from intracellular stores. Fugimoto et al. (Brain Res. 202: 21, 1980) have reported that electron-dense deposits indicating Ca^{2+} localization can be detected using electron microscopic techniques. We have used the method of Fugimoto et al. to study the effects of theophylline on Ca^{2+} localization in sympathetic ganglia of bullfrog. The IXth and Xth paravertebral sympathetic ganglia of bullfrogs were dissected and incubated in vitro in a Ringer's solution containing 20 mM Ca^{2+} for 1-1.5 hrs. The ganglia were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.3. After 3-3.5 hr. in fixative, the ganglia were postfixed in a 2% solution of osmium tetroxide for 1.5 hr. Subsequently, the ganglia were dehydrated in a graded series of ethanol solutions and embedded in Epon 812. Thin sections (600-700 A) were stained with uranyl acetate for 30 min. and with lead citrate for 6 min. Electron microscopic examination of the tissue revealed electron-dense deposits in the plasma membrane, subsurface cisternae and mitochondria of sympathetic neurons. In presynaptic terminals, deposits were found on the membrane of synaptic vesicles. The addition of 10 mM theophylline greatly reduced the deposits in plasma membrane, and electron-dense deposits were not observed in subsurface cisternae. Deposits remained, however, in the matrix of mitochondria. In presynaptic terminals, synaptic vesicles tended to aggregate after treatment with theophylline and electron-dense deposits were frequently observed at points where adjacent vesicles were apposed. If the electron-dense deposits provide an indication of Ca^{2+} localization, the results suggest that theophylline may release Ca^{2+} associated with subsurface cisternae and plasma membrane.

50.7 LIGHT AND ELECTROMICROSCOPICAL IMMUNOCYTOCHEMISTRY OF ASTROCYTES IN THE RAT DENTATE GYRUS. M.J. Rickmann* and B.D. Boss (SPON: U. Bellugi). The Salk Institute and the Clayton Foudation for Research-California Division, La Jolla, CA 92037.

Antibodies against the S100 and glial fibrillary acidic (GFAP) proteins have been used to analyze the morphology and distribution of astroglial cells in the dentate gyrus of rats and their relationship to the laminar arrangement of the afferents that terminate within the molecular layer. In adult animals, both antigens have been found to be confined to astrocytes and at the same time all astrocytes seemed to contain both GFAP and S100. However, whereas GFAP is largely restricted to major processes, S100 appears to be distributed throughout the entire cell. Within and deep to the granule cell layer are the bodies of astrocytes with radial processes that extend across the entire thickness of the molecular layer. The inner third of the molecular layer is mainly occupied by star-shaped astrocytes, while the outer two-thirds contain primarily candelabra-like astrocytes with processes extending superficially towards the pia or the hippocampal fissure. As previously reported, there is a slight increase in the number of astroglial cell bodies near to the junction between the inner third and outer two-thirds of the molecular layer but by itself this does not clearly delineate the border between the zones of termination of the commissural/associational and entorhinal afferents. However, in semithin sections of material reacted for S100 the density of smaller processes shows this demarcation quite clearly, since the inner third of the molecular layer contains appreciably fewer stained processes. This differential distribution of astrocytic processes is found in all parts of the dentate gyrus and has also been confirmed by postembedding staining of semithin sections. Electron microscopy confirms that the S100 antigen is exclusively located in astrocytes where it seems to be primarily associated with microtubules and the plasma membrane. There are no detectable differences in the completeness or intensity of astroglial immunoreactivity for S100 in the various sublaminae of the molecular layer; the differential distribution of S100 staining in the molecular layer thus appears to be due to differences in the density of astrocytic processes and not to different cellular concentrations of the antigen. Although the distribution of astrocyte perikarya is complete by postnatal day 20 (P20), and while at this stage the cells have acquired their mature form, the sublaminar distribution of small processes typical of the adult molecular layer is not evident until about P50.

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50.8 IN VITRO RECOMBINATION OF NEURONS AND ASTROGLIA PURIFIED FROM NORMAL AND WEAVER MOUSE CEREBELLUM. M.E. Hatten, R.K.H. Liem and C.A. Mason. Department of Pharmacology, N.Y.U. School of Medicine, New York, New York 10016.

The weaver mouse, an autosomal recessive genetic mutation, suffers a loss of granule neurons subsequent to their failure to migrate along the processes of Bergmann's astroglia into their proper position in the cerebellar cortex. In vitro studies have revealed a defect in neuron-astroglial interactions between weaver granule neurons and astroglia, as well as the persistence postnatally on weaver cerebellar cells of the embryonic cell surface characteristics of agglutinability with the lectins WGA and ConA. Here we report experiments on cultures of purified P4 normal and weaver astroglia to which P4 normal or weaver neurons have been added.

When normal neurons were recombined with normal astroglia at a ratio of 4:1, two forms of astroglial cells were seen and these appeared to organize the arrangement of the neurons in the culture. When weaver neurons were recombined with weaver astroglia, extensive granule neuron death occurred and astroglia had flatter shapes with stunted processes often filled with glial filaments. Most weaver granule neurons did not associate with weaver astroglia. When normal neurons were added to purified weaver astroglia, little neuronal cell death was seen and weaver astroglia had longer processes and more complex shapes than those observed when they were co-cultured with weaver granule neurons; the vast majority of normal neurons associated with weaver astroglia. When weaver neurons were added to purified normal astroglia, although few neurons died and the vast majority of weaver neurons bound to normal astroglia, normal astroglia had flattened shapes with fewer, shorter arms than their counterparts co-cultured with normal neurons.

These results suggest that the granule neuron is a site of action of the weaver gene and that disrupted neuronal-glial interactions, in turn, lead to impaired astroglial differential. Supported by NIH grants NS 15429 (MEH) NS 15182 (RKH) and NS 16951 (CAM).

50.9 IMMUNOCYTOCHEMICAL LOCALIZATION OF REGULATORY PROTEINS IN CULTURES OF EMBRYONIC CHICK SENSORY GANGLIA. K.J. Doane*, F.J. Roisen and F.J. Wilson* (SPON: W. Nicklas). Dept. of Anatomy, UMD-Rutgers Medical School, Piscataway, NJ 08854.

The interaction of actin and myosin is controlled by divalent cations through separate regulatory proteins such as troponin and tropomyosin in striated muscle or by modification of the contractile proteins themselves as in the myosin light chain kinase system of smooth muscle. Nonmuscle cell movements may also depend on these mechanisms. This study investigates the immunocytochemical localization of calmodulin (CM) and troponin (TN) in neurons and non-neuronal cells of cultured embryonic chick dorsal root ganglia (DRG) and compares their distributions with tropomyosin (TM). Antisera directed against the TN complex, each of its three subunits (TN-C, TN-I, TN-T) and TM were prepared and characterized previously. Anti-CM was obtained commercially. Organized and trypsin-dissociated cultures of 8.5 day embryonic chick DRG were grown for 48h prior to treatment. Anti-CM was localized in the perinuclear regions of the non-neuronal cells. Little reaction was observed in the peripheral areas of the cells or along filament bundles within the cytoplasm. This antibody appeared in discrete packets within the neurites and growth cones. The staining pattern observed with the antisera against each TN component was markedly different from anti-CM. The reaction of these antibodies within non-neuronal cells was especially intense along filament bundles and periodic in distribution. Anti-TN was observed in neurites as discrete globules. Reaction with anti-TM elicited an intense, periodically distributed fluorescence along prominent filament bundles in non-neuronal cells. In contrast, no staining was observed in neuronal perikarya or processes. Although both CM and TN were found in neurons and non-neuronal cells, their distributions were not identical. Antibodies to TN and TM were deposited along filaments in regions previously shown to be rich in actin and myosin, whereas CM was distributed in the perinuclear area apparently independent of filaments. The different location of CM and TN suggests the presence of two separate mechanisms which regulate cell motility and intra-axonal movement.

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50.10 TRANSIENT MIDLINE RAPHE GLIAL STRUCTURE IN THE DEVELOPING RAT. C. Van Hartesveldt, B.K. Hartman, B.W. Moore*, and S.H.C. Hendry. Department of Psychology, University of Florida, Gainesville, FL 32611, and Departments of Psychiatry and Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

A major glial structure is present during development within the midline raphe of the midbrain, hindbrain, and cervical spinal cord of the rat. It is composed of great numbers of glial cell bodies lying immediately ventral to the cerebral ventricular system, and their large radial processes extending from these cells toward the ventral surface of the brain roughly within the midsaggital plane. There is also a smaller group of glial cells on the dorsal surface of the aqueduct and the central canal whose glial processes extend to the dorsal surface of the brain. The entire structure exhibits an intensely positive immunoreactivity with the antibody to the S-100 protein thus making possible a clear visualization of the extent, magnitude, and continuity of this structure from at least embryonic day 15, the first age examined, until postnatal days 7-8 when it is no longer visible by this technique. This glial structure is not reactive with the antibody to GFAP nor does it stain with cresyl violet.

This glial structure has several prominent morphological characteristics. During prenatal and early postnatal development the fibers comprising the ventral aspect of the structure in the midbrain and hindbrain are formed into two parallel plates on either side of the midline with S-100-negative tissue between the plates. As development progresses, S-100-positive fibers are continually added so that the plates become thicker at the expense of the non-staining intervening area. By postnatal day 4 only a single midline plate of fibers is visible, occupying the entire midline raphe. Preliminary electron microscopic studies have shown that these glial processes have specialized contacts with one another. In the region of the pontine flexure the entire structure takes on a distinctly pleated configuration. A curious "sine wave" appearance results when the plane of section crosses these vertical pleats. At postnatal day 5 the structure begins to disappear in a patchy fashion and it is no longer visible by postnatal days 7-8.

50.11 DISSOCIATED CELL CULTURES PREPARED FROM THE VISUAL CORTEX OF POSTNATAL HOODED RATS. J. E. Huettner and R. W. Baughman, Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115

Dissociated cell cultures of neocortical neurons traditionally have been prepared from embryonic animals. The mammalian neocortex is known to contain a variety of different neuronal cell types, however, it has not been possible to conclusively identify many of these cell classes in dissociated embryonic cultures. We have developed techniques for preparing dissociated cell cultures from the occipital cortex of postnatal hooded rats and have succeded in obtaining labelled cell populations in culture by prelabelling cells in vivo with retrograde fluorescent markers.

Single cell suspensions are obtained by enzymatic and mechanical dissociation of rectangular blocks of cortex comprising area 17 and portions of areas 18 and 18a. For newborn to five day old pups, tissue blocks are incubated for 15 minutes in 0.025% trypsin; for 5 to 15 day old animals, tissue samples are treated with papain (20 units/ml for 1.5 hours). Cells are plated onto collagen coated glass or plastic coverslips or onto a confluent layer of astrocytes. The culture medium consists of Eagle's MEM supplemented with 5% rat serum. Proliferation of nonneuronal cells is inhibited by the addition of 5 uM cytosine arabinoside several days after plating. Under these culture conditions neurons from newborn to 15 day old animals generate an extensive network of processes and survive for three to four weeks. Antisera recognizing glial-fibrillary acidic protein and the 140KD subunit of neurofilaments have been used to identify astrocytes and neurons respectively. In addition, subpopulations of neurons can be stained using antisera against glutamic acid decarboxylase and vasoactive intestinal polypeptide. Electrophysiological recordings revealed that neurons in these cultures generate action potentials when stimulated by current injection and exhibit frequent spontaneous epsps and ipsps.

In order to study particular identified classes of cortical neurons we have begun to label specific cell populations in vivo by retrograde transport using fluorescent latex microspheres as a tracer (Katz, Burkhalter and Dreyer, 1984). Preliminary experiments indicate that cortical neurons that project across the corpus callosum retain the fluorescent microspheres following dissociation and can be readily identified in culture for physiological and immunocytochemical studies.

50.12 E.M. HISTOCHEMICAL LOCALIZATION OF THIAMINE PYROPHOSPHATASE IN RAT HIPPOCAMPAL NEURONS AND SUBCELLULAR MICROSOMAL FRACTIONS. M.B. Laskowski, D.B. Bennett*, J.W. Spain* and C.J. Coscia. Dept. of Physiology and E.A. Doisy Dept. of Biochemistry, St. Louis University School of Medicine, St. Louis, MO 63104

Previous binding studies revealed the subcellular enrichment of opiate and other receptors in both microsomal and synaptic plasma membrane fractions. Electron microscopic evidence showed fine structural differences between these fractions (Roth et al., J. Biol. Chem. 256:10117, 1981). In a continuing effort to identify the organelles associated with microsomal fractions enriched in opiate receptors, we have used the E.M. enzyme histochemical reaction for thiamine pyrophosphatase (TPPase) on both intact brain tissue and isolated brain microsomal fractions. TPPase is located in trans-Golgi elements.

Adult rats were perfused with 1% paraformaldehyde-1% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4. Hippocampi were removed, and 40 um sectons were cut on a Vibratome. The TPPase incubation medium and procedures were similar to those of Novikoff et al. (J. Cell Biol. 50:859, 1971). Microsomal fractions were obtained from bovine hippocampus or rat forebrain and incubated under identical conditions. After incubation, all tissues were processed for electron microscopy.

In intact hippocampal tissue pyramidal cells of the CA1 region and granules cells were examined. TPPase reaction product was found in most cells, but the amount of product varied greatly even in adjacent cells. Segments of tubules oriented parallel with the nucleus were filled with reaction product. In some cases the reaction product filled long parallel rows of lamellae, in others discontinuous 50 nm segments of an hexagonal network were filled. Lamellae filled with reaction product were adjacent to vesicles of 40-100 nm diameter, some of which were coated vesicles. Microsomal fractions contained reaction product in association with lamellae or vesicles of 200 to 500 nm. Particle size of the reaction product on these vesicles was 13 nm. These results indicate that rat hippocampal pyramidal and granule cells contain Golgi elements with TPPase activity. They further show TPPase activity in isolated subcellular microsomal fractions and that this can be identified at the ultrastructural level. Differential histochemical staining will be needed to determine which segments of the Golgi-GERL system are abundant in fractions enriched in opiate receptors. (Supported by NSF Grant BNS-81-14947).

51.1 STATE-RELATED CONTROL OF RESPIRATORY SINUS ARRHYTHMIA IN YOUNG AND AGED CATS. V.L. Schechtman, R.M. Harper, M.H. Chase, and D. Taube*. Neuroscience Program, Brain Research Institute, and the Department of Anatomy, UCLA, Los Angeles, CA 90024.

We have previously found that aged cats show significantly more respiratory sinus arrhythmia (RSA) during quiet sleep than do young adult cats (Harper et al., Soc. Neurosci. Abs., 9:105, 1983). This finding was inconsistent with human data which shows diminished waking RSA in geriatric patients, a discrepancy which may be due to state-related differences in RSA. In the present study, we examined RSA during both quiet sleep and waking states in aged and young cats. Five aged (9-19 years) and 5 young adult (1.5-2.5 years) cats were anesthetized with Nembutal and electrodes were inserted for recording heart rate, EEG, respiration, and postural EMG. The physiological parameters were continuously recorded for up to 5 days from each cat. One minute heart rate epochs were selected during each state from all cats, a rate curve was calculated from R-R intervals, and these series were subjected to fourier transforms to determine the amount of cardiac variation at the respiratory frequency. RSA was found to be significantly diminished during waking as compared to sleep in both young and aged cats. Analysis of waking data showed no significant age-related differences in RSA, although there was much enhanced RSA during quiet sleep in aged over young cats. The results suggest that changes in RSA during aging may be state-dependent.

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51.2 PERIPHERAL DETERMINANTS OF THE EXPRESSION OF THE PRESSOR REFLEX EVOKED BY MUSCLE CONTRACTION. G. A. Iwamoto and B. R. Botterman. Dept. of Cell Biology and Moss Heart Ctr., Univ. Texas Health Sci. Ctr., Dallas, TX. 75235.

The effects of muscle tension, active muscle mass and fiber-type composition on the pressor reflex evoked by muscle contraction were examined in decerebrate and anesthetized cats. Muscle contraction was induced by stimulating the cut distal ends of the L_7 and S_1 ventral roots with 0.1 ms duration pulses, 3X motor threshold at various frequencies. Arterial blood pressure was monitored via one carotid artery. The experiments were designed to isolate the variable under study as much as possible and included the use of selectively denervated preparations to limit muscle contraction either to all or parts of the triceps surae muscle group, which was monitored for tension. It was found that altering the evoked tension by varying the resting muscle length of the triceps surae both in intact and selectively denervated hindlimbs had commensurate effects on the pressor reflex (greater evoked tension caused a larger reflex). In addition, it was found that the changing amount of active muscle mass by comparing pressor responses obtained on activation of both heads of the gastrocnemius with the response to activation of one head of the gastrocnemius caused similar changes in the reflex (the greater the muscle mass, the larger the reflex). Finally, it was found that contrary to other accounts, small pressor reflexes could be evoked by selective activation of the slow-twitch muscle soleus, composed only of slow-twitch fibers. This small reflex could be potentiated by using stimulation of the contralateral L_7 and S_1 ventral roots as a conditioning stimulus. The response of the isolated soleus preparation was greatly attenuated by administering 75 mg/Kg α-chloralose.

In summary, these results indicate that the pressor reflex evoked by muscle contraction demonstrates characteristics which indicate that spatial summation occurs in its circuitry and that furthermore that the level of central excitability plays an important role in its overall expression.

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51.3 SOURCES OF TONIC EXCITATION OF SPLENIC, RENAL AND CARDIAC NERVES IN CATS AFTER HIGH CERVICAL SPINAL CORD TRANSECTION. R. Meckler and L. Weaver. Dept. Physiol., Mich. St. U., E. Lansing, MI 48824.

Stimulation of visceral receptors can lead to unequal responses in splenic, renal and cardiac nerves. Splenic nerve activity often can be more excited or less inhibited than that of renal or cardiac efferent nerves. This study was done to quantify differences in resting discharge among these 3 nerves during the first 2 h after C1 spinal cord transection. Potential sources of tonic nerve excitation also were evaluated. Experiments were done in chloralose-anesthetized, sinoaortic-denervated cats. Multifiber activity of splenic, renal and cardiac nerves, heart rate and aortic blood pressure were recorded. Potential sources of tonic excitation evaluated were: 1) high $PaCO_2$, 2) changes in blood pressure, 3) tonic excitation by spinal afferent nerves and 4) tonic excitation by spinal preganglionic nerves. The ability of mesenteric receptors to initiate reflex responses of splenic and renal nerves was also evaluated after acute decentralization of the solar plexus.

Before spinal transection, splenic, renal and cardiac nerve discharge rates were not different from each other. After C1 transection, renal and cardiac nerve discharge rates were significantly depressed by more than 50%, whereas splenic nerve activity was not significantly affected. Increased blood pressure, systemic hypercapnia or thoracolumbar dorsal rhizotomy did not produce responses specific to splenic, renal or cardiac nerves. Acute decentralization of the solar plexus markedly decreased but did not abolish activity of splenic or renal nerves. Stimulating intestinal receptors by injecting capsaicin into the circulation of the vascularly isolated small intestine of these cats caused excitation of activity of splenic and renal nerves.

Conclusions: 1) activity of splenic nerves is less dependent upon supraspinal sources of tonic excitation than that of renal or cardiac nerves; 2) ongoing discharge of splenic, renal and cardiac nerves in spinal cats is not the result of hypercapnia, hypoxia, low arterial blood pressure or of tonic activity of afferent nerves entering the spinal cord through dorsal roots; 3) some of the ongoing discharge of splenic and renal nerves does not depend upon tonically active preganglionic nerve activity and 4) reflex excitation of splenic and renal nerve activity can be elicited after acute decentralization of the solar plexus. Supported by HLBI Grant 21436-07.

51.4 REFLEX RESPONSES OF SPLENIC AND RENAL NERVES TO INFLUENCES FROM VISCERAL RECEPTORS AND BARORECEPTORS. J. Tobey and L. Weaver. Dept. Physiol., Mich. St. U., E. Lansing, MI 48824.

Buffering influences of baroreceptors on excitatory splenic and renal sympathetic responses to splenic receptor activation were investigated in anesthetized cats. Receptors of the vascularly-isolated spleen were stimulated by capsaicin (CAPS), bradykinin (BK) or warm physiological saline (PS). CAPS-stimulation of splenic receptors produced excitation of integrated splenic nerve activity (SNA) (+112%), no significant change in renal nerve activity (RNA) (-8%), increased systemic arterial pressure (SAP) (+27 mmHg) and splenic venous pressure (SVP) (+6 mmHg) and unchanged heart rate (HR) (+3 bpm). BK-stimulation produced increased SNA (+32%), unchanged RNA (+2%) and increased SAP (+7 mmHg), HR (+5 bpm) and SVP (+8 mmHg). PS-congestion of spleen increased SNA (+28%), did not change RNA (+7%) or HR (+2 bpm) and increased SAP (+10 mmHg) and SVP (+33 mmHg). All 3 stimuli excited SNA significantly more than RNA.

Potential contributions of arterial baroreceptors and cardiopulmonary vagal afferents to the observed sympathoinhibition and suppression of excitation were investigated next. Responses to increased arterial pressure were investigated in 4 states of innervation: 1) all baroreceptors intact (Baro-intact); 2) vagal afferents intact, denervated sinus and aortic baroreceptors (Vagi-intact); 3) carotid sinus and aortic nerves intact, vagotomized (CSN/ADN intact) and 4) vagi, carotid sinus and aortic depressor nerves sectioned (Baro Den). Dextran-induced small increases in SAP (15-21 mmHg) caused significant, equivalent inhibition of SNA and RNA in Baro-intact group (-34% SNA, -31% RNA), in Vagi-intact group (-15% SNA, -20% RNA), in CSN/ADN intact group (-12% SNA, -21% RNA) and no change in SNA and RNA in Baro Den group. Norepinephrine-induced large increases in SAP (50-66 mmHg) caused significant inhibition of SNA and RNA in Baro-intact group (-64% SNA, -95% RNA), in Vagi-intact group (-39% SNA, -86% RNA) and in CSN/ADN intact group (-51% SNA, -80% RNA). RNA was inhibited significantly more than SNA. In Baro-Den group the large SAP increase caused significant equivalent inhibition of SNA (-17%) and RNA (-19%). Results indicate: 1) Excitatory renal and splenic sympathetic responses to splenic receptor stimulation are not buffered equivalently by inhibitory inputs from cardiovascular pressoreceptors; 2) redundant baroreceptor inhibition exists; 3) high intensity stimuli are more likely to produce unequal sympathetic responses.

51.5 CHANGES IN SOMATOSYMPATHETIC REFLEXES AFTER OCCLUSION OF THE MIDDLE CEREBRAL ARTERY IN THE CAT. V. C. Hachinski, J. Ciriello, K. E. Smith* and F. Bihari*. Departments of Physiology and Clinical Neurological Sciences, University of Western Ontario, London, Canada, N6A 5C1.

Focal cerebral ischemia has been shown to result in an increase in plasma norepinephrine and cardiac arrhythmias (Stroke 12:200, 1981; Stroke 10:548, 1979). This evidence suggests that cerebral ischemia results in the activation of the sympathetic nervous system. However, the mechanisms involved in the activation of the sympathetic nervous system are not known. In the present study, experiments were done in chloralosed, paralyzed and artificially ventilated cats to determine the effects of occluding the left middle cerebral artery (MCA) on the reflex sympathetic activity during activation of sensory afferent fibers. Electrical stimulation (0.5-1.5 mA; 2 ms, 1 Hz) of the superficial radial (SRN) and sciatic (ScN) nerves evoked an early and late reflex response in either the T_2 or T_3 white ramus with peak latencies of 25-50 ms and 120-200 ms, respectively. Occlusion of the MCA resulted in a significant decrease in the peak-to-peak amplitude of the early reflex response (n=8) and an increase in the peak-to-peak amplitude of the late reflex response (n=9) during stimulation of the SRN. Similarly, the peak-to-peak amplitude of the late reflex response evoked by stimulation of the ScN (n=9) was significantly increased. These data suggest that a sudden withdrawal of inputs from the damaged cerebral cortex to cardiovascular regulating centers in the brain stem and spinal cord results in a change in the functional properties of sympathetic preganglionic neurons.

(Supported by the Ontario Heart Foundation)

51.6 BLOOD FLOW TO THE HYPOTHALAMUS INCREASES IN RESPONSE TO HYPOVOLEMIC HYPOTENSION IN THE NEWBORN LAMB. J.T. O'Neill*, S.M. Golden*, and E.R. Alden* (SPON: G. Mueller) Department of Pediatrics, Uniformed Services University, Bethesda, MD 20814.

The minimum pressure necessary to assure perfusion of the neonatal brain has not been established. We, therefore, examined the cerebral vascular response of neonatal lambs (< 7 days old) to hemorrhagic hypotension (n=6). Lambs were anesthetized with chloralose and urethane, paralyzed with succinylcholine and placed on a ventilator. Respiration was controlled to maintain constant arterial P_{O2} and P_{CO2}. $NaHCO_3$ was given to counter the development of acidosis. Total and regional cerebral blood flow (CBF) was measured with radioactive microspheres injected into the left ventricle via a catheter advanced from the left femoral artery. Arterial blood samples and pressure measurements were taken from catheters placed in the axillary arteries. Cerebral venous samples and pressure measurements were taken from a catheter placed in the dorsal sagittal sinus. Blood pressure (BP) was reduced in three steps from control (97 mmHg) to 50, 40 and 30 mmHg with a reservoir attached to the right femoral artery. CBF measurements were taken at each BP. Total CBF was not changed by decreasing BP. Total CBF was 37 $\pm$ 4, 40 $\pm$ 4, 43 $\pm$ 7, and 35 $\pm$ 3 ml/min/100g at the respective BP's. None of the 19 regional CBF's measured decreased significantly when BP was decreased. However, blood flow to the hypothalamus increased from 20 $\pm$ 2 to 29 $\pm$ 4, 36 $\pm$ 6 and 35 $\pm$ 4 ml/min/100g ($P<0.05$). These results indicate that there may be a unique mechanism in the hypothalamus of the newborn which operates not only to maintain blood flow but to increase it with hypovolemic hypotension. This flow change may have been a result of increased metabolism in the hypothalamus due to the cardiovascular stress of hypotension.

51.7 **THE ACTIONS OF LEUKOTRIENES (LT's) C_4, D_4, E_4 AND F_4 ON CEREBRAL VASCULAR RESISTANCE IN THE ANESTHETIZED PIG.** **M. Cirino and L.G. Letts*. Merck Frosst Canada Inc., Montreal, Quebec**

LTC_4, D_4 and E_4 have potent actions on porcine vascular resistance in vivo as observed in the blood perfused hind limb (Cirino, M. and Letts, L.G., Can. J. Physiol. Pharmacol., 62, 1984). To compare the effects of the synthetic LTs in another porcine vascular bed we have studied their actions on cerebral vascular resistance. Pigs were anesthetized with sodium pentobarbital (2-4 mg/kg/hr, i.v.). Systemic arterial pressure (SAP) was measured using a catheter-tip pressure transducer inserted into the right femoral artery and the heart rate (HR) monitored. Left carotid arterial blood was pumped at a constant flow into the right carotid artery to perfuse the cerebrovascular bed. In each experiment flow rate (95 to 140 ml/min) was adjusted so that perfusion pressure (PP) equalled mean SAP. Injection of bolus doses of LTC_4 and LTD_4 produced dose-dependent increases in PP (see Table). When the higher doses of each LT passed into the systemic circulation moderate changes were recorded in SAP and HR. LTF_4 produced dose related reductions in PP. The administration of indomethacin (**Indo**, 5 mg/kg, i.v.) and methysergide (**Meth**, 2.0 mg/kg, i.v.) increased the baseline PP approximately 15mmHg and enhanced the PP effects of LTC_4, LTD_4 and LTF_4.

	PP (mmHg ± sem)		
	Before	After	After
dose µmol/kg	Indc + Meth	Indo + Meth	FPL55712
LTC_4			
1.6×10^{-10}	5.8±0.8 (n=7)	10.5±2.2 (n=6)	
3.2×10^{-10}	9.8±1.7 (n=7)	18.2±3.4 (n=6)	
8.0×10^{-10}	19.9±3.6 (n=9)	25.6±5.3 (n=8)	16.8±1.7 (n=4)
LTD_4			
2.0×10^{-10}	8.3±1.4 (n=7)	17.5±2.9 (n=6)	
4.0×10^{-10}	14.0±2.2 (n=7)	24.7±3.1 (n=6)	
1.0×10^{-9}	25.0±3.3 (n=9)	29.6±2.8 (n=7)	11.4±2.9* (n=5)
LTF_4			
1.7×10^{-10}	-8.0±2.3 (n=7)	-15.8±3.6 (n=6)	
3.4×10^{-10}	-15.9±3.4 (n=9)	-23.3±2.5 (n=8)	
8.6×10^{-10}	-26.1±4.5 (n=7)	-35.8±3.6 (n=6)	-26.3±4.0 (n=4)

Infusion of FPL-55712 (0.1 mg/kg/min) into the extracorporeal line reduced the pressor effects of LTC_4 and LTD_4 (*$p\leq0.05$), but did not affect the dilator response of LTF_4. LTE_4 did not produce any changes in PP at doses up to 1.1×10^{-8} µmol/kg. It is concluded that LT's can modulate porcine cerebral vascular resistance.

51.8 CEREBRAL VASCULAR REACTIVITY VIEWED DIRECTLY WITH FLUORESCENCE MICROSCOPY. Rong-Sheng Lee and Paul F. McDonagh. Department of Physiology, Texas Tech Univ. Hlth. Sci. Ctr., Lubbock, TX 79430.

Recently we described a technique to view the pial microcirculation directly by intravital fluorescence microscopy. It is our objective to use this model to examine neurogenic regulation of the cerebral circulation; however, it was necessary first to determine whether the pial microvessels in this model were vasoactive. Accordingly, the reactivity of pial arterioles to local application of either acidic (pH = 6.87) artificial CSF solution or 5% $BaCl_2$ solution was studied in rats anesthetized with sodium pentobarbital. After fronto-parietal craniotomy, the dura mater was reflected and the pial microcirculation superfused with mock CSF solution (pH = 7.34). Pial arterioles were viewed directly by intravital fluorescence microscopy. First, a fluorescent albumin conjugate (FITC-BSA) was injected (i.v., 0.2 ml of 5 g%/100 g); then the microvessels were illuminated and observed through a Zeiss fluorescence microscope. Arteriolar diameter was measured directly from the TV image by a video analyzer (Vista Electronics). The arterioles examined ranged from 15-76 µm in diameter. We found that topical application of the acidic CSF solution caused a 32.3 ± 2.8% (S.E.) increase in pial arteriolar diameter ($P < 0.01$). The 5% $BaCl_2$ solution elicited a 27.5 ± 2.8% decrease in pial arteriolar diameter ($P < 0.01$). It appeared that the smaller arterioles (20-40 µm dia) were more responsive to the acid challenge than the larger arterioles (40-60 µm) studied. The smaller arterioles demonstrated a 39 ± 3% increase in diameter, whereas the larger arterioles demonstrated only a 23 ± 3% increase in diameter ($P < 0.05$). These results indicate that under fluorescence microscopy, rat pial arterioles are indeed vasoactive. The response to acid CSF compared favorably with that reported by Kontos et al. (Stroke 8: 358, 1977). It appears that intravital fluorescence microscopy can be employed to study the vasoactivity of cerebral microvessels. The cerebral dilator effect caused by decreased pH was most dramatic in the smallest terminal pial arterioles. (Supported in part by a grant from Tarbox Parkinson's Disease Institute of Texas Tech University School of Medicine.)

51.9 ADRENAL GLANDS PARTICIPATE IN THE METABOLICALLY-LINKED CEREBROVASODILATION ELICITED BY STIMULATION OF THE DORSAL MEDULLARY RETICULAR FORMATION IN RAT. C. Iadecola, M.D. Underwood*, T. Ishitsuka* and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Electrical stimulation of the dorsal medullary reticular formation (DMRF) globally increases regional cerebral blood flow (rCBF) and metabolism (Iadecola et al., Brain Res. 272:101,1983). Transection of the spinal cord at C1 with maintenance of arterial pressure (AP) by phenylephrine infusion reduces, but does not abolish, the increase in rCBF. Conceivably, the release of substances from the periphery contribute to the metabolic vasodilation. In this study, we sought to determine: (a) whether adrenal hormones contribute to the increase in rCBF elicited from DMRF stimulation, and, if so (b) whether the cerebrovascular effects can be attributed to global disruption of the blood brain barrier (BBB). Rats were anesthetized, paralyzed and artifically ventilated. AP was monitored and blood gases were kept in physiological range. DMRF was stimulated (50 Hz; 50uA; 1 sec on/1 sec off) and AP kept in the autoregulated range for rCBF. rCBF was measured by the ^{14}C-iodoantipyrine technique in 13 brain samples removed by dissection. BBB permeability was determined in dissected brain samples by measuring the blood-brain influx constant (Ki) of aminoisobutyric acid (AIB). In unstimulated intact rats (n=5), rCBF ranged from 59 $\pm$ 7 (ml/100g x min) in corpus callosum to 114 $\pm$ 10 in sup. colliculus. Acute adrenalectomy (n=5) did not significantly change rCBF. In intact rats (n=4), DMRF stimulation elicited a global increase in rCBF with maximal responses in cerebral cortex (frontal (Fcx), 200% of control; occipital (Ocx), 187%; $p<0.001$). In contrast, after adrenalectomy (n=4), DMRF stimulation increased rCBF only in Pcx and Ocx (to 135% of control; $p<0.05$). Therefore, adrenalectomy reduces the increases in rCBF to a greater extent than spinal cord transection. In controls (n=4), Ki for AIB ranged from 2.5 $\pm$ 0.03 (ml/g x sec x 10^{5}) in caudate n. to 11.2 $\pm$ 2.1 in hypothalamus. During DMRF stimulation (n=4), Ki values did not different from control ($p>0.05$), indicating that the BBB was not disrupted. We conclude that adrenal hormones, presumably catecholamines (CAs), substantially contribute to the metabolically-linked cerebrovasodilation elicited by DMRF stimulation. The mechanism is obscure since CAs do not cross the BBB. Conceivably, DMRF stimulation might activate a specific mechanism for brain uptake of CAs resulting thereby in increased metabolism and rCBF, or alternatively they might activate a trigger zone located outside of the BBB.

51.10 BILATERAL LESIONS OF NUCLEUS TRACTUS SOLITARII IN RAT GLOBALLY IMPAIRS CEREBROVASCULAR AUTOREGULATION. T. Ishitsuka*, C. Iadecola, M.D. Underwood* and D.J. Reis (SPON: D.J. Reis). Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

We sought to determine whether bilateral lesions of the nucleus tractus solitarii (NTS) producing acute hypertension, would affect regional cerebral blood flow (rCBF) and its autoregulation in rat.

Rats were anesthetized (chloralose 40 mg/kg), paralyzed and artificially ventilated. Arterial pressure (AP) was monitored and blood gases were kept in physiological range. rCBF was measured in 13 brain areas by the ^{14}C-iodoantipyrine technique with regional dissection (Nakai et al., Am. J. Physiol. 243:H266, 1982).

Bilateral electrolytic lesions of NTS elevated AP from 124 $\pm$ 4 to 171 $\pm$ 3 mmHg and increased rCBF globally from 190% of control in hippocampus to 330% of control in parietal cortex (Pcx) ($p<0.005$, n=5). Passive elevation of AP to the same level (170 $\pm$ 3 mmHg) by phenylephrine (n=5) increased rCBF in all regions ($p<.05$). However, in nine areas, rCBF increases were significantly less than those associated with NTS hypertension. To determine if NTS lesions abolished autoregulation, rCBF was measured at steady-state values of AP (90,125,140,170 mmHg) in unlesioned controls or after NTS or cuneate n. lesions. In unlesioned rats (n=16) or after cuneate n. lesions (n=16), rCBF did not change at AP values from 90 to 140 mmHg (i.e., rCBF was autoregulated), but was elevated at 170 mmHg. In contrast, after NTS lesions (n=20), autoregulation was substantially impaired in all regions so that rCBF was less than control at 90 mmHg and greater than control at values over 125 mmHg ($p<0.05$; analysis of variance). To establish if cerebrovascular reactivity was impaired by the NTS lesions, rCBF response to inhalation of 5% CO_2 was examined in rats with (n=5) and without (n=6) NTS lesions. NTS lesions did not reduce the cerebrovaso-dilatory response to CO_2 in any region. We conclude that: (a) bilateral NTS lesions increase rCBF globally in association with hypertension; (b) the increases in rCBF after NTS lesions are greatest in cerebral cortex and exceed those obtained by increasing AP passively to a comparable extent; (c) NTS lesions markedly perturb cerebrovascular autoregulation globally in brain; (d) the effect upon autoregulation of the NTS lesions is anatomically specific and not due to vasoparalysis. Thus, cerebrovascular autoregulation, in addition to local vascular myogenic mechanisms, is dependent upon neural pathways, some of which may be represented in NTS. (Supported by Grant HL18974).

51.11 ARE THERE TWO CONTROL SYSTEMS WITHIN THE FASTIGIAL NUCLEUS OF THE RAT, ONE AFFECTING THE SYSTEMIC AND ANOTHER THE CEREBRAL CIRCULATION. S. Mraovitch, E. Pinard* and J. Seylaz*. Lab. of Physiologie et Physiopathologie Cérébrovasculaire, C.N.R.S. E.R.A. 361, I.N.S.E.R.M. U. 182, Paris, France.

The fastigial nucleus (FN) of the cerebellum plays an important role in the regulation of the systemic (Mura and Reis, Am. J. Physiol. 219, 1330, 1970) and the cerebral (Nakai et al., Am. J. Physiol. 243, H226, 1982) circulation. Electrical stimulation of the rostral FN will increase arterial pressure (AP), heart rate (HR) and cerebral blood flow (CBF) without affecting cerebral metabolism. In the present study we sought to examine in rat whether increase in CBF elicited by FN stimulation is related or not to variations of AP.

Rats were anesthetized (pentobarbital) and two probes were stereotaxically implanted in right and left cortex (areas 2, 2a, 13 and 14, Krieg, 1946) to allow for repetitive and quantitative measurements of CBF by mass spectrometry (Seylaz et al., Am. J. Physiol. 245, H513, 1983). Two weeks later the rats were reanesthetized (chloralose), paralyzed (tubocurarine) and artificially ventilated. In all animals AP, HR and body temperature were continuously monitored and blood gases were measured and controlled. The FN was electrically stimulated with intermittent trains (1 sec on/1 sec off) at 50 Hz and with an intensity up to 50 μA. At all times AP was maintained within the autoregulatory range ($<$ 150 mm Hg) for rat CBF. In all animals histological localization of stimulated sites were carefully reconstructed.

The FN stimulation induced increases in CBF which is in good agreement with previous findings (Nakai et al. Am. J. Physiol. 243, H 226, 1982).

A systematic exploration of FN for an augmentation of CBF in 6 rats revealed that out of 100 stimulated sites, 22 sites giving bilateral increase of cortical flow (up to +249%) lie within the ventrolateral region of the rostral FN. Stimulus intensity (10-50 μA) was carrefully adjuasted in order to minimally change AP. The influence of FN stimulation on the relationship between AP and CBF was analysed by using linear regression analysis. Correlation coefficient of $r = 0.62$ indicates that increase in CBF is not significantly dependent on AP.

We tentatively conclude that effects of FN stimulation upon blood pressure and CBF may be mediated by two distinct neural systems. While the exact anatomical localization of the neurons subserving the cerebral circulation remains to be elucidated, the present results demonstrate that they may lie in the proximity to those regulating the systemic circulation.

52.1 EFFECTS OF VASOPRESSIN AND DES-GLY VASOPRESSIN ON THE ACQUISITION AND EXTINCTION OF A SPATIAL LEARNING TASK IN RATS. Aaron Ettenberg and Mark Packard*. Dept. Psychology, University of California, Santa Barbara, CA 93106.

It is well established that administration of the neurohypophyseal hormone, vasopressin (AVP), can improve conditioned test performance in lab animals. However, the precise mechanism of action for this effect remains controversial. Some investigators have stated that AVP directly acts on the neural substrates of memory. Others have suggested that improved test performance occurs only indirectly as a consequence of the endocrinological (i.e. arousing) properties of the peptide. A means of dissociating the central from peripheral effects of AVP is to employ an AVP analog (e.g. des-gly-vasopressin; DGAVP) lacking peripheral endocrinological actions. Improvements in conditioned test performance with this analog would provide strong support for the notion of a direct central mode of AVP action.

In the present study hungry rats were individually placed on an 8-arm radial maze for 10 min on each of 3 successive days. During this period no food was present in the maze. After each day the rats received a 5ug/kg s.c. injection of AVP or DGAVP or an equal volume of saline (n=7/group). On the 4th day, two 45mg food pellets were placed at the endpoints of each of the 8 arms. Each rat was permitted to choose 16 arms before being removed from the maze and returned to its home cage. The number of errors (returning to an already visited arm) was recorded for the first 8 choices for every animal on every trial.

After 18 days of acquisition, an extinction procedure was instituted. With no food in the maze, every animal was permitted to visit each arm once. The rats were then injected with the same treatment they had received during training. Two hours later, each animal was replaced on the maze and the total number of arms chosen in 10 min was recorded. Three additional extinction trials were conducted at 2-hr intervals. In this task, improved memory would be indicated by a potentiation of extinction i.e. rats should more readily learn to cease responding since food was longer available in the maze.

Neither AVP nor DGAVP produced any reliable effects during acquisition. However, during extinction, AVP-treated animals ceased responding far more rapidly than saline control rats. Conversely, DGAVP reliably increased activity during the 1st two extinction tests, an effect which is inconsistent with a prediction of memory facilitation. These results support the position that peripheral endocrinological actions may be necessary to demonstrate the memory-improving actions of AVP.

52.2 STRESS MIMICS EFFECTS OF VASOPRESSIN ON BEHAVIOR. G.F. Koob, R. Dantzer, F. Rodriguez, F.E. Bloom, and M. Le Moal. Div. Preclinical Neuroscience and Endocrinol. Scripps Clinic and Res. Fdn. La Jolla, CA 92037. Lab. Psychobiol. Comportements Adaptatifs.U 259 INSERM and Lab. Neurobiol. U 176 INSERM, 33077 Bordeaux, France.

Substantial work exists to show that administration of arginine vasopressin (AVP) can improve the retention of learned responses in addition to its classical physiological functions. Although a direct neural subsrate involved in this effect on memory consolidation has been hypothesized, our recent work has emphasized the role of peripheral visceral signals in some of these effects. The demonstrated efficacy of a pressor antagonist of AVP (1-deaminopenicillamine - 2-O methyltyrosine arginine vasopressin - dptyr(Me)AVP) to reverse these behavioral effects of exogenously administered AVP provides a powerful tool by which to examine the possible functional significance of endogenously released AVP.

Male, Wistar rats weighing 150-220gms were trained to avoid shock in a pole jump avoidance test. A 5 sec conditioned stimulus (light illumination) preceeded 0.3-0.5 MA foot shock. Following 3 days of acquisition (10 trials/day), the rats were subjected to a fourth day of extinction testing with the shock turned off. Here, four blocks of 10 trials each were run and after the first 10 trials each rat was treated with 0.5 ml subcutaneous (s.c.) injection of 0.9 % saline or intraperitoneal (i.p.) injection of 0.25-2.0 molar hypertonic saline in a volume of 2.0 ml. Extinction testing continued for 3 more blocks of ten-trials, one block every 2 hours. In a second experiment, the procedure was identical except that the rats received two injections : either 0.9 % saline or dptyr(Me)AVP (s.c.), and then 1.0 or 1.5 M NACL (i.p.).

The i.p. injection of hypertonic saline prolonged extinction of active avoidance similar to that observed with 1 ug/rat AVP and this prolongation of active avoidance was blocked by 5 and 10 ug/rat of the vasopressin antagonist, dptyr(Me)AVP. The hypertonic saline also had aversive effects similar to AVP (acted as an unconditioned stimulus in a taste aversion test) and these aversive effects also were blocked by dptyr(Me)AVP. These results show that a severe osmotic stress can mimic the effects of AVP on learned behavior presumably by release of AVP, and suggest a role for endogenously released AVP in adaptive behavior.

52.3 AVERSIVE STIMULUS PROPERTIES OF AVP ARE CAUSED BY ITS HYPERTENSIVE EFFECT. M.Le Moal, R.M.Bluthé, R.Dantzer, and P.Mormède. Inserm U259, Psychobiologie des Comportements Adaptatifs, rue Camille Saint-Saëns, 33077 Bordeaux, France.

Arginine vasopressin(AVP) has been shown to induce conditioned taste aversion(CTA) at behaviorally active doses(Dantzer et al.1983, Ettenberg et al.1983). In the present experiments, we tested the hypothesis that the aversive stimulus properties of AVP are caused by its hypertensive effect using the vasopressor antagonist of AVP, dPTyrMe-AVP (AAVP) and by testing the generalization of the stimulus properties of AVP to another hypertensive agent Angiotensin (AII).

In the first experiment, 20 rats were trained to drink their daily amount of water in a 30min session. On day 1, they were exposed to a solution of 0.1 saccharin instead of water and then injected with both 10ug/kg AVP and 50ug/kg AAVP. Three other groups received saline, 10ug/kg AVP or 50 ug/kg AAVP alone(n=5). The same procedure was repeated on day 3. Preference for saccharin or water was measured on day 5 in a two bottle test. AVP treated animals avoided saccharin (32.6 % saccharin preference) in comparison to saline treated animals (68.5 %) and this effect was reversed by AAVP (55. 82 %) which had no effects on its own (67.34 %) ($F(1,16)=4.52$ $p<.05$).

In the second experiment, 36 rats were submitted to the same CTA paradigm using a 3 by 3 factorial design : before conditioning, rats were treated either with daily injections of saline(SAL) or with AVP 2ug/kg, 6ug/kg and 10ug/kg, each dose being injected for 2 successive days, or with AII 100ug/kg, 150ug/kg and 200ug/kg. These treatments were administered separate from the daily sessions of water intake. Each group was subdivided into 3 subgroups receiving, respectively; saline, 6ug/kg AVP, or 150ug/kg AII after drinking saccharin on days 1 and 3. Preference for saccharin was assessed in two test sessions run on days 5 and 6. Pretreatment with AII blocked the aversive effect of AVP(SAL-AVP 30.3 % saccharin preference, SAL-AII 31 %, SAL-SAL 86.1 % AVP-SAL 81.4 %, AVP-AVP 51.8 %, AVP-AII 55.6 %, AII-SAL 76.1 %, AII-AII 79.8 %, AII-AVP 62.8 %, $F(4,25)=5.59$ $p<.01$).

The results of these experiments support the hypothesis that hypertension is a critical factor in the aversive properties of AVP and suggest that high circulating levels of AVP may have important visceral discriminative properties.

52.4 ARGININE VASOTOCIN (AVT) CAUSES A REDUCTION IN THE GILL WITHDRAWAL REFLEX (GWR) OF APLYSIA BY NARROWING THE SENSORY NEURON ACTION POTENTIAL. William F. Colmers, J. Edstrom*, and Ken Lukowiak*, Department of Medical Physiology, University of Calgary, Calgary, Alberta, T2N 1N4, Canada.

The GWR of Aplysia californica undergoes associative and non-associative learning in vitro. Recently, some endogenous substances have been found to modulate the properties of this reflex. The neuropeptide AVT, which has been found in the central nervous system (CNS) of Aplysia, causes an increase in the CNS suppressive control over the GWR. The central component of the reflex is thought to be mediated via LE cluster sensory neurons (SN) of the abdominal ganglion, which form a monosynaptic excitatory connection with the gill motoneurons of that ganglion. Many of the plastic properties of the GWR are thought to reside at this synapse.

We have investigated the mechanism of AVT's effect on this synapse. Isolated abdominal ganglia were desheathed and superfused at a constant 2ml/min flow rate at a constant temperature. Shielded, 20 - 30 Megohm electrodes were used to impale the somata of LE cluster SN's. Action potentials (AP's) were evoked by brief pulses of inward current, passed via bridge circuit. The width of the AP was measured at the midpoint voltage of the repolarization phase of the AP. The superfusion of 10mM TEA in the seawater causes an increase in the AP width of 35%. The addition of 1 uM AVT to the TEA seawater causes a decrease in the AP width of 10 - 20%. The AP width increases to the earlier value after subsequent superfusion of seawater containing TEA only. In addition, AVT caused a decrease in the frequency-dependent spike broadening (FDSb, Edstrom and Lukowiak, Neuroscience, in press) in SN's. FDSb occurs due to a progressive inactivation of an outward potassium current, unmasking an underlying calcium inward conductance. Thus, the action of AVT may presumably be to decrease a calcium conductance in these neurons.

A decrease in the width of the AP, caused by a decreased calcium influx would presumably lead to a decrease in transmitter release at the excitatory synapse. AVT has previously been shown to cause a decrease in evoked monosynaptic EPSP's from SN's to gill motoneurons (Goldberg, 1983). We have therefore shown a plausible mechanism for the action of AVT on the GWR.

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52.5 OXYTOCIN AND ARGININE-VASOTOCIN ENHANCE GROOMING IN THE RAT. F. Drago*, J. Caldwell*, C.A. Pedersen, and A.J. Prange, Jr. Dept. Psychiatry, Biological Sciences Research Center, Univ. North Carolina School of Medicine, Chapel Hill NC 27514, and Inst. Pharmacology, Univ. Catania, Italy.

We have shown that intracerebroventricular (ICV) infusion of oxytocin (OXY) will increase the incidence of maternal behavior and the frequency of lordosis behavior in female rats. In this study, we demonstrate that ICV injections of OXY and associated peptides will induce grooming behavior in male and female rats.

Animals were observed in novel Plexiglas cages beginning 25 minutes after ICV injection of various peptides or saline vehicle (5 µl). Grooming was scored as the total number of 15 second intervals in which grooming occurred over a 30-minute observation period (i.e., maximum possible score was 120). In intact female rats, grooming behavior increased in a dose-dependent manner following administration of OXY doses from 0.1 in 10 µg (from 37 ± 5 to 86 ± 11 versus 8.1 ± 1 for saline injected controls). Intact males demonstrated similar increases in grooming following OXY doses of 1 and 5 µg (57 ± 4 and 85 ± 5 respectively, vs. 9 ± 2 for saline injected controls). In females, grooming behavior remained elevated for 45 minutes during a prolonged observation period. Arginine-vasotocin in a dose equimolar to 500 ng of OXY was very effective in inducing grooming (84.4 ± 3.5), whereas doses of tocinoic acid, MIF-1 and lysine vasopressin equimolar to 1 µg OXY were less effective (15.2 ± 3, 23.7 ± 5, and 38.7 ± 7, respectively).

When injected intraperitoneally one hour before behavioral observations, the dopamine receptor antagonist, haloperidol (0.5 mg/kg) totally suppressed (8 ± 2) and the opioid antagonist, naloxone (1 mg/kg), attenuated (43 ± 6) the grooming induced by 1 µg OXY. Ovariectomy (OVX) had no effect on the quantity of grooming induced by 1 µg OXY (sham OVX = 56 ± 4, OVX = 52 ± 5). A small but significant increase in grooming was seen following SC injection of 100 and 200 µg OXY.

We conclude that OXY increases grooming behavior, the effect is not sex or steroid dependent and may involve dopamine and/or opioid neurotransmission.

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52.6 OXYTOCIN FACILITATES LORDOSIS BEHAVIOR IN ESTROGEN-TREATED RATS. J. D. Caldwell*, C. A. Pedersen*, and A. J. Prange, Jr. (SPON: M.A. Lipton). Dept. Psychiatry, Biological Sciences Research Center and Neurobiology Program, Univ. North Carolina School of Medicine, Chapel Hill, NC 27514.

Intracerebral infusion of a number of peptides will result in changes in female receptivity. Peptides such as Gn-RH, ACTH, CRF, and a-MSH and certain of their fragments will enhance lordosis behavior while vasopressin, β-endorphin, and longer ACTH fragments reduce female receptivity. We have found that the nonapeptide oxytocin (OXY), when injected intracerebroventricularly (ICV) will greatly enhance lordosis behavior in ovariectomized (OVXed) estrogen-treated rats.

Previously untreated animals were injected SC for three days with 0.5 µg estradiol benzoate (EB). On the fourth day, animals were tested with sexually active male rats before and 20, 40, and 90 minutes after ICV infusions of OXY in 5 µl normal saline or normal saline alone. OXY doses of 800, 1000, 1200, and 2000 ng significantly ($p < .01$, .02, .01 and .005, respectively with t-test on ranks) increased lordosis quotients (LQs) over vehicle treated animals. Oxytocin (1000 ng) increased LQs significantly ($p < .005$) compared to an ICV infusion of an equimolar dose of Gn-RH.

In OVXed animals previously (one week or more) given a SC dose of 200 µg EB for the purpose of maternal behavior testing, doses of 0.15, 0.2, or 0.25 µg EB x three days were sufficient to sensitize animals to the lordosis-enhancing effects of 800 ng OXY ($p < .01$ vs. saline injected controls). Animals primed with corn oil vehicle alone showed no enhancement of receptivity following ICV OXY. In animals primed with 0.25 µg EB x three days, a dose of neurotensin equimolar to 800 ng of OXY was ineffective in enhancing lordosis response.

We conclude that OXY enhances lordosis behavior, that this effect is estrogen dependent and, under our testing conditions, somewhat specific to OXY.

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52.7 OXYTOCIN REVERSES NOVELTY INHIBITION OF ONSET OF OVARIAN STEROID-INDUCED MATERNAL BEHAVIOR. C.A. Pedersen, J.D. Caldwell* and A.J. Prange, Jr. Dept. of Psychiatry and Biol.Sci. Research Center, Univ. North Carolina, Chapel Hill, NC 27514

We first compared the effects of a novel cage environment on the onset of maternal behavior in ovarian steroid-treated nulliparous rats and on the reemergence of maternal behavior in primiparous rats separated for 24 hrs from pups. Nulliparous Sprague Dawley rats from Zivic Miller Laboratories (200-250 grams) were given SC one Silastic capsule containing 4.4 mg of 17 β-estradiol eight days after ovariectomy (OVX) and three capsules each containing 40 mg of progesterone ten days after OVX. Progesterone capsules were removed on the 20th day after OVX, 24 hrs before the introduction of three rat pups (1-5 days of age). Primiparous Sprague Dawley rats from Charles River Breeders were allowed 4-6 days of postpartum contact with pups and then separated from pups for 24 hrs. Some animals were allowed to habituate (habituated) to observation cages starting 16-24 hrs prior to introduction or reintroduction of pups. Other animals were introduced or reintroduced to pups at the same time they were placed in a novel observation cage (nonhabituated). Habituated ovarian steroid-treated nulliparous animals displayed a significantly ($p < .005$, Fisher's exact probability) higher incidence of full maternal behavior (FMB) in the first two hrs of pup contact (8/10) than did nonhabituated ovarian steroid-treated nulliparous animals (1/9). All primiparous animals, whether habituated or nonhabituated, displayed FMB within minutes of being reunited with pups.

Another group of nonhabituated, ovarian steroid-treated nulliparous rats were given intracerebroventricularly either 800 ng of oxytocin in 10 µl of normal saline or normal saline alone just before being introduced to pups in novel observation cages. Oxytocin significantly ($p < .01$) increased the incidence of FMB in the first two hrs of pup contact (15/18) compared to saline (5/16).

Yet another group of nonhabituated ovarian steroid-treated nulliparous rats were given naloxone (2 mg/kg) or vehicle alone IP one hr prior to being placed in a novel cage with pups. Naloxone did not increase the incidence of FMB in the first two hrs of pup contact.

Our results suggest that novelty may prevent the release of endogenous OXY and thereby delay the onset of maternal behavior. Endogenous opiates do not appear to mediate the inhibitory effects of novelty on the onset of ovarian steroid-induced maternal behavior.

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52.8 EFFECT OF PEPTIDES ON FLANK GLAND GROOMING FOLLOWING MICROINJECTION INTO THE MEDIAL PREOPTIC AREA OF GOLDEN HAMSTERS C.F. Ferris* and H.E. Albers Dept of Physiology, University of Massachusetts Medical Center, Worcester, MA 01605 and Worcester Foundation of Experimental Biology, Shrewsbury, MA 01545.

The microinjection of vasopressin (VP) into the medial preoptic area (MPOA) of golden hamsters triggers a complex stereotypic behavior, flank marking, a type of scent marking involved in olfactory communication (Ferris et al., Science in press). Accompanying the flank marking is a grooming behavior which we have called "flank gland grooming". This behavior consists of the animal vigorously rubbing its eyes and nose with its forepaws followed by a licking and chewing of the flanks creating large areas on both sides that are matted a soaked with saliva. Various peptides were microinjected into the MPOA and their effect on flank gland grooming observed. Hamsters were anesthetized with pentobarbital and a 26 gauge guide cannula implanted stereotaxically into the MPOA, the ventromedial or lateral hypothalamic areas (VM-LH), or directly into the lateral ventricle (LV). Animals received microinjections within three days after the guide cannulae were implanted. Four hamsters were microinjected into the MPOA with VP (50ng) angiotensin II (AII) (50ng), neurotensin (NT) (50ng), oxytocin (OXY) (50ng). All peptides were dissolved in 0.9% NaCl and given in a volume of fifty nanoliters. Each hamster was injected with each of the four peptides over the course of four days. There was a significant difference ($p < .01$) in the time spent flank gland grooming between the peptides tested. In a 10 min observation period the time spent flank gland grooming in response to VP and OXY were similar, 3.7 ± 1.05 and 3.3 ± .83 mins, respectively. Over 50% less grooming was observed following the microinjection of AII (1.59 ± .82 min) and NT (0.91 ± .57 min). There was a significant difference ($p < .05$) in the time spent flank gland grooming depending upon whether VP was injected into the MPOA, LV or VM-LH. Hamsters (n=4) microinjected with VP into the LV spent 1.88 ± .28 min grooming while animals (n=7) injected into the VM-LH spent 0.8 ± .28 min grooming. In these studies its was particularly interesting that VP and OXY had similar effects on flank gland grooming; since, only VP is effective in eliciting the flank marking component of the entire behavioral sequence.

52.9 VASOPRESSIN PRESSOR ANTAGONIST INJECTED CENTRALLY, REVERSES PERIPHERALLY BEHAVIORAL EFFECTS OF VASOPRESSIN BUT ONLY AT DOSES THAT REVERSE INCREASES IN BLOOD PRESSURE. C.Lebrun, M.Le Moal*, G.F.Koob and F.E.Bloom. (SPON: Judith Nyquist). Div. Preclin. Neurosci. & Endocrin., Scripps Clinic and Res. Fdn., La Jolla, CA; *INSERM, Unite 259, Bordeaux, France.

Previous work (Le Moal et al. Nature 291, 491, 1981) has established that the prolongation of extinction of active avoidance produced by subcutaneously (sc) injected arginine vasopressin (AVP) could be prevented by pretreatment of rats with a vasopressin analog (deaminopenicillamine 2.0 methyl tyrosine arginine vasopressin; [dPtyr (Me) AVP]) that antagonizes AVP pressor effects. Much smaller doses of AVP (1 nanogram/rat) injected intracerebroventricularly (icv) also prolonged the extinction of active avoidance (Koob et al. Regulatory Peptides 2, 153, 1981) and the latter effect could be antagonized by dPtyr(Me)AVP injected sc. The purpose of the present study was to determine if peripherally administered AVP acts via a peripheral blood pressure effect or by a direct action in the central nervous system. To test this we examined the effects of the antagonist injected icv on the prolongation of active avoidance extinction and on blood pressure effects of sc injected AVP. Male Wistar rats were equipped with a chronic cannula aimed above the lateral ventricle. A few days following surgery, the rats were trained to jump on a pole to avoid shock in a series of 10 trials on each of 3 successive days. On the fourth day, series of extinction tests were conducted every two hours. Following the first ten trials of extinction, each rat was placed into one of 3 groups and received either a icv injection of SAL and a sc injection of SAL (SAL/SAL group); or a icv injection of SAL and a sc injection of AVP (SAL/AVP group); or a icv injection dPTyr(Me)AVP and a sc injection of AVP (dPtyr(Me)AVP/AVP group). The same division was used for the blood pressure experiment. Results showed that dPtyr(Me)AVP (6 µg; icv) blocked both the behavioral and the pressor effect of systemically injected AVP; 0.2 µg of antagonist icv did not block either effect. These results show that peripherally injected AVP acts on peripheral systems and support our hypothesis that the peripheral visceral actions of AVP contributes significantly to its behavioral effects (Supported by NINCDS NS 20912 and NSF INT-8215308 to GFK.)

52.10 APPROACH AND AVOIDANCE BEHAVIOR OF VASOPRESSIN-DEFICIENT ROMAN HIGH AVOIDANCE RATS. J.P. Herman, D.M. Gash, G.J. Thomas and C.T. Hansen*, Dept. of Anatomy and Center for Brain Research, University of Rochester, Rochester, NY 14642, and Veterinary Resources Branch, National Institutes of Health, Bethesda, MD 20205.

Recent evidence has suggested that genetically vasopressin (VP)-deficient Brattleboro rats (LE/DI rats) from different colonies may manifest divergent approach behavior, probably reflecting differences in the genetic background of the respective colonies. Similarly, differences in approach behaviors have been observed between normal Long Evans control animals of different colonies. In an attempt to control for extraneous genetic variability in the examination of behavioral concomitants of VP-deficiency, we have chosen to use the newly-developed VP-deficient Roman High Avoidance (RHA/DI) strain in our behavioral experiments. The inbred RHA/DI substrain is congenic with the normal RHA (RHA/NO) strain, differing from the parent strain only by the gene responsible for VP deficiency.

RHA/NO and RHA/DI animals were run in an approach-avoidance conflict test, allowing for the examination of neophobia, approach strength, and avoidance behavior in a single paradigm. Training consisted of a) six days of goal box (GB) placements, where food-deprived animals were placed in the GB of a straight runway and allowed access to wet mash for 60 sec.; b) ten days of approach trials, where the animals was required to traverse the straight runway to receive wet mash reward; c) one shock trial on the tenth day, where a 2.5 mA shock was delivered upon contact with the food dish; d) ten days of post-shock trials, where the animal was given the opportunity to once again traverse the runway to receive reward.

As expected, there were no differences between RHA/NO and RHA/DI groups in either the neophobic response to eating in the novel GB or in preshock approach latency. These results suggest that differences in approach behavior observed between Long Evans DI and NO animals are due to the genetic incompatibility of the experimental groups. Unexpected, however, was the observation the RHA/DI rats extinguish their post-shock avoidance response more rapidly than RHA/NO animals ($p<0.05$, two-way ANOVA). This result was not observed in animals from either colony of LE/DI rat run previously in an identical paradigm. It is theorized that the attenuated extinction in the RHA/DI substrain is reflective of a differentially attenuated emotional reactivity inherent to VP-deficiency, masked by polygenic factors in the LE/DI strain.

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52.11 SUPPRESSION OF LATERAL HYPOTHALAMIC SELF-STIMULATION BY VASOPRESSIN-RELATED PEPTIDES IN RATS. W.H. Simmons*, G. Meisenberg and S.A. Lorens. Dep. of Biochemistry, Loyola Univ. Med. School, Maywood, IL 60153.

Oxytocin (OXY) and vasopressin (AVP) occur in various brain areas thought to be important in reinforcement. Therefore, the effects of OXY, AVP, and some synthetic analogs on lateral hypothalamic self-stimulation (LHSS) were determined in rats. The animals were tested daily for 10 minutes in an operant conditioning chamber. Each lever press delivered a 0.2 sec train of biphasic square waves (100 Hz) on a continuous reinforcement schedule. The current-intensity was adjusted individually to yield approximately half-maximal response-rates. On drug days, the animals were tested before and 10-20 minutes after and, in one experiment, 40-50 min after the intracerebroventricular (i.c.v.) injection of a peptide- or vehicle-solution. After i.c.v. injection, AVP (100ng), OXY (400ng or 2.0ug), and 1-deamino[6-carba,8-ornithine]vasopressin (D-COVP) suppressed LHSS, while 1-mercapto-,'-cyclopentamethylenepropionic acid 2-(O-methyl) tyrosine,8-arginine]vasopressin (PMP-AVP) (1.0ug) and 8-lysine,9-desglycinamide]vasopressin (DG-LVP) (1.0ng) were inactive. D-Covp was the most potent peptide, with a dose-dependent suppression of LHSS being observeable at doses between 6.25 and 50ng. In this dose range, only slight changes of spontaneous motor activity were observed. The effects of AVP (100ng) and OXY (400ng and 2.0ug) could be observed 10-20, but not 40-50 minutes after the injection, whereas the metabolically stable analog D-COVP (12.5ng) was still significantly active 40-50 minutes after the injection. A combination of PMP-AVP (1.0ug) and D-COVP (12.5ng) did not significantly reduce LHSS, suggesting that PMP-AVP, an antagonist of the pressor effect of AVP, antagonizes the LHSS-reducing effect as well. The inactivity of DG-LVP suggests that the LHSS-reducing effect is independent of previously described memory-effects of AVP, and the considerable potency of OXY suggests that it is unrelated to the convulsant properties that these peptides show at higher doses. The short durations of action of AVP and OXY and the more prolonged effect of D-COVP suggest that biological inactivation is an important determinant for the effects of these peptides on LHSS.

The authors are greatly indebted to Dr. T. Barth and K. Jost from the Czechoslovak Academy of Sciences for their generous gift of D-COVP.

53.1 FACILITATION OF LORDOSIS BY INFUSION OF SUBSTANCE P IN THE MIDBRAIN CENTRAL GRAY. Wayne A. Dornan*, Charles W. Malsbury, Memorial University, Dept. of Psychology, St. John's, Newfoundland, Canada, A1B 3X9

It seems clear that the induction of sexual receptivity in rodents by gonadal hormones is mediated at least in part by ventromedial nucleus (VMN) projections to the midbrain central gray (MCG). It also seems likely that peptides synthesized in the VMN region and transported to terminals in the MCG are acting at this site to facilitate lordosis. It is not known, however, which neurotransmitters or neuromodulators synthesized in the VMN facilitate lordosis. Recently, LHRH and prolactin have been found to facilitate lordosis when infused into the MCG. However, whether prolactin or LHRH-containing cell bodies are present in the VMN is not clear.

In contrast, immunocytochemical studies report numerous Substance P (SP) containing cell bodies in the VMN. In addition, nerve terminals, as well as receptors for SP, have been located in the MCG, particularly in its dorsal segment (an area in which electrical stimulation has been reported to facilitate lordosis).

In light of these findings, we investigated the role of SP in the modulation of female sexual receptivity. We were interested in comparing SP with LHRH, a peptide previously reported to facilitate lordosis when infused into the MCG.

60 female Sprague Dawley rats (250-300g) were ovariectomized, and after a one week recovery period, were stereotaxically implanted with 28 gauge bilateral cannulae directed at the dorsal segment of the MCG. Following a week recovery, animals were injected with 5µg of EB and tested 96 hours later for sexual receptivity. Only animals which displayed moderate levels of lordosis were subsequently infused with either LHRH (50ng/.5µl), acidified SP (see Hall and Stewart, Peptides, 4, 1983, for rationale), or acidified saline. All animals in the SP group received three different doses of SP (either 50ng, 500ng, or 1µg/.5µl), in counterbalanced order.

Infusion of both acidified SP and LHRH led to a prompt facilitation of lordosis, evident at 5 minutes post infusion, and for as long as 2 1/2 hours. SP also produced a dose related facilitation of lordosis. Moreover, doses of 500ng and 1µg of SP yielded a greater facilitation at 5, 10, 30, and 90 minutes post infusion compared to LHRH.

53.2 COMPARISON OF THE SPINAL LEVEL ACTIONS OF SUBSTANCE P AND RELATED PEPTIDE FRAGMENTS IN A MOUSE BEHAVIORIAL MODEL. J. Heym, M.R. Boucher*, L.S. Reynolds*, D.A. Clark* and C.J. Pazoles. Central Research Division, Pfizer Inc., Groton, CT 06340

Substance P (SP) is a proposed peptide neurotransmitter that is present in vertebrate spinal cord. Although SP is found throughout the spinal cord, attention has focused largely on its localization within the dorsal horn and its possible role in mediating or modulating the transmission of nociceptive information. When SP is injected intrathecally (i.t.) into the lumbar spinal cord of mice a dose-dependent bite/scratch behavior ensues which presumably reflects nociception (Hylden and Wilcox, Brain Res. 217, 212, 1984). We have utilized this behavioral model to characterize the spinal level agonist activity of SP and related C-terminal fragments.

SP is a potent compound for inducing bite/scratch behavior after i.t. injection with an ED_{50} of 4.5 pmoles/mouse. Hexa- and heptapeptide C-terminal fragments of SP were equipotent to SP for eliciting the bite/scratch syndrome. However, C-terminal fragments consisting of less than 6 amino acids were considerably less potent as evidenced by the relative inactivity of the C-terminal pentapeptide. $pGlu^6Pro^9$-SP(6-11), reported to be a "superagonist" for SP_2 receptors in the spinal cord (Piercey et al., Neurosci. Abstr. 9, 171, 1983), was no more potent than $pGlu^6$-SP(6-11) or SP as a bite/scratch agonist. However, the Pro^9 substitution significantly increased the duration of the behavioral effect and raised the level of maximum response by 50%. This suggests that the "superagonist" properties of $pGlu^6Pro^9$-SP(6-11) observed in vivo may be the result of increased efficacy due to metabolic stability rather than enhanced potency. In contrast to findings in rat brain where $pGlu^5MePhe^8Sar^9$-SP(5-11) is a more stable and functionally equipotent analog of SP (Eison et al., Science 215, 188, 1982), methylation of Phe^8 dramatically reduced the spinal level activity of heptapeptide fragments.

These data demonstrate that the mouse bite/scratch model is a useful assay for studying structure-activity relationships of SP agonists. C-terminal fragments as small as hexapeptides are as potent as SP for eliciting this behavior after i.t. injection. Interestingly, spinal level activity of SP agonists may not parallel their observed activity in brain.

53.3 STRUCTURE-ACTIVITY STUDIES OF SUBSTANCE P: EFFECTS ON MOTOR BEHAVIOR. M.E. Hall* and J.M. Stewart, Dept. Biochem., University of Colorado Medical School, Denver, Colo. 80262.

Substance P (SP) alters open field behavior in mice. Intraventricular injections of SP significantly increased grooming, scratching, rearing, sniffing, cage licking and locomotion in Swiss/ Webster males. Injections of the C-terminal hexapeptide fragment of SP, thought to be essential for biological activity, fully reproduced the effects of SP on grooming, scratching, licking and locomotion, but caused a decrease, rather than an increase, in rearing and sniffing. Treatment with the N-terminal heptapeptide SP(1-7), thought to be biologically inactive, fully reproduced the effects of SP on rearing and sniffing, but not on grooming, scratching, licking or locomotion. Thus, both the N- and C- terminal regions of SP are required to produce the full range of SP effects on motor behavior.

Additional information regarding the structural requirement for producing N-terminal (increased rearing and sniffing) and C-terminal (increased grooming, scratching, and licking, and decreased rearing and sniffing) motor effects was obtained from tests of N- and C-terminal fragments of varying lengths. Increased rearing was seen with SP(1-6) and SP(1-7). SP(1-4) was also effective, but less potent than SP(1-6) or SP(1-7). SP(1-2) and SP(1-8) were inactive. SP(2-7) was effective, but SP(3-7) was not. C-terminal effects were seen with SP(4-11), pyroglutamyl-SP(6-11) and pyroglutamyl-SP(7-11). The short C-terminal fragments SP(7-11) and SP(8-11) were inactive on grooming, scratching, licking and locomotion, but still reduced rearing and sniffing.

These studies reveal that three separate biologically active regions of the SP molecule are involved in mediating effects of SP on open field behavior. These findings should influence the development of SP antagonists, metabolically stable agonists, and studies of the enzymatic degradation of SP.

53.4 NEUROTENSIN SUPPRESSES LOCOMOTOR HYPERACTIVITY INDUCED BY AMPHETAMINE BUT NOT BY SCOPOLAMINE. K.M. Skoog, S.T. Cain, J. Kenner*, and C.B. Nemeroff, Dept. Psychiat., Duke Univ. Med. Ctr., Durham, NC 27710.

Neurotensin (NT) administered intracisternally (IC) markedly attenuates the locomotor hyperactivity in mice and rats induced by d-amphetamine, methylphenidate and cocaine (J. Pharmacol. Exp. Ther. 225: 337, 1983). In the present study, the effect of IC NT on scopolamine-induced hyperactivity was compared with its effect on d-amphetamine-induced hyperactivity in order to evaluate the specificity of NT-induced suppression of hyperactivity induced by psychomotor stimulants. Scopolamine (a muscarinic antagonist) produces hyperactivity by a mechanism independent of DA-containing neural systems (Psychopharmacology 73: 311, 1981).

Sixty to 70 day old Sprague-Dawley rats were habituated to photocell activity cages (Opto-Varimex, Columbus Instruments) at least one day prior to testing. On test days rats were briefly anesthetized with ether and injected IC. Eighteen rats received 30 µg IC NT followed immediately by intraperitoneal (IP) injections of 2 mg/kg scopolamine (n=7) or 2 mg/kg d-amphetamine (n=11). Twenty-four rats received IC isotonic saline followed immediately by IP injections of 2 mg/kg scopolamine (n=7), 2 mg/kg amphetamine (n=9) or isotonic saline (n=8). Following a 10 min. recovery period photocell interruptions by ambulatory and stereotypic behaviors were counted in activity cages at 10 min. intervals over a 3 hr. period.

Both d-amphetamine and scopolamine induced significant elevations of ambulatory ($p<.05$) and stereotypic ($p<.05$) behaviors within the first hour (ANOVA Student-Newman-Keuls). NT significantly attenuated d-amphetamine-($p<.05$) but not scopolamine -induced ambulatory behavior and had no effect on the stereotypy induced by either drug (Student's t test). Ambulatory behaviors induced by DA-releasing drugs are believed to be largely mediated by the mesolimbic DA system, whereas stereotypic behaviors induced by these drugs are believed to be mediated by the nigroneostriatal DA system. Thus, these data, are consistent with previous biochemical and behavioral findings which indicate that NT modulates activity of the mesolimbic, but not the nigroneostriatal, DA system. These results also demonstrate that NT does not induce a non-specific locomotor impairment.

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53.5 EFFECTS OF NEUROTENSIN ON THE HYPERACTIVITY INDUCED BY INTRA-ACCUMBENS ADMINISTRATION OF ADTN AND DIBUTYRYL CYCLIC AMP. F.B.Jolicoeur,R.Rivest,M.Dumais and M.A. Gagné. Dept.of Psychiatry,Faculty of Med.,University of Sherbrooke, Sherbrooke,Quebec,Canada,J1H 5N4.

Neurotensin has been shown previously to affect hyperactivity but not stereotypy induced by various dopamine stimulating drugs, thus pointing to a specific action on mesolimbic dopaminergic processes (Jolicoeur et al. Neurosci. Biobehav.Rev.7:385,1983). To further investigate this selective influence, we have examined the effects of both intraventricular and intra-accumbens administration of neurotensin on the hyperactivity produced by bilateral intra-accumbens injections of 12.5 µg ADTN, a potent and long acting dopamine agonist, and of 20.0 ug dibutyryl cyclic AMP, a drug thought to act beyond the dopamine receptor. Both intraventricular and intra-accumbens administration of neurotensin significantly decreased the hyperactivity induced by ADTN. In both cases the reduction in activity was maximal at 20 min and dissipated gradually over a 2hr period following neurotensin injections. However, important differences in doses required to produce this effect were noted between the two routes of administration. Whereas intraventricular injections of doses as low as 50 ng were sufficient to reduce significantly ADTN induced hyperactivity, bilateral intra-accumbens administration of at least 1.8 µg were needed to replicate this effect. Intraventricular administration of neurotensin also significantly decreased the motor activity produced by dibutyryl cyclic AMP. The inhibitory action of neurotensin on hyperactivity induced with dibutyryl cyclic AMP was more prominent and persistent than that seen with ADTN. Preliminary results also indicate that intraventricular administration is more efficient than intra-accumbens injection of the peptide in decreasing dibutyryl cyclic AMP induced hyperactivity. Taken together these results demonstrate that neurotensin can affect motor activity elicited by either direct activation of dopamine receptors or stimulation of events beyond these receptors. The exact mechanisms of action underlying the inhibitory action of the peptide remains to be clarified but the observed greater efficacy of intraventricularly administered neurotensin suggests an influence of the peptide on extra-accumbens regions, probably on efferent outputs of mesolimbic stimulation. (Supported by the Medical Research Council of Canada).

53.6 INTRANIGRAL NEUROTENSIN INDUCES PERSISTENT ALTERATIONS IN BEHAVIOR AND DOPAMINE FUNCTION. K.L. Hulebak, T.C. Napier, S.G. Emerick*, E.L. Edwards* and G.R. Breese. Biological Sciences Research Center, UNC School of Medicine, Chapel Hill, NC 27514.

Neurotensin (NT) was microinjected (2 or 5 µg/.5µl) into substantia nigra zona compacta of unanesthetized male Sprague Dawley rats previously implanted with bilateral guide cannulas. The effects of this neuropeptide were determined by characterization of specific behaviors following bilateral infusions and by quantifying circular locomotion in animals unilaterally injected. Some animals were sacrificed following nigral NT microinjection, the brains removed, placed on ice and the caudate nucleus (CN) dissected. The remaining brain was immediately frozen and the globus pallidus (GP) was punched out of 600 µ sections with a 13 gauge tube. Quantification of dopamine (DA) and its primary metabolites was performed by high performance liquid chromatography.

Bilateral administration of NT did not cause noticeable behavioral changes up to an hour postinjection, even though DA and its metabolites were increased in both the CN and GP. However, 20 hours after NT was microinjected, the animals demonstrated a hyperactivity which included increased sniffing, rearing, and locomotion. DA and its metabolites also remained increased over control levels at 20 hours postinjection.

Unilaterally administered NT did not cause rotational behavior (as measured for 120 sec every 5 min for 45 min). Subsequent administration of low doses of amphetamine, 0.5 and 1 mg/kg ip, generally caused the animals to rotate contralaterally, approximately 2 turns/min (in agreement with Everist et al., Neurosci. Abst. 9:716, 1983). Contralateral circling of comparable rates was exhibited even when amphetamine was administered 20 hours after NT. Animals which received unilaterally-infused NT but not amphetamine did not demonstrate rotational behavior after 20 hours.

These results indicate that DA functions are altered in not only the striatum following intranigral NT infusions but also in the GP. These results also indicate that the NT-induced alterations may persist for an unusually long period of time and likely outlast the presence of the neuropeptide itself. (Supported by AD-0720, F32-NS07247, HD-03110 and MH-36294)

53.7 AVERSIVE PROPERTIES OF BOMBESIN IN THE CONDITIONED PLACE-PREFERENCE PARADIGM IN RATS. G. Meisenberg, S.A. Lorens, W.H. Simmons* and Y. Sayeed*. Dep. of Biochemistry, Loyola Univ. Med. School, Maywood, IL 60153.

After intracerebroventricular (i.c.v.) injection in mice or rats, the amphibian skin peptide bombesin induces a behavioral syndrome characterized by excessive grooming and scratching behavior. Therefore, we tested this peptide for aversive properties in a "biased" place-preference paradigm. The conditioning chamber consisted of two compartments (34 x 25 cm each), which were separated by a guillotine door. One compartment had black walls and a smooth floor, the other had white walls and a grid floor. The rats were implanted with an i.c.v. cannula and behavioral testing was initiated 7 - 12 days after the implantation. During the first three days of behavioral testing, the animals were placed in the center of the conditioning chamber with subsequent access to both compartments for 15 minutes in order to determine their baseline-preference. Those rats which spent 5 - 50% of their time in the black compartment were subjected to conditioning-sessions for the following four days: Once daily they were connected to a microinjection device and injected i.c.v. with 10ul of bombesin-solution or vehicle (artificial CSF) in the previously preferred white compartment. They were confined to this compartment for the subsequent 20 minutes. They also received a daily sham-injection in the non-preferred black compartment with subsequent confinement to this compartment for 20 minutes. On day 3 of behavioral testing, the rats were given access to the whole apparatus for 15 minutes and their preference for the two compartments was determined. While the CSF-injected rats did not significantly change their preference, doses of 80ng, 400ng, and 2.0ug bombesin induced a strong aversion to the previously preferred compartment. At these doses, bombesin induced enhanced grooming (80ng) or grooming and scratching (400ng and 2.0ug). This result suggests that bombesin-induced grooming/scratching behavior is accompanied by an aversive state and is compatible with the hypothesis that this peptide stimulates nociceptive mechanisms. Bombesin-like immunoreactivity had been described in brain areas thought to be involved in nociception (Peptides 2, 75-79, 1981), but the chemical identity of this material has not yet been elucidated.

53.8 PET-DIRECTED STUDY OF NEUROPEPTIDE Y IN ALZHEIMER'S DISEASE. N.L. Foster*, C.A. Tamminga, T.L. O'Donohue and T.N. Chase. (SPON: W. Carpenter). Experimental Therapeutics Branch, NINCDS, Bethesda, MD 20205.

Alzheimer's disease is a progressive dementing disorder associated with neuronal degeneration in the cerebral cortex and certain subcortical structures. Recently, positron emission tomography (PET) following F-18-deoxyglucose administration has shown substantial overall reductions in cortical glucose metabolism, but more severe depression in temporoparietal regions (centered in area 39) and relative sparing of frontal cortex. Although these metabolic changes are assumed to reflect diminished synaptic activity of local neurons, no similar changes in neurotransmitter levels have been identified. We measured neuropeptide Y, which is found in exceptionally high concentrations in cerebral cortex, to see if its distribution in Alzheimer's disease could account for the regional changes observed in glucose metabolism.

Cortex was dissected at -20°C from Brodmann areas 10 (frontal pole), 39 (angular gyrus) and 40 (supramarginal gyrus) in 10 brains with pathologically confirmed Alzheimer's disease and 8 normals matched for age and postmortem conditions. While cold, tissue was diced, mixed and then a portion sonicated in distilled water (5 ml/mg) before being extracted in .5N acetic acid. Neuropeptide Y-like immunoreactivity was measured using a radioimmunoassay.

	Neuropeptide Y Concentration mean pg/µg ± SEM	
	Normal	Alzheimer's
Area 10	18 ± 2.6	15 ± 2.7
Area 39	12 ± 1.7	10 ± 1.8
Area 40	13 ± 2.3	16 ± 2.9

In each of the areas tested there was no significant difference in neuropeptide Y in Alzheimer's disease as compared to normals. No significant difference in frontal vs. temporoparietal distribution was seen.

Neuropeptide Y does not appear to be decreased in Alzheimer's disease and does not explain the regional changes in glucose metabolism seen on PET scanning. Thus it is unlikely to contribute to the cognitive dysfunction found in patients with this disorder.

53.9 BEHAVIORAL EFFECTS OF INTRASTRIATAL INFUSIONS OF SOMATOSTATIN AND SOMATOSTATIN ANALOGUES. J.S. Fink and J.B. Martin. Department of Neurology, Massachusetts General Hospital, Boston, MA 02114.

Somatostatin (SOM) is present in the striatum of rats by radioimmunoassay. Using immunohistochemical methods SOM has been localized to both cell bodies and fibers within the striatum. The physiologic function of SOM in the striatum, however, is unknown. In these experiments we sought to determine the behavioral effects of intrastriatal infusions of SOM and SOM analogues in awake rats. Bilateral infusions of peptides or 0.9% NaCl (1 ul each side over 1 min) were made through indwelling cannulae located in the anterior caudate-putamen of awake rats. Over the next 30 mins locomotor activity was recorded with photocells and behavioral ratings were made at 1 min intervals.

Infusions of cyclic SOM-14, in doses of 0.1, 1.0 and 10 ug, produced brief dose-related vigorous sniffing, weak sniffing stereotypy and weak circling responses. The more potent SOM analogue D-Trp^{8}-SOM (1 ug) and the long-acting SOM analogue SMS-201995 (1 ug; Sandoz) produced increased photocell counts, sniffing stereotypy and circling; these behavioral responses were particularly robust after the SMS-201995 infusion. Behavior after infusion of the inactive SOM analogue Ala^{8}-SOM (1 ug) was indistinguishable from 0.9% NaCl infusions. Unilateral infusion of SOM-14 (10 ug) produced weak contralateral circling, leaning or posturing. In rats in which both cannulae were not localized within the striatum, behavioral responses after peptide infusion were blunted or absent.

The behavior produced by infusions of SOM-14 or active SOM analogues was qualitatively similar to the behavior produced by systemic administration to rats of dopaminergic agonists such as amphetamine or apomorphine. Other data from this laboratory has shown that intrastriatal infusions of SOM-14 or SMS-201995 produces increases in dopamine turnover within the striatum (Beal and Martin, Neuroscience Letters 44:271, 1984). Taken together, these data suggest that intrastriatal infusions of SOM-14 or active SOM analogues produce behavioral activation by enhancing dopaminergic activity within the striatum.

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NEUROPEPTIDES AND BEHAVIOR III

54.1 PERIPHERAL CHOLECYSTOKININ OCTAPEPTIDE (CCK-8) ENHANCES HEAD TWITCHES BUT NOT LOCOMOTION PRODUCED BY L-5-HTP IN MICE. K. J. Simansky, N. Streisfeld* and J. Friedberg*. Depts. of Pharmacology and Medicine (Endocrinology), Medical College of Pennsylvania, Philadelphia, PA 19129.

CCK-8 is a mammalian brain-gut peptide that displays pharmacologic properties characteristic of neuroleptic drugs in behavioral assays (e.g., Nair et al,Prog. Neuro-Psychoph. & Biol. Psychiatr., 1982, 6:509-512; Van Ree et al, Eur. J. Pharm., 1983, 93:63-78). Interest in the neuropharmacologic mechanisms for CCK actions has focused on interactions of this peptide with opiatergic and dopaminergic systems (e.g., Skirboll et al, Neuroscience, 1981, 6:2111-2124). We examined the effects of peripheral CCK-8 on a behavior thought to be mediated by a tryptaminergic mechanism at the S-2 class of neuroleptic receptor: viz., head twitches produced by L-5-HTP in mice. Effects of CCK-8 on locomotor and other activity were also determined to assess the behavioral selectively of the peptidergic actions.

Male Swiss-Webster albino mice were injected with carbidopa (25 mg/kg,ip) followed 30 min later by L-5-HTP. Head twitches were counted for 5 min every 20 min for 2 h after 5-HTP; crossing (i.e., locomotor) and noncrossing (other) activity were counted automatically throughout the 2 h. An initial study with 7 doses of 5-HTP (0, 50, 100, 141, 200, 400 and 560 mg/kg,ip; n=8/grp) yielded dose-dependent increases in head-twitches after all doses of 5-HTP compared to vehicle (0 dose), with a maximum at 400. 5-HTP produced a biphasic effect on locomotor activity, with a maximum at 200, and a monotonic increasing curve for nonlocomotor activity with a maximum at 200.

Administration of CCK-8 (30 or 300 ug/kg,sc) prior to 5-HTP (200 mg/kg,ip) produced dose-related increases in head twitches (vehicle, 188 ± 10; 30, 239 ± 22*; 300, 302 ± 12**; Dunnett's test, *p<.05, **p<.01) without significantly changing locomotor (185 ± 32; 200 ± 64; 219 ± 24) or other activity. These data suggest that peripherally administered CCK selectively enhances stereotypic behavior mediated by serotonergic or other mechanisms at a neuroleptic receptor.

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54.2 THE EFFECTS OF INTRAVENTRICULAR NEUROTENSIN AND CHOLECYSTOKININ ON THE DEAFFERENTATION SYNDROME IN THE RAT. J. Ovelmen-Levitt, E. Rossitch‡ and B.S. Nashold,Jr. Div. Neurosurgery, Duke U. Med. Ctr.

A deafferentation syndrome is produced in rats by dorsal root ganglionectomies. The syndrome consists of scratching and/or biting of the anesthetic limb, sometimes to the point of amputation. Neurotensin (NT) is a neuropeptide, present in the local interneurones of laminas I, II, III, which produces an analgesia not reversed by naloxone. Cholecystokinin (CCK-8) exerts a dose dependent biphasic effect. Low doses antagonize opiate actions, while higher doses have been reported to produce analgesia. A total of 34 Sprague-Dawley rats underwent unilateral C5-T2 dorsal root ganglionectomies. Of these, 24 had a cannula placed into one lateral ventricle which was connected to a subcutaneously implanted Alzet 2002 osmotic minipump. Ten of these rats received lactated ringers (LR) via the pump; ten others were given NT (1.5 ug NT/hr X 2 wks), and 4 were given CCK-8 (25 ng CCK/hr X 2 wks). Through daily observations, the onset of scratching and biting was noted, and the behavior quantified using a scale that assigned a numerical value to the severity of biting. All animals with severe biting were sacrificed immediately. Rats that underwent ganglionectomy only fell into two groups with respect to biting: one with early onset, less severe biting, and a second with later onset, less severe biting. Ratsthat received either LR or NT also fell into these two groups. The onset of scratching varied for each experimental group. At day eight, the percentage of rats that had scratched in each group was as follows: deafferentation only- 0%, LR- 20%, NT -70%, and CCK-8 - 0%. These results indicate that NT accelerates the onset of the scratching component of the deafferentation syndrome. The presence of the minipump and cannula also accelerates the scratching component, although to a lesser extent than NT. Neither group seemed to change the biting component. On the other hand, preliminary results indicate that CCK-8 may delay the onset of scratching. The effect of CCK-8 on the course of the biting component are yet to be assessed.

54.3 DOSE RELATED EFFECTS OF CCK-8 ON ATTENTION, APOMORPHINE-INDUCED STEREOTYPY AND EPIDURAL EEG. L. H. Miller*, B. A. Turnbull, A. Sudilovsky and L. J. Traficante*. Department of Biobehavioral Sciences, Boston University Medical Center, Boston, MA 02118 and The Squibb Institute for Medical Research, Princeton, N. J. 08540

There is at present considerable evidence implicating an interaction of cholecystokinin octapeptide (CCK-8) with brain dopaminergic systems. To further examine this proposed interaction we investigated the effect of various doses of CCK-8 on three paradigms in which dopaminergic agents are known to have significant effects.

The effects of CCK-8 on apomorphine-induced stereotypy in male Sprague-Dawley rats (N=10) were assessed following intraperitoneal (ip) administration of apomorphine HCl 2.0 mg/kg alone or concurrently with 50, 125 or 250 µg/kg of CCK-8 ip. Stereotyped behavior was rated using a 0-3 point method of scoring during 10-second segments every 15 minutes for 75 minutes after injection. CCK-8 at doses of 100 and 250 µg/kg significantly attenuated apomorphine-induced stereotypy.

Delayed response performance, a measurement of visual stimuli retention was assessed in male, Long-Evans rats (N=6) administered ip with either saline +,0 1M acidic acid (vehicle); CCK-8, 50, 100 or 250 µg/kg; chlorpromazine (CPZ), 1.0 mg/kg; or CCK-8 50 µg/kg + CPZ 1.0 mg/kg. Evaluations were made 45 minutes post-injection at periods of delay ranging from 0 to 12 seconds. CCK-8 at doses of 100 and 250 µg/kg significantly impaired delayed response performance. While CCK-8, 50 µg/kg and CPZ, 1.0 mg/kg did not differ from control their combination resulted in delayed response performance that was significantly poorer than control.

Effects of CCK-8 on epidural EEG were recorded using bipolar electrodes placed above the hippocampi in male Sprague-Dawley rats (N=10) administered with either vehicle or CCK-8, 50, 100 or 250 µg/kg ip. EEG segments (10 seconds) were recorded approximately 45 minutes after injection. Spectral analysis yielded a dose related increase in output in the 2-5 Hz range.

Thus, while the behavioral effects of CCK-8 in the present study seem to mimic those found with dopamine blocking agents, its effects on EEG activity are opposite to those reported after neuroleptic treatment. This suggests that not all the effects of CCK-8 on the central nervous system are mediated by dopamine receptor blockade.

54.4 ANALGESIC PROFILE OF CCK-8: DIFFERENTIATION FROM OPIATES AND NON-OPIOID PEPTIDES. W.L. Autry*, B.S.Barbaz, F.G. Ambrose* and J.M. Liebman. Neuroscience Res., Res. Dept., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

The sulfated octapeptide of cholecystokinin (CCK-8) has been reported to yield analgesia upon systemic (Zetler, Neuropharmacology 19:415, 1980) and/or intracerebroventricular (i.c.v.) administration (Jurna and Zetler, Eur. J. Pharmacol. 73:323, 1981). Zetler (1980) found this effect to be naloxone-reversible. The release of beta-endorphin is enhanced by CCK-8 (Matsumura et al., Neuroendocrinology 36: 443, 1983), and other studies have documented other CCK-8 opioid interactions (Faris et al., Science 219:310, 1982). The present experiments systematically compared the analgesic profile of CCK-8 with that of morphine and met^5-enkephalin. In addition, the analgesia induced by other non-opioid neuropeptides (neurotensin and bombesin) was compared with that induced by CCK-8.

Analgesia was assessed in the mouse hot plate (HP) test (53 ± 0.5° C), using jump latencies as a measure of nociception, and in the mouse phenylquinone-induced writhing (PQW) test. Intracerebroventricular administration of CCK-8 elevated HP latencies. The dose that doubled jump latencies from control values (the ED_{200}) was estimated to be approximately 2 µg. Higher doses of CCK-8 (ED_{50} = 4.5 µg) were required to suppress PQW. In contrast, neurotensin suppressed PQW at doses (ED_{50} = 0.01 µg) considerably lower than the ED_{200} for HP latencies (approx. 0.65 µg). Despite its lower potency, met^5-enkephalin showed a profile similar to that of neurotensin (ED_{50} = 26 µg; ED_{200} = 424 µg in HP). Morphine was also more potent in PQW than in HP. Unlike these substances bombesin had no effect on HP latencies at doses up to 30 µg i.c.v. Bombesin was, however, remarkably effective in suppressing PQW (ED_{50} = 0.002 µg).

The effects of intracerebroventricular CCK-8 and neurotensin on HP latencies were reversed by doses of 0.5 mg/kg s.c. naloxone. However, naloxone at doses of 0.5 and 3.0 mg/kg failed to block the suppression of PQW by CCK-8, neurotensin and bombesin. Naloxone (0.5 mg/kg s.c.) readily antagonized the effects of morphine and met^5-enkephalin on HP and PQW.

The results indicate that CCK-8 has a profile of analgesic activity different from that of other neuropeptides or opioids. The analgesic activity of CCK-8 is unlikely to be mediated solely by opioid mechanisms.

54.5 PERIPHERAL AND CENTRAL EFFECTS OF CHOLECYSTOKININ-OCTAPEPTIDE ON OPERANT RESPONDING IN RATS. S.L. Cohen*, M. Knight, C.A. Tamminga and T.N. Chase. Department of Psychology, Bloomsburg University of Pennsylvania 17815; Experimental Therapeutics Branch, NINCDS, Bethesda, MD 20205

Exogenous cholecystokinin-octapeptide (CCK8) has multiple effects on behavior. It is well known that CCK8 inhibits food-intake. Systemic injections of CCK8 also produce sedative-like symptoms (ptosis, reduced locomotion, depressed rearing, and prolonged hexobarbital sleep), antagonize opiate-mediated footshock analgesia, increase passive avoidance latency, and decrease exploration. We have previously shown that peripherally administered CCK8 retards signaled and Sidman avoidance learning and has a partial inhibitory effect on apomorphine-induced stereotypy. Central injections of CCK8 have been shown to antagonize β-endorphin analgesia and TRH-induced activity, produce hyperglycemia and hypothermia, and retard acquisition of active avoidance. Direct comparisons of central and peripheral CCK8 administration in nonfood-related behaviors are lacking. Therefore, we compared these two modes of administration using an operant conditioning paradigm. We have previously shown that systemic administration of CCK8 suppresses operant responding in water deprived rats. However, it remains unclear whether the response suppression of water-reinforced lever pressing following CCK8 was centrally or peripherally mediated. To examine this issue, rats were trained to press a lever for water in an operant conditioning chamber under a variable-interval schedule of reinforcement. After response rate stabilized, injections were administered; response suppression was measured by comparing injection response rate to baseline rate. Intracerebroventricular injections of CCK8 reduced lever pressing but at relatively high doses (5 and 50 µg). In a direct comparison of central and peripheral administration, intraperitoneal injections of CCK8 (20 µg/rat) reduced responding significantly more than injections into the lateral ventricle (20 µg/rat). The suppressive effects of CCK8 (0 to 300 µg/kg, i.p.) were blocked by complete abdominal vagotomy. The effects of CCK8 (30 µg/kg, i.p.) were partially blocked by preinjection with the specific competitive CCK8 antagonist dibutyryl cyclic GMP (70 and 140 mg/kg, i.p.), but no blocking effect was observed by preinjections of the acetylcholine antagonist atropine (0 to 10 mg/kg, i.p.). These data suggest that suppression of operant lever pressing in water deprived rats is primarily mediated by vagal afferent fibers.

54.6 THYROTROPIN-RELEASING HORMONE: BEHAVIORAL EFFECTS IN RATS OF CHRONIC HIGH-DOSE TREATMENT; LACK OF TOXICITY; AND MODIFICATION BY METHYSERGIDE OR CYPROHEPTADINE. C.K. HAUN[1], W.K. ENGEL[2], and I. TAN[1]. (SPON: B.L. NEWMAN) Depts. of Anatomy & Cell Biology[1] and Neurology[2], USC School of Medicine, Los Angeles, CA 90033.

TRH at high dosage (2.2 mg/kg/d, sc) reduces spasticity and improves strength of patients with amyotrophic lateral sclerosis (Neurology, 1982, 34:3, Suppl. 1, 238). Long-term treatment necessitates high-dose toxicity studies in animals. Weanling Holtzman rats of both sexes were given sc injection of TRH (Abbott) 5d/wk, for 12 wks. Groups received 2.2, 22 or 110 mg/kg, or saline vehicle, without visible enduring toxic effects.

Growth curves of both sexes receiving 2.2 or 22 mg/kg TRH matched their saline controls; the 110 mg/kg-treated rats of both sexes gained weight at a non-significantly lower rate. Daily water consumption was unaffected. Sexual development (opening of vagina and cyclicity) proceeded identically in TRH-treated and control females. Behavioral effects were observed directly and videotaped for review by 2 observers. The behaviors were mostly "exaggerations" of normal--such as oral and oral-lingual movements, licking of paws, grooming of face and body, scratching of neck, ear or shoulder, all being done very rapidly and in a stereotyped manner, for seconds-to-minutes, and repeated at brief intervals. These behaviors were both dose-dependent and reproducibly characteristic of individuals. Most unusual were high-frequency shaking of the forepaws and "wet dog shakes" (WDS), at repetition rates that sometimes exceeded 100/10 min., and a repeated vigorous pulling on the fore- or hind-claws with the teeth; these displays usually began within 1 min. of injection and continued for 1-3 hours after the 22 and 110 mg/kg doses (males responded more intensely and with a higher repetition rate). Pilo-erection occurred almost invariably in both sexes throughout most of the displays, as did an early-onset "whole-body tremor", that persisted for several minutes.

Methysergide (0.1 mg/kg, ip, 5 min. pre-TRH) diminished WDS but exacerbated the whole body tremor and caused a frenzied combination of the TRH-behaviors (not seen with methysergide alone). Cyproheptadine (10 mg/kg, ip, 5 min. pre-TRH) "slow-motioned" the animals' TRH responses and brought on eating and sleeping (at 20-30 min.), behaviors not seen in rats under the acute influence of TRH alone for 1-3 hrs. Another effect of chronic high-dose TRH treatment was an occasional transient asthma-like wheezing in a "sensitive" animal. (Supported by the Muscular Dystrophy Association.)

54.7 BIOLOGICAL PROPERTIES OF THYROTROPIN-RELEASING HORMONE AND SELECTED CONGENERS.
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Thyrotropin-releasing hormone (pGlu-His-Pro-NH_2;TRH) is an endogenous tripeptide that produces a variety of biological effects. We observed that [^{3}H]pGlu-3-MeHis-Pro-NH_2 ([^{3}H]MeTRH) binds specifically to membranes derived from several rat brain regions including the amygdala. Specific [^{3}H]MeTRH binding was selectively displaced in the amygdala by chlordiazepoxide and diazepam. Other benzodiazepines and drugs from numerous other classes did not affect binding. Conversely, TRH, MeTRH and a major metabolite of TRH, cyclo-His-Pro, did not displace specific [^{3}H]flunitrazepam binding in the amygdala, frontal cortex, hippocampus or cerebellum. These TRH congeners also did not displace specific [^{3}H]diazepam binding to amygdala membranes. In addition, the ability of GABA to stimulate [^{3}H]flunitrazepam binding was not affected by TRH, MeTRH, or cyclo-His-Pro in the brain regions tested.

TRH, MeTRH and cyclo-His-Pro were tested in a variety of CNS pharmacological protocols. In a conflict procedure in rats, which predicts anxiolytic activity, TRH but not MeTRH or cyclo-His-Pro increased punished responding. All three were effective in inhibiting depressed posture in a stress-induced depression test in rats whereas only TRH and MeTRH enhanced apomorphine-induced climbing in mice. All three peptides were inactive in their ability to alter the syndrome produced by 5-hydroxytryptophan in rats or antagonize tetrabenazine-induced depression or ptosis in mice. In addition, these congeners did not block pentylenetetrazol-induced convulsions in rats. These studies suggest that TRH/anticonflict interactions do not occur via classical benzodiazepine receptors, and behavioral support is garnered for TRH/catecholaminergic interactions.

54.8 POTENTIATION OF ANALGESIC RESPONSES FOLLOWING CENTRAL BUT NOT PERIPHERAL ADMINISTRATION OF THYROTROPIN RELEASING HORMONE.
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Thyrotropin releasing hormone (TRH) exerts differential effects upon analgesic responses, reducing neurotensin analgesia, potentiating the analgesia produced by acute exposure to 20 or 80 footshocks, yet failing to alter opioid analgesia. These effects occur despite the failure of TRH to alter basal pain thresholds itself. The present experiments assessed whether central or peripheral administration of TRH would alter opioid-mediated front paw shock analgesia, nonopioid-mediated hind paw shock analgesia, and cold-water swim analgesia. Three groups of eight rats received intracerebroventricular (icv) injections of 0, 10, or 50 ug TRH respectively 20 min prior to front paw shock (1.6 mA for 90 sec). Tail-flick latencies were assessed for up to 20 min after shock. Pretreatment with the 50 ug, but not the 10 ug, TRH dose significantly potentiated the duration and magnitude of front paw shock analgesia as compared to vehicle-pretreated rats. An identical paradigm was repeated three weeks later except that shock was delivered to the hind paws. While hind paw shock produced significant analgesia in all three groups, neither TRH dose potentiated this analgesic response. In the second experiment, two groups of eight rats received intravenous (iv) injections of vehicle or TRH (2 mg/kg) respectively, immediately before front paw shock. No difference in the duration or magnitude of front paw analgesia was observed between groups, indicating a central mechanism of action for TRH. In a third experiment, two groups of eight rats received either vehicle or TRH (50 ug, icv) 20 min prior to a no swim condition and subsequent 3.5 min swim conditions at water temperatures of 21, 15, 8, and 2°C. A one-week interval separated each swim condition. TRH significantly potentiated the magnitude of swim analgesia on the tail-flick test in all swim conditions, and potentiated the magnitude of swim analgesia on the jump test following the 15°C bath. These potentiations in swim analgesia by TRH were not accompanied by thermoregulatory changes. A fourth experiment revealed that TRH (2 mg/kg, iv) failed to alter swim analgesia, again indicating a central mechanism of action. (Supported by PSC/CUNY Grant 6-63210).

NEUROPEPTIDES AND BEHAVIOR IV

55.1 ENDOGENOUS MODULATION OF PEPIPHERAL LEU-ENKEPHALIN (LE) SYSTEMS AFFECTS AVOIDANCE CONDITIONING.
J. L. Martinez, Jr., P. Conner*, R. Dana*, C. Chavkin, F. Bloom and J. de Graff*. Psych. Dept., Univ. of Calif. Berkeley, CA, 94720; Pharmacol., Dept., Univ. of Calif., Irvine, CA, 92717; Scripps Clinic and Research Fdn., La Jolla, CA, 94720; Organon Internatl., Oss, The Netherlands.

First it was determined whether naloxonium, a peripherally acting opiate antagonist, (sal, 2.7, 8.1, or 27.2 µmol/kg, i.p.), given 5 min before training, would affect acquisition of a one-way active avoidance response in mice. Mice were placed in the shock compartment of a two-compartment alley and given 10 sec to shuttle into the safe compartment. If no avoidance occurred in 10 sec, a shock (800 µA) came on, which terminated when the mouse escaped. Fourteen trials were given; the ITI was 20 sec. The number of avoidances the mice made on Trials 6-12 was the measure of acquisition. A dose of 8.1 µmol/kg enhanced acquisition, as determined by Dunnett's procedure ($t(2,72) = 2.37$, $p < .025$). In the second experiment LE injected i.p.(180 nmol/kg) impaired acquisition ($t(33) = 2.12$, $p < .05$) in comparison to a control group, and this impairment was attenuated by naloxonium (8.1 or 27.2 µmol/kg), given as a cocktail with the LE (ts (3,33) < 1.44, ps > .05). The final behavioral study examined a 3 log unit dose response function (1.75 -.0175 mg/kg protein) of LE antiserum (Peninsula Labs.) given i.v., 4 hours before training. The control group was injected with equal amounts of preimmune serum. The 1.75 mg/kg dose enhanced acquisition of the response ($t(3,154) = 2.51$, $p < .025$).

The antibody was characterized by a radioimmunoassay for specificity and capacity. Three dilutions were assayed (1:2000, 1:1000, 1:500), each in duplicate against 10 trace dilutions (250 µM-500 nM) of (tryosyl-3,5,-^{3}H) enkephalin (5-L-Leucine). Binding data were analyzed by binding saturation and Scatchard analysis. The antisera binding capacity was 6.5 +/-0.6 nmol of LE bound/µl undiluted sera. Each mouse (from the 1.75 mg/kg dose group) was then injected with 124.1 nmol/kg potential LE binding activity. Thus, endogenous modulation of peripheral LE systems affects the strength of a conditioned response. Both naloxonium and the LE antibody produce behavioral effects that are in the same direction and the opposite of that produced by LE. The attenuation of LE-induced impairment by naloxonium suggests that a peripheral receptive site is involved (Supported by ONR Contract N00014-83-K-0408 to JLM).

55.2 THE EFFECTS OF VARIOUS OPIATE RECEPTOR AGONISTS AND MORPHINE ON SPONTANEOUS BEHAVIOUR FOLLOWING INTRAHIPPOCAMPAL ADMINISTRATION IN THE RAT.
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The application of a variety of exogenous opiates to various regions of the hippocampus has been shown to produce, with few exceptions, an increase in its electrical activity. Anatomically, the presence of various types of both opiate receptors and ligands has been demonstrated within the hippocampus, although contrary to what might be expected on the basis of the neurophysiological data, they are not uniformly distributed within it. That is, µ-receptors predominate in the pyramidal layer especially in CA1 and CA2, δ-receptors are most numerous in CA2 and κ-receptors have been localized exclusively within the dentate. In addition, endogenous ligands have been localized within fibre systems which do not necessarily project to areas containing the greatest concentrations of the corresponding receptor types. That is, using radioimmunoassay, enkephalin (a presumed δ-receptor agonist) has been identified as present within the perforant path and mossy fibres, which project to dentate and CA3 respectively, and dynorphin (a presumed κ-agonist) has been localized solely to the mossy fibres and therefore would presumably have an effect on cells of CA3.

This experiment was designed to address these apparent anatomical discrepancies and to study further the possible function of endogenous opiates within the hippocampus since it has been difficult to identify the conditions under which they are released. A range of doses of prototypic µ-, δ-, and κ-receptor agonists and morphine were applied to the hippocampal CA3 region of the rat via chronic indwelling cannulae. Spontaneous behaviours including grooming, teeth-chattering, yawning, wet-dog shakes, "flaccid-immobility" and seizures were subsequently recorded during a 45-minute observation period. Agonists used were morphiceptin (4, 20, 100 nmol, as the µ-agonist), DSLET (4, 20, 80 nmol), as the δ-agonist), U50,488 (2, 20 and 200 nmol, as the κ-agonist) and morphine (2, 20 and 200 nmol).

DSLET, the δ-receptor agonist was found to be the most potent agent in CA3, eliciting wet-dog shakes at the lowest dose, while morphine and morphiceptin did so only at the highest dose. The presumed κ-agonist, U50,488, was relatively ineffective. These results will be compared with the effects of the same doses of these compounds when applied to the dentate region, with an apparently different receptor population, and to a control area of the brain also rich in various ligand and receptor types.

55.3 **EFFECT OF ANTIPSYCHOTICS ON IN VITRO CENTRAL β-ENDORPHIN PROCESSING IN THE RAT.** T. P. Davis, H. Schoemaker and A. J. Culling*. Dept. of Pharmacol., Univ. Arizona, Coll. of Med., Tucson, AZ 85724.

Central metabolism of β-endorphin (β E 1-31) by membrane associated peptidases not only results in the deactivation of the parent compound but at the same time leads to the formation of specific α-endorphin (β E 1-16) and γ-endorphin (β E 1-17) fragments which can produce behavioral effects in rats and man. Based on the amphetamine-like activity of α-type endorphins and the neuroleptic-like activity of γ-type endorphins, previous studies have suggested that endorphins may function in brain homeostasis and in a variety of behavioral adaptive processes (De Wied et al, Life Sci. 26:1275, 1980).

A previous study in our laboratory reported evidence that the putative neuroleptic peptide des-enkephalin-γ-endorphin (DEγE; BE 6-17) is produced at a three-fold higher rate in twice washed membrane-bound enzyme homogenates of post-mortem human putamen from patients diagnosed as having schizophrenia versus sex and age matched controls (Proc. West. Pharm. Soc. 26:89, 1983). These data suggested that there was a dysfunction in β-endorphin metabolism in post-mortem putamen from schizophrenic subjects that may be due to an alteration in the activity of an endogenous peptidase due to the disease state or could result from antimortem drug treatment. To address the effect of drug treatment we studied the effect of two commonly prescribed antipsychotic drugs, haloperidol and chlorpromazine in an eight day infusion study in rats.

Drugs were administered in male Sprague-Dawley rats (134-185 g; n=5 ea) using ALZET Model 2001 osmotic minipumps as follows: haloperidol (3.5 mg/kg/day) and chlorpromazine (4.2 mg/kg/day). Control rats received the complete surgical treatment but no drug. After the eight day period the rats were sacrificed, and the whole brain minus the cerebellum was removed and placed on ice. β -endorphin (10 μM) was incubated in sterile PBS buffer at 37°C with twice washed membrane preparations of the whole brain. After 5-90 min incubations, samples were boiled for 15 min and centrifuged for 20 min at 15,000 xg. The resultant supernatant was assayed for β-endorphin related peptides by means of a selective and quantitative HPLC procedure previously described (J. Pharm. Exp. Ther. 227:499, 1983). Animals receiving haloperidol showed an increase in DEγE formation from 159.5 ± 28.5 ng/mg protein to 333.6 ± 12.2 ng/mg protein and chlorpromazine infusion showed a similar increase to 253.7 ± 42.2 ng/mg protein after a 90 minute incubation. Increases in the production of other neuroleptic-like peptides were similar showing nearly a two-fold increase in γ-endorphin and des-tyrosine γ-endorphin (DγE; β E 2-17). No effect was noted in the formation rates of the amphetamine-like peptides (i.e. α-type endorphins) at any of the time points.

These results demonstrate that the infusion of centrally acting antipsychotic drugs selectively affects the formation of γ-type endorphins and may help explain the increase in DEγE noted in the post-mortem brain tissue from schizophrenic patients. These results also raise the possibility that the mechanism of action of antipsychotics may include γ-type endorphins (Supported by NIH Grant AG04439).

55.4 AUTORADIOGRAPHY OF OPIATE RECEPTORS FOLLOWING SCHEDULED DRINKING. M.J. Blake[1], E.A. Stein[1] and D.A. Czech[2]. Depts. of Biology[1] and Psychology[2], Marquette Univ., Milwaukee, WI 53233.

It is becoming apparent that one or more of the endogenous opioid peptides plays, as yet, an unspecified role in the mechanism related to consumatory behavior. Opioid antagonists have been shown to suppress feeding and drinking when administered either peripherally or directly into the brain. We have previously demonstrated that naloxone will attenuate water consumption in water-deprived animals when injected centrally into the LH, POA, and ZI. In contrast, no significant alterations were noted with injections into the NAS, LV, cortex, or SN. It is hypothesized that the attenuation in water intake occurs as a result of naloxone competitively blocking the receptor activation of an endogenously released opioid. Utilizing this hypothesis, we have applied the technique of in vivo autoradiography of opiate receptors to drinking behavior to further delineate those areas which show opioid alterations after this behavior.

Male Holtzman rats were implanted with indwelling jugular catheters. Following a 1-wk recovery period, rats were either placed on a 23-hr water deprivation schedule with one hr water access given daily between 1400-1700 hrs, with food available ad lib or they were provided with both food and water ad lib. Following at least one wk on this schedule, and just prior to sacrifice, the water-deprived group was further divided into a group which received water for 15 min and one which did not. They were then injected with H^3-Diprenorphine (192 μCi/kg, 0.002 mg/kg) and, following a 20-min period to allow for washout of nonspecific binding, rats were decapitated and brains removed. Portions of cerebellum, medulla and liver were also dissected out, counted in a liquid scintillation counter, and crossmatched to insure equivalent injections of H^3-Diprenorphine. Immediately after removal, brains were frozen and stored until sectioned. Sections (16 μ) were cut in a freezing cryostat, thaw mounted onto glass slides, then dried on a slide warmer. Slides were then placed in standard x-ray cassettes and exposed to tritium-sensitive x-ray film (LKB Ultrofilm) for 4-6 wks.

Initial visual analysis indicated several slight changes in a number of brain areas. Many of these areas are those previously shown to be involved with both drinking and putative reinforcement circuitry. These data will be discussed with respect to a possible modulation of drinking by opioid mechanisms. Supported in part by NIDA grant DA02234 to EAS.

55.5 NALOXONE SENSITIVITY OF STIMULATION SITES IN THE CENTRAL GRAY EFFECTIVE IN MODULATING FELINE PREDATORY AND AFFECTIVE AGGRESSION. C. Pott*, S. Weiner*, S. Kramer* and A. Siegel. UMDNJ, Newark, N.J. 07103 and Seton Hall Univ., S. Orange, N.J. 07079.

Recent studies in our laboratory suggest that the central gray (CG) and hypothalamus mediate both affective and predatory aggression. Furthermore, attention has recently been focussed on CG with respect to the presence of opiate receptors and endogenous opioids. The present studies were undertaken to assess the role of opiate mechanisms within CG in modulating hypothalamically-elicited aggressive behaviors.

Adult cats were implanted with chronic electrodes aimed at the ventromedial hypothalamus (VM) and lateral hypothalamus (LH), which when stimulated, elicited affective hissing and attack directed at conspecifics (VM) or predatory attack (LH). Each animal was then implanted with CG cannula-electrodes. Response modulation was determined by dual stimulation of CG and hypothalamus alternated with stimulation of the hypothalamus alone in an A-B-B-A sequence. 5 ul (1ug) of naloxone (in saline) was injected through each CG cannula and response latencies were again determined. Eight electrodes sites within CG modulated VM-elicited hissing. Three sites were found to be inhibitory; 5 were facilitatory. The inhibitory sites were topographically located within the rostro-dorsal CG. Intracerebral naloxone injections into these sites completely blocked stimulation-induced inhibition. The facilitatory sites were located within the caudo-ventral region of CG. However, intracerebral naloxone did not block stimulation-induced facilitation of affective defense.

Predatory attack was modulated from 5 stimulation sites in CG. Four sites were inhibitory; one was facilitatory. Only the facilitatory site was blocked by naloxone. Inhibitory sites were located more caudally than the facilitatory electrode. In 2 cases, both predatory and affective attack were inhibited from the same electrode. However, only inhibition of affective aggression was sensitive to naloxone.

These studies suggest distinct topographic regions within CG responsible for inhibition and facilitation of both predatory and affective aggression in the cat with separate opiate-mediated mechanisms of modulation.
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55.6 REWARDING EFFECTS OF OPIOID PEPTIDES: EVIDENCE FOR μ RECEPTORS ACTIVATION. M. Amalric*, J.L. Martinez, Jr.†, N. Ling‡ F.E. Bloom, and G.F. Koob. (SPON: H. RIGTER). Div. Preclin. Neurosci. Endocrinology, Scripps Clinic & Res. Fdn., La Jolla, CA 92037. †Dept. of Psychology, Univ. Calif. Berkeley, CA 94720. ‡Lab of Neuroendocrin., Salk Inst., La Jolla, CA 92037.

The rewarding properties of psychomotor stimulants and opiate drugs are well known. The rewarding effect of endogenous opioid peptides has yet to be extensively explored. The present experiment was designed to elucidate the role of β-endorphin (β-END) and dynorphin (DYN)) systems as possible mediators of reinforcement using a conditioned place preference.

The paradigm paired an intracerebroventricular injection of an opioid peptide with one distinctly different environment and saline with another on alternate days, for six days (training). Peptide injections were paired with the least preferred environment based on the preference of the rats in a pretraining session; however there were no group differences preference. βEND (1.5, 2.5, 5.0, 10.0 μg/rat) and DYN (2.5, 5.0, 10.0, 20.0 μg/rat) were injected intracerebroventricularly (ICV 2 μl volume) immediately before the rat was placed in the training box (for 30 min). Control rats were injected with either saline, morphine (10 μg ICV) or heroin (0.5 mg/kg sc). After training, each rat was tested drug-free in a double-environment box where each end was identical to the training environments, with a smaller grey neutral area in the center. Time spent in each end of the test box was recorded over a 10 min period.

Heroin, a synthetic opiate known to be rewarding produced strong evidence for places of prior heroin injection (0.5 mg/kg sc). Rats also showed dose-dependent place-preference for the environment paired with either βEND or DYN (significant dose for βEND = 2.5 μg and DYN = 10.0 μg). Rats injected with the higher doses of the 2 peptides showed no preference for their environment but did show catalepsy and immobility with β-END and bizarre postures with barrel rotations with DYN.

These results demonstrate positive reinforcing properties for these endogenous opioid peptides in the central nervous system (CNS). The potency of βEND versus DYN indicates that this rewarding property may involve Mu receptor activation, and suggests a possible specific opiate neurochemical substrate for opiate reinforcement in the CNS. (Supported by NIDA grant 03665 to FEB and ONR contract N00014 83 K-0408 to JLM.)

55.7 OPIOID MECHANISMS IN BRYOZOA. Nestor Rosales,* Rita Colon-Urban* and George B. Stefano. Biological Sciences Program, SUNY/College at Old Westbury, Old Westbury, New York 11568.

Behavioral studies in our laboratory have shown that in several species of the phylum Bryozoa (Bugula turrita, Scrupocellaria diegensis) addition of morphine (0.08-0.16 μM) to the bathing media resulted in immobilizing lophophore retraction in a dose dependent manner. Naloxone alone (0.19-0.21 μM) caused the lophophore to open and close rapidly for about 45 seconds followed by a period of immobility. Concomitment application of naloxone (0.21-0.08 μM) and morphine resulted in a blockade of the morphine-induced behavioral response. Furthermore, data from binding studies demonstrates the presence of a high affinity binding site for opioid neuropeptides in the tissues of bryozoans.

H-D-ala met-enkephalin (20 nM) bound to 10 pmol/g protein of bryozoan tissues. The binding was reduced by sodium (46%), an effect that is reversed by mangaese. In addition, dopamine caused the stimulation of the lophophore extention-retraction cycle, an effect which can only be blocked by haloperidol (5 M). Phentolamine and naloxone were ineffective in blocking the dopamine stimulating effect. These results strongly suggest the presence of opiate as well as dopamine receptors in these organisms. (Supported by NIH Grants MH 17138 & RR 0118-02.)

55.8 BEHAVIORAL EFFECTS OF INTRACEREBRAL VENTRICULAR oCRF ARE INDEPENDENT OF EFFECTS AT THE PITUITARY. D.R. Britton, M. Varela*, A. Garcia* and J. Rivier*., Physiology Dept., Univ. of New Mexico School of Medicine, Albuquerque, N.M. 87131.

As a continuation of previous studies, we have examined various aspects of the sensitivity of rats to the behavioral effects of intracerebral ventricular (icv) administered oCRF. Consistant with our previous reports, we have found that icv CRF (0.5 or 1.0 ug) produces a marked suppression of eating by fasted animals when they are tested in their home cages. This suppression was associated with significant increases in the amount of grooming and the amount of locomotor activity. All three effects were present within 10 min. following the administration of the peptide and were still present 180 min. later. These effects could not be mimiced by the iv administration of oCRF (0.5 - 8.0 ug) at doses which have been show to produce profound activation of the pituitary adrenal axis by the release of ACTH and corticosterone. When administered i.v., CRF did result in an initial suppression of eating which lasted up to 90 min. This initial suppression of eating was followed at about 150 min. post-injection by a phase of increased eating compared to saline injected controls. There was also a decrease in locomotor activity compared to either saline injected controls or to animals injected icv with oCRF.

Pretreatment (4 hrs. prior to testing) of animals with dexamethasone (100 ug, iv) which has been shown to suppress the pituitary ACTH response to CRF failed to alter the behavioral response to icv oCRF. These data suggest that the observed behavioral effects of CRF (decreased food consumption, increased grooming) are not due to stimulation of the pituitary adrenal axis. In addition, we fail to find evidence that the behavioral effects of centrally administered oCRF are sensitive to steroid feedback inhibition.

55.9 CRF-INDUCED AGGRESSIVE BEHAVIOR AND SEIZURES: FACILITATION OF AMYGDALA KINDLING

Susan R.B. Weiss*, Robert M. Post, Philip W. Gold*, George Chrousos*, Tim L. Sullivan*, David Walker*, and Agu Pert Biological Psychiatry Branch, NIMH, and Developmental Endocrinology Branch, NICHD, Bethesda, Md. 20205

Rats were surgically implanted with bipolar electrodes in the amygdala for stimulation and recording, and with guide cannulae placed 1.5 mm. above the left lateral cerebral ventricle for intracerebroventricular (icv) injections. Corticotropin releasing factor (CRF) (100 μg) or vehicle (10 μl) was administered for five consecutive days icv. On days 1 or 2 all CRF-treated rats exhibited the late onset (2-5 hours) of major motor seizures. Behaviorally and electrographically, these seizures were indistinguishable from those produced by electrical kindling of the amygdala. By the fifth day of CRF injections no seizures occurred, suggesting the development of tolerance. This is further supported by a second study in which rats received increasing doses of CRF (10-100 μg) for six days. Only 1 of 6 animals displayed seizures at the highest dose. In CRF treated rats, fierce aggressive behaviors were observed intermittently several hours after the injections and were dose-related.

Following chronic administration of CRF in the first study, the animals were electrically stimulated in the amygdala. The CRF-treated rats were found to develop amygdala-kindled seizures approximately twice as fast as vehicle injected controls ($p<.03$). Thus, while tolerance developed to the seizure-inducing effects of CRF, an enhancement of electrical kindling of the amygdala nevertheless resulted.

In another series of studies, rats were implanted with chronic indwelling cannulae guides aimed for structures high in CRF content. CRF (25-50 μg) injected directly into the amygdala, hippocampus, septum, anterior hypothalamus, medial thalamus or periaqueductal gray area (PAG) produced seizures in only 3 of 39 rats with delays of 3-5 hours. None of the animals with amygdala implants, and only one animal with a hippocampal implant (N=7 in each group) showed seizures. This suggests that diffusion or transport of CRF from the ventricular system to these brain regions is an unlikely explanation of the late onset of CRF seizures. All eight rats with PAG implants and 5 of 7 rats with medial thalamic implants showed increased aggression.

These data raise the possibility that an endogenous, stress-related neuropeptide may, in pathological conditions, alter convulsive and aggressive responsivity.

55.10 ACTH PEPTIDES DIFFERENTIALLY ACCELERATE MATURATION OF MOTOR ACTIVITY IN HABITUATED VS NAIVE INFANT RATS. J.A. King* and F.L. Strand. Biology Dept. New York University, Washington Square, N.Y. 10003

Infant rats were administered ACTH 4-10 (10ug/kg); ACTH 4-9, a trisubstituted analogue, Org 2766 (0.01 ug/kg); or saline SC from the day of birth to day 14. Individual components of spontaneous activity, and habituation of this activity, were determined daily during this 2 week period using an Opto-Varimex animal monitor. Total Activity (TA) was subdivided into Total Very Active Behavior (TVA) which is mainly ambulations and grooming, and Total Slight Activity (TSA) which is predominantly sniffing and headwaving, using observational methods. The response of naive animals was tested on days 7, 9, 11 and 13 and compared to that of habituated animals of the same age.

Both peptides, ACTH 4-10 and 4-9, increased the percent TVA exhibited by the animals. However, ACTH 4-9 treated rats showed significantly more TVA and TSA. Habituation of spontaneous activity was demonstrated by all animals tested including controls. Habituation of TSA was achieved by 7-8 days in all animals tested. This may be interpreted as an acceleration of motor activity initiated by the increased activity of the pups exposed to repeated testing.

The peptide treated habituated animals exhibited a higher level of activity than the peptide treated naive animals at the same time in their development. Peptide treatment increases motor activity in neonatal rats (Saint-Come, Acker and Strand, Peptides 3:439-449) but this increase can be utilized only by the habituated animals during the course of their early postnatal development. This may explain the higher TA of the habituated rats as compared to the naive animals.

56.1 OVERLAPPING PROJECTIONS TO THE CAUDATE NUCLEUS FROM THE FRONTAL CORTEX AND CAUDAL INTRALAMINAR THALAMUS IN THE DOG. L.G. Isaacson* and D. Tanaka, Jr., Dept. of Anatomy, Michigan State University, East Lansing, Michigan 48824

Recent studies have examined in detail the relationships between neostriatal projections from individual cortical areas. Also, there have been a number of investigations of thalamostriate projections from the intralaminar nuclei. However, there have been few studies that have compared the topographical organizations of specific corticostriate and thalamostriate projections. In this study, we mapped thalamostriate projections from the centrum medianum (CM) and parafascicular (Pf) thalamic nuclei and compared them with those from the orbitofrontal (OF) and primary motor (MI) cortical areas.

Injections of lectin conjugated horseradish peroxidase (WGA-HRP) into the lateral part of the caudate nucleus resulted in retrogradely labeled neurons located primarily in the dorsal part of CM and throughout the lateral-medial extent of MI. Labeled neurons in MI were most numerous in layer III but were also scattered throughout layers II-VI.

Injections of WGA-HRP into the medial part of the caudate nucleus resulted in labeled neurons located mainly in OF and Pf. Neurons labeled from dorsomedial injections of the caudate nucleus were localized more dorsally in Pf than were neurons labeled by ventromedial injections. In OF, wide bands of labeled neurons, located primarily in layer III, alternated with regions containing minimal numbers of labeled cells. Separate injections of tritiated amino acids into OF and Pf showed patchy labeling within the medial part of the caudate nucleus.

These data indicate that MI and CM both project to the lateral part of the caudate nucleus while OF and Pf project to the more medial part of the nucleus. A similar segregation of inputs has been reported in primates in which the prefrontal cortex and Pf project to the caudate nucleus while MI and CM project to the putamen. Taken together with data from other studies reporting projections from MI to CM and from OF to Pf, these results suggest that overlapping patterns of afferents from anatomically related cortical and thalamic regions may demarcate distinct anatomical and functional regions of the neostriatum.

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56.2 CORTICAL AFFERENTS TO THE NUCLEUS ACCUMBENS (NA) IN CATS. A.JAYARAMAN, Dept.of Neurology, Louisiana State University School of Medicine, New Orleans, La. 70112.

The NA is a constituent of the "limbic striatum". To identify the cortical afferents to NA, 0.05 ul of 2% WGA-HRP was injected into NA of 12 cats. Injections restricted to medial NA led to labeling of CA 1 and subicular neurons. Injections of ventral NA resulted in labeling of cells in infralimbic and prelimbic cortex, medial and lateral entorhinal cortex, areas 35 and 36, and insular cortex, whereas injections into adjacent olfactory tubercle resulted in profuse labeling of the anterior olfactory nucleus, prepyriform and the insular cortex and a less intense labeling of cells in the infra and prelimbic and the lateral entorhinal cortex. Lateral NA injections resulted labeling of lateral entorhinal cortex, areas 35 and 36 and the insular cortex. Injections of WGA-HRP in dorsomedial areas of the head of the caudate nucleus resulted dense labeling of neurons of area 6, the anterior cingulate and the anterior sylvian gyri and less profusely to the posterior cingulate and the retrosplenial cortex. Most of the labeled neurons were in layer V, but in some cortical areas cells of layers II and III were also labeled. In addition to the predominantly ipsilateral cortical projections, NA also receives afferents from homotypical areas of contralateral cortex.

These results suggest that NA can be divided into two subdivisions, a medial division receiving CA 1 and the subicular afferents and a ventrolateral division which receives projections from the anterior limbic, entrorhinal and insular cortex and areas 35 and 36. The study also suggests that the dorsal limits of lateral NA may extend to an area which is traditionally considered to be medial regions of the caudate nucleus.

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56.3 INTRACELLULAR RECORDING AND HRP INJECTION IN BILATERALLY PROJECTING CORTICOSTRIATAL NEURONS IN THE RAT. C.J. Wilson. Dept. Anat., Univ. Tenn. Ctr. Hlth. Sci., Memphis, TN 38163.

Electrical stimulation of agranular frontal cortex evoked large EPSPs in neostriatal spiny projection neurons on both sides of the brain. Responses recorded on the contralateral side appeared to arise from a population of bilaterally projecting corticostriatal neurons that have no axonal branches decending beyond neostriatum on either side. This conclusion is based on the following evidence: (1) The maximal amplitude of the EPSP was not significantly reduced by acute cortical ablation ipsilateral to the recording in neostriatum, (2) The maximal EPSP amplitude was halved after chronic hemidecortication , (3) Stimulation of contralateral neostriatum evoked an EPSP identical to that evoked from contralateral cortex, (4) Stimulation of the contralateral internal capsule at the level of the globus pallidus was not effective at evoking the EPSP. The EPSP evoked from contralateral cerebral cortex had a constant latency within cells and varied in latency between cells from 3 to 13 ms (mean= 8.3, SD=2.1, N=46). These observations suggest that antidromic activation from contralateral neostriatum should allow identification of a large population of corticostriatal neurons uncontaminated by stimulation of axons of passage or by cortical cells giving rise to both decending projections and neostriatal axon collaterals.

Intracellularly stained crossed corticostriatal neurons in agranular frontal cortex identified by antidromic activation from contralateral neostriatum were small pyramidal neurons located in the superficial 1/3 of layer V. They had thin apical dendrites with extensive arborizations in layer I. Their local axonal arborizations were mostly restricted to layers III and V. The main axon of the cells branched in the subcortical white matter, giving rise to medially and laterally running branches of approximately equal diameters. The laterally coursing branch was in some cases followed into the ipsilateral neostriatal neuropil. The other branch continued medially toward the corpus callosum. Antidromic action potentials in these neurons ranged in latency from 5.0 to 22.8 ms (mean=10.3, SD=5.1, N=32). This latency range is similar to that obtained for orthodromic crossed corticostriatal EPSPs. Stimulation of ipsilateral cerebral peduncle or thalamus evoked EPSP-IPSP sequences in crossed corticostriatal neurons. These responses were simular to those observed previously in rat PT neurons contributing collateral axonal arborizations to the neostriatal neuropil.

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56.4 DIFFERENTIAL CONNECTIONS OF CAUDATE NUCLEUS AND PUTAMEN IN MONKEY. A. Parent, Y. Smith and C. Bouchard*. Lab. of Neurobiology, Fac. Med., Laval Univ., Québec, Canada.

Experiments with multiple fluorescent retrograde tracers have revealed that striatal neurons projecting to globus pallidus (GP) in squirrel monkeys (Saimiri sciureus) are mainly confined to putamen (PUT) whereas those projecting to substantia nigra (SN) occur mostly in caudate nucleus (CD). Only about 10% of striatal neurons were found to be double-labeled after GP and SN injections, suggesting that the striatopallidal and striatonigral projections exist largely as two distinct fiber systems in primate (Parent et al., Brain Research, in press).

In the present study large WGA-HRP injections were made in PUT on the left side and in CD on the right side in 8 squirrel monkeys, and the anterograde and retrograde transport of this tracer was studied using the TMB method. The anterograde tracing of WGA-HRP reveals that putaminofugal fibers terminate massively in the ventral two-thirds of GP, where they display typical band-like arrangement, and much less abundantly in lateral third of SN. In contrast, caudatofugal fibers occupy only the dorsal border of GP but terminate densely in medial two-thirds of SN. In SN itself the anterogradely labeled fibers are distributed according to a very complex pattern. Although dense networks of labeled fibers and granular HRP material (suggestive of terminals) are found in pars reticulata (SNr), granular HRP reaction product also covers neurons of pars compacta (SNc). In SNc clusters of retrogradely labeled cells are closely intermingled with clusters of unlabeled neurons. The labeled-cell clusters are particularly dense on PUT injection side but more loosely organized on CD injection side. Striatofugal fibers terminate almost exclusively on clusters composed of retrogradely labeled cells, suggesting a precise interrelationship between the nigral and striatal neuronal aggregates. On the other hand, the retrograde cell labeling observed in thalamus is strikingly asymmetric. For instance, strong labeling of neurons in nucleus centralis superior lateralis is seen on CD but not on PUT injection side. In Centre median-parafascicular complex (CM/Pf), the retrograde cell labeling is largely confined to Pf n. on CD side and to CM (except its lateral border) on PUT injection side. The neurons in lateral border of CM appear to project only to cortex. These various findings suggest that the striatum in primate, as in other species, is a highly heterogeneous structure. (Supported by the MRC of Canada).

56.5 STRIONIGRAL PROJECTIONS IN THE OPOSSUM. J.C. Hazlett, T.P. Ma, A. Dunst* and G.R. Penny. Depts. of Anatomy, Wayne State Univ., Detroit, MI 48201 and Univ of Tennessee, Memphis, TN 38163.

Horseradish peroxidase injected into the caudate nucleus and/or putamen of 25 adult opossums resulted in the retrograde labeling of ipsilateral substantia nigra neurons and the orthograde labeling of strionigral axons. Large numbers of labeled pars compacta neurons together with a scattering of reactive pars reticulata and pars lateralis cells were observed. The orthogradely labeled striofugal fibers, upon descending to the level of the substantia nigra, were located between the medial border of the cerebral peduncle laterally and the pars compacta medially. From this position variable sized fascicles of labeled axons arose from the main bundle and coursed laterally into and through the pars reticulata where they formed a dense meshwork of labeled fibers. Much of this plexus appears to be limited to the pars reticulata. Furthermore, immunocytochemical analysis would suggest that both the main descending bundle and much of the fiber meshwork were GAD positive. We observed a moderate number of reactive and many non-reactive pars reticulata neurons embedded within the meshwork of labeled strionigral axons. However, a few labeled neurons were also found in the overlying tegmentum and adjacent pars lateralis. While neither of these two latter groups were located within the plexus described above, serial reconstructions of their labeled dendrites revealed branches extending into the zone of labeled axons (pars reticulata). Moreover, Golgi preparations of the opossum nigra revealed that many pars lateralis neurons possess long dendritic branches which enter the ventromedially adjacent pars reticulata. Although the proximal portions of these dendrites are usually spine free, many of the distal branches, upon entering the reticulata, exhibit sessile and pedunculated appendages. From this material we conclude that the pars reticulata neurons, regardless of their projection target, are located in a position which maximizes the potential for contact by strionigral terminals. In contrast, the pars lateralis contains many neurons, a few of which project to the neostriatum, whose dendritic arborizations include the presumed terminal zone of the striatal projections in the adjacent pars reticulata.

56.6 QUANTITATIVE EVALUATION OF CROSSED AND UNCROSSED BASAL GANGLIA PROJECTIONS TO THE CAT THALAMUS. K. Kultas-Ilinsky, A. Rosina and I.A. Ilinsky . Dept. of Anatomy, University of Iowa, Iowa City, IA 52242 and Istituto di Fisiologia dei Centri Nervosi del CNR, 20131 Milano, Italy.

The existence of a crossed component in nigro- and pallidothalamic pathways was demonstrated recently by several authors (Gerfen et al., 1981; Beckstead and Frankfurter, 1982; Nakano et al., 1983). These findings indicated, however, that the basal ganglia projections to the contralateral thalamus were very sparse when compared to the ipsilateral projections. To better understand the potential functional significance of these contralateral projections quantitative evaluation of crossed and uncrossed components of nigro- and pallidothalamic pathways was undertaken in the present study. Retrograde tracers (HRP, true blue and diamidino yellow) were injected either uni- (HRP) or bilaterally (fluorescent dyes) into thalami. After appropriate survival times, retrogradely labeled cells in the entopeduncular nucleus (EPN) and substantia nigra pars reticularis (SNr) were charted on X-Y plotter from serial sagittal sections at 500 μ intervals. The number of retrogradely labeled cells was then compared to the total number of cells present after counterstaining of the same sections. The number of retrogradely labeled cells in the EPN and SNr varied depending on the extent of involvement of the nigral and pallidal projection zones in the region of active tracer uptake. The maximum number of ipsilaterally projecting cells in the EPN was found to be 88% and in the SNr 49.4%. In the EPN 6.4% of total cell population was found to project to the contralateral thalamus and in the SNr such cells comprised 3% of the total population. The number of bilaterally projecting neurons was found to be negligible in both nuclei. In EPN and SNr such cells comprised 0.1% and 0.6% of total cell population respectively and 13.5% and 7.4% of the total number of contralaterally projecting cells.

These quantitative data confirm and document the qualitative observations of others that contralaterally projecting cells in the EPN and SNr represent only a small fraction of the cell population in each of these nuclei. The EPN and SNr cells projecting to ipsi- and contralateral thalamus appear to represent distinct cell populations since no significant amount of axonal branching is evident.

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56.7 ULTRASTRUCTURAL MORPHOLOGY OF AXONS AND INTRINSIC AXON COLLATERALS OF NIGROTHALAMIC AND NIGROTECTAL CELLS. I. Grofova, B. Spann and S. T. Kitai. Depts. of Anatomy, Mich. State Univ., E. Lansing, MI 48824-1316 and Univ. of Tenn., Memphis, TN 38163.

In a previous light microscopic study on the morphology of nigrothalamic and nigrotectal neurons intracellularly stained with HRP we have reported that axons of these cells often emitted several collaterals during their course through the substantia nigra (SN). The collaterals coursed for distances of several hundred μm extending far beyond the dendritic fields of the parent cells and distributing to both the pars reticulata (SNR) and pars compacta (SNC) of the SN (Grofova et al., 1982).

The electron microscopic data presented here are based on examination of HRP-labeled axons and intrinsic axon collateral branches of two nigrothalamic, one nigrotectal and one branched nigrothalamic/nigrotectal neurons. The axons arose from the somata or large proximal dendritic trunks and rapidly attained a constant diameter of 0.7 μm. The axon hillocks were occasionally contacted by large en passant boutons containing numerous mitochondria and clusters of small pleomorphic synaptic vesicles, but the initial segments (30-50 μm long) were always ensheathed by glial processes. The myelin sheath increased the axon diameters in 1.2 μm. The axon collaterals commonly originated from the Ranvier's nodes and often coursed unbranched for long distances. The initial collateral branches averaged 0.5 μm in diameter and they were covered by a thin layer of myelin. A delicate myelin sheath was also observed on major secondary and tertiary branches which gave rise to a profusion of thin-elongated en passant boutons which contained small pleomorphic synaptic vesicles and established symmetrical synapses with unlabeled medium-sized dendrites in both parts of the SN.

These observations offer the first positive ultrastructural identification of axons and intrinsic axon collateral branches and terminals of SNR projection neurons. They further support the contention that the axon collaterals are involved in a complex integration of different output neurons. Supported by N.I.H. Grant NS 19483.

56.8 AN EXTRACELLULAR MICROELECTRODE STUDY OF GLOBUS PALLIDUS AND CEREBELLAR PROJECTIONS TO THE THALAMUS IN THE MONKEY. R.D. HUFFMAN, L.P. FELPEL AND J. LUM*. Dept. Pharmacology, Univ. of Texas Health Science Center, San Antonio, Texas, 78284.

Several investigators have reported convergence of input from globus pallidus (GP) and the deep cerebellar (CB) nuclei onto single ventral lateral and ventral anterior thalamic neurons in cat. In addition, GP and/or entopeduncular stimulation has frequently been reported to excite these same neurons. Uno and Yoshida (Br. Res. 99:377, 1975), however, only reported inhibition of thalamic neurons and found no evidence of convergence of the two systems. Since the GP primate is a well defined nucleus and not embedded in the internal capsule as is the feline entopeduncular nucleus, we decided to employ primates to characterize the pallidothalamic system and to assess the possible degree of convergence of the pallidothalamic and cerebellothalamic systems. Adult Macaca mulatta (5) or Macaca fascicularis (6) monkeys anesthetized with either chloralose-urethane or pentobarbital were employed in this study. Bipolar concentric (6) and unipolar (5) stainless electrodes were employed to stimulate GP and the contralateral dentate nucleus of the CB, respectively. Two barrel glass micropipettes were employed for recording and for glutamate iontophoresis. CB stimulation evoked a short latency response (2.04±0.12msec) that could be recorded from VPLo, area X and the more caudal portions of VLc. GP stimulation, on the other hand, evoked a powerful inhibition of neuronal discharge in VLm, VLo and subthalamic nucleus. The latency to inhibition was estimated to be approximately 5msec, while the duration of inhibition ranged from 50msec to 500msec. No cells in any of the 7 regions studied were excited by pallidal stimulation. Excitatory responses were only observed when the stimulating electrodes strayed into the adjacent interval capsule. The effects of both CB and GP stimulation were tested on 171 diencephalic neurons and no cells were found that were affected by both input sources. These results of these stimulation studies are summarized in the following table.

	VPLo	X	VLc	VLo	VLm	ZI	Subth
CB Evoked	44/51	35/45	11/37	0/16	0/14	0/15	0/10
GP Inhibited	0/52	0/43	1/28	6/26	10/19	0/19	10/10
Both	0/51	0/39	0/28	0/16	0/14	0/13	0/10

In summary, our electrophysiological studies have provided no evidence for convergence of CB and GP input onto any of the populations of thalamic neurons we have studied and thus are in agreement with recent anatomical evidence that suggests a segregation of these two sources of diencephalic input.

56.9 THE PLACE OF PREFRONTAL CORTEX IN THE EXTRAPYRAMIDAL MOTOR SCHEME: NIGRO-THALAMO-CORTICAL CONNECTIONS IN THE RHESUS MONKEY. I.A. Ilinsky, M.L. Jouandet and P.S. Goldman-Rakic. Section of Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

The present study provides evidence that the nigral--recipient nuclei of the monkey thalamus project widely to prefrontal association cortex as well as to portions of the premotor cortex. The method of autoradiography was employed for tracing nigral efferents while thalamocortical projections were studied by means of double or triple retrograde labeling with the fluorescent dyes Fast Blue, Diamidino Yellow and Propidium Iodide injected simultaneously into different regions of the frontal lobe.

Autoradiographic analysis revealed that the main targets of nigral afferents in the monkey thalamus are the magnocellular part of the ventral anterior nucleus (VAmc) and the paralaminar subdivision of the mediodorsal nucleus (MDmf of Olszewski, Karger, 1952). In addition, a number of small patchy nigral terminal areas were observed scattered throughout the mediodorsal nucleus (MDmc and MDpc). Analysis of the thalamus in cases with fluorescent dye injections in the principal sulcus (Walker's area 46), the anterior bank of the arcuate (Walker's areas 45 and 8A), the lateral orbital gyrus (Walker's area 11) and medial frontal cortex (Walker's areas 6 and 8B) revealed labeled cells in VAmc from all areas injected and in MDmf mainly from Walker's areas 8A and 45. Moreover, the thalamocortical projections of the VAmc are topographically organized: the medial part of the VAmc projects to the dorsolateral and orbital prefrontal cortex, while the lateral part of the nucleus projects to the frontal eye fields and the medial premotor cortical areas. These findings together with recent data from other laboratories indicate that the substantia nigra, like the globus pallidus and deep cerebellar nuclei, is the origin of a parallel feedback loop to the cerebral cortex with wide targets in the frontal association and premotor cortex. (Supported by NIMH and NIH.)

56.10 SUBSTANTIA NIGRA LESIONS ABOLISH THE THALAMIC RESPONSE PRODUCED BY STRIATAL DOPAMINERGIC STIMULATION. P. Patiño* and M. García-Muñoz (SPON: R. A. Sjodin). Center of Research in Cellular Physiology, U.N.A.M., México D.F. 04510.

The ventromedial nucleus of the thalamus (VMT) receives inputs from the two major outputs of the basal ganglia: the globus pallidus (GP) and the substantia nigra (SN). The aim of this work was to observe how striatal dopaminergic stimulation affects VMT cell activity and to study the participation of the output nuclei in this response. Male albino Wistar rats were anesthetized with halothane vapour (0.8% in 95% O_2-5% CO_2) and extracellular activity recorded in VMT. A guide cannula was implanted in the striatum. Apomorphine 400 ng/1µl/5 min was administered into the striatum after the basal VMT firing rate was determined. After the injection the firing rate increased from 1.8 ± 0.02 to 7.4 ± 0.1; 5 ($\bar{X}$ ± SEM;n). A kainic acid lesion (2.5µg/0.5µl) 8-20 days prior de recording experiment in GP did not alter VMT cell activity: 1.5 ± 0.05 to 9.7 ± 0.6; 5. A lesion in the SN abolished VMT response: 1.0 ± 0.06 to 1.0 ± 0.06; 5.
The participation of the entopeduncular nucleus is being studied.
So far it can be suggested that the SN conveys the information about dopamine receptor stimulation to VMT.

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56.11 OPPOSING INFLUENCE OF THE STRIATONIGRAL FEEDBACK LOOP ON APOMORPHINE-INDUCED INHIBITION OF DOPAMINE NEURON FIRING. B. S. Bunney and A. A. Grace, Depts. Psychiatry & Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510 and Dept. Physiology & Biophysics, New York Univ. Med. Ctr., New York, NY 10016.

In this study, we used in vivo intracellular and extracellular recording from dopamine (DA) and non-DA zona reticulata (ZR) cells to determine the mechanisms by which the striatonigral pathway and DA cell autoreceptor stimulation modulate DA cell activity. Stimulation of the striatum elicited in DA cells a short latency (1.8-2.2 msec) IPSP with an amplitude of 3-4 mV which was followed by a rebound depolarization of sufficient intensity to trigger additional spikes. Based on its reversal potential (-67 mV) and inversion to depolarization by intracellular chloride ion injection, this IPSP was determined to be a conductance increase to chloride ions. Despite the existence of this direct inhibitory projection, stimulation of the striatum with trains of low intensity pulses typically increased the firing rate of DA cells.

Striatal stimulation also elicited in non-DA ZR cells IPSPs which had a similar latency, reversal potential, and also reversed to a depolarization with chloride ion injection. However, the IPSPs in ZR cells were always larger in amplitude and longer in duration than those observed in DA cells. In addition, stimulation of the striatum at the same intensities which caused an increase in DA cell firing rate inhibited ZR neurons. We had demonstrated previously that a ZR interneuron inhibited DA cells and was more sensitive to GABA than were DA cells. Furthermore, systemic administration of the GABA agonist muscimol increased DA cell firing rate by preferentially inhibiting these ZR cells. Thus, in a similar manner, trains of stimulation delivered to the striatum also preferentially inhibited these ZR cells, and resulted in an increase in DA cell firing rate through disinhibition.

Nigral DA cells respond to low, autoreceptor specific (10-20 ug/kg iv) doses of apomorphine (APO) with a hyperpolarization and inhibition of firing as well as an increase in input resistance. The inhibition is due to direct inactivation of the slow depolarization which triggers spiking in DA neurons. Transection of striatonigral feedback pathways eliminated only the effect of APO on input resistance. Low doses of APO also inhibited ZR interneurons in the presence of an intact feedback pathway. Thus, i.v. administration of autoreceptor specific doses of APO appears to elicit the following sequence of events: 1) DA cells are inhibited directly, thus 2) removing DA inhibition of striatonigral cells. The increased firing of striatal cells then 3) preferentially inhibits ZR interneurons, thereby 4) removing GABA inhibition of DA cells and increasing their input resistance. (Supported by USPHS MH-28849, MH-25642 and the State of CT)

56.12 REINNERVATION OF DOPAMINE-DENERVATED STRIATUM BY SUBSTANTIA NIGRA TRANSPLANTS: IMMUNOCYTOCHEMISTRY AND ELECTROPHYSIOLOGICAL CORRELATES Olson, L.*[1], Strömberg, I.*[1], Johnson, S.[2] and Hoffer, B.[2] (Sponsor: G. Kindt) [1]Karolinska Institute, Stockholm, Sweden and [2]Dept. of Pharmacology, UCHSC, Denver, CO 80262

This study further evaluated whether or not fetal substantia nigra (SN) tissue, grafted to striatum lesioned previously with 6-hydroxydopamine(6-OHDA), provides functional dopaminergic reinnervation of striatum. Sprague-Dawley rats were given unilateral injections of 6-OHDA into the brain parenchyma and subsequently screened by measurement of apomorphine-induced rotation. Some animals then received SN transplants into "delayed cavities" placed just dorsal to the striatum.

Falck-Hillarp histochemistry and immunofluroescent staining for tyrosine hydroxylase demonstrated extensive networks of nerve fibers which extended 1-1.5 mm from the nigra graft into striatal tissue.

Multibarrel micropipettes were used to record neuronal action potentials and test electrophysiological responses to phencyclidine (PCP), an indirect dopamine agonist, applied locally by micropressure ejection. "Distal" neurons, defined as those striatal neurons more than 2.0 mm from the nigral grafts, fired at an average spontaneous rate of 13.4 spikes/sec and were relatively insensitive to the effects of locally applied PCP. Similar changes were seen in animals which received the 6-OHDA treatment without subsequent grafting. In contrast, "proximal" neurons, defined as those neurons less than 1.0 mm from nigral grafts, fired at a significantly lower average rate of 4.9 spikes/sec, and were much more sensitive than distal neurons to the effects of PCP. Properties of proximal neurons resembled those of neurons in normal caudate.

These results suggest that fetal substantia nigra grafts can provide functionally significant reinnervation of striatum lesioned previously with 6-hydroxydopamine. (Supported by the Swedish Medical Research Council and USPHS grants NS09199 & MH00289.)

56.13 DEMONSTRATION OF MONOAMINE RELEASE FROM TRANSPLANT-REINNERVATED CAUDATE NUCLEUS BY IN VIVO ELECTROCHEMICAL DETECTION Gerhardt, G.*[1], Rose, G.[1,2], Strömberg, I.*[3], Olson, L.*[3] and Hoffer, B.[1] [1]Dept. of Pharmacology, UCHSC, [2]Medical Research, VAMC, Denver, CO and [3]Karolinska Institute, Stockholm, Sweden

Previous studies have shown that fetal substantia nigra (SN), grafted to cavities overlying dopamine(DA)-denervated striatum, can reverse a number of behavioral abnormalities induced by the denervation. While some histochemical and physiological evidence suggests that this reversal is the result of a functional DA input from the transplant to the host brain, there is little direct evidence for actual transmitter release from ingrowing graft nerve fibers. In this study, we sought to determine if local micropressure ejection of K^+ could induce monoamine transmitter release, using the technique of in vivo electrochemistry.

Nafion-coated electrodes (Gerhardt et al.,Brain Res.,290,390) were employed to minimize any signal derived from ascorbate or acidic metabolites. Animals were injected unilaterally with 6-hydroxydopamine (6-OHDA) into the SN and screened by measuring apomorphine-induced rotation. Some were then given SN grafts using a "delayed cavity" just dorsal to the lesioned striatum. The morphometric relationships between all striatal recording sites and the transplants were verified histologically for each animal. The intact sides were studied with each electrode to provide "control" release values.

Releases from striatal sites within 1 mm of the SN grafts were slightly, but not significantly, less than those obtained from the intact sides (3.2 ± 0.41 (S.E.M.)um vs. 4.2 ± 0.40 um). In contrast, releases from striatal sites further distal were markedly reduced, to about 30% of control values. These were similar to those obtained in 6-OHDA treated animals which did not receive SN grafts.

These data lend further evidence to the postulate that SN grafts ameliorate lesion-induced behavioral disfunctions by providing specific DA input to the host brain. (Supported by USPHS grants NS09199 & MH00289, grants from the Swedish MRC, and the VA Medical Research Service.)

56.14 ULTRASTRUCTURE OF HOST-GRAFT CONTACTS IN STRIATUM REINNERVATED BY DOPAMINERGIC NEURONS OF TRANSPLANTED SUBSTANTIA NIGRA. T. J. Mahalik, T. E. Finger, L. Olson*, and I. Strömberg*. Dept. of Anatomy, Univ. Colo. Med. Sch., Denver, CO and Dept. Histol., Karolinska Inst., Stockholm, SWEDEN

A number of recent experiments indicate that intracerebral grafts of embryonic substantia nigra can ameliorate the behavioral effects of 6-hydroxydopamine (6-OHDA) lesions of the medial forebrain bundle. Fluorescence histochemistry has shown that catecholaminergic (CA) cell bodies develop in the transplant and innervate the nearby portions of the host striatum. The purpose of the present study was to examine the ultrastructure of the host-transplant contacts within the reinnervated striatum.

Injections of 6-OHDA were made into the medial forebrain bundle of adult Sprague-Dawley rats. Approximately two months later, the animals were screened for the success of striatal denervation by a test of apomorphine-induced rotation. In the well denervated cases, the ventral mesencephalic tegmentum from an E-19 rat fetus was transplanted so as to lie just dorsal to the lesioned striatum. After 10 months, these host animals were fixed and the brain and transplant processed for immunocytochemical localization of tyrosine hydroxylase. These immunostained preparations confirm the virtually complete destruction of the CA cells of the host substantia nigra.

At the light microscopic level, tyrosine hydroxylase-like-immunoreactive (THLI) fibers could be seen coursing out of the graft and into the dorsal 20% of the host striatum. Occasionally, THLI fibers even extend 1 to 2 mm into the adjacent host neocortex. In contrast, virtually no THLI fibers are present in the ventral half of the striatum. At the electron microscopic level, immunoreactive boutons which synapse onto unlabeled dendritic processes are present in the host striatum. More importantly, THLI processes within the dorsal striatum are postsynaptic to non-immunoreactive terminal boutons. The immunoreactive post-synaptic neurites are similar to dendrites in terms of containing microtubules and possessing post-synaptic specializations characteristic of asymmetric axo-dendritic synapses.

The present results suggest that the nigral tissue graft establishes functional dopaminergic synapses onto host neurons, and raise the additional possibility that host tissue neurons synapse onto the ingrowing processes of the transplanted neurons. This would provide an avenue by which the host tissue may regulate the activity of the dopaminergic neurons of the nigral graft.

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56.15 ASCENDING AND DESCENDING PROJECTIONS OF THE NUCLEUS TEGMENTI PEDUNCULOPONTINUS IN THE RAT. B. Spann and I. Grofova. Dept. of Anatomy, Mich. State Univ., E. Lansing, MI 48824.

The nucleus tegmenti pedunculopontinus(NPP) is the brainstem target of descending connections of various basal ganglia nuclei including striatum, globus pallidus(GP), entopeduncular nucleus(EN), subthalamic nucleus(STN) and substantia nigra(SN). It has been established that the major efferent projections of the NPP ascend toward the GP, EN, STN, SN and thalamus (Jackson and Crossman, '83). It has also been suggested that the NPP gives rise to fibers descending to the spinal cord which may mediate the basal ganglia influence on motor performance.

In the present study three series of experiments were carried out to determine the distribution of the cells of origin of ascending and descending NPP projections and to test the presence of collateralization. In the first two series of experiments multiple injections of HRP were made bilaterally either in the targets of ascending NPP efferents or in the lower cervical cord. Following injections involving GP, EN, STN, SN and post. thalamus we observed large numbers of densely labeled cells in the entire region of NPP. The labeled cells were either fusiform or multipolar in shape and varied in sizes from 14.4 to 38 um along the longest axis. Injections involving the cervical cord resulted in light labeling of a relatively small number of NPP cells throughout the nucleus. The third series of experiments were performed to determine whether the axons of some NPP cells divided into ascending and descending collaterals. Multiple injections of Granular Blue(GB) were made into the cervical cord and Diamidine Yellow Dihydrochloride(DYD) was injected into the forebrain targets of NPP efferents. Cells labeled from the forebrain with DYD considerably outnumbered those labeled with GB from the cervical cord and there was no distinct separation of the two populations of NPP projection neurons. A few double-labeled neurons were observed. The GB-labeled NPP cells exhibited faint fluorescence in contrast to other adjacent brainstem nuclei which project to the spinal cord and which indeed exhibited very bright fluorescence. Similar results were obtained when the injections of fluorescent dyes were reversed. These results confirm previous observations that the NPP gives rise to prominent ascending connections. In addition, we demonstrate conclusively projections of the NPP to the spinal cord. The majority of both ascending and descending fibers arise from separate populations of NPP neurons which are intermingled throughout the nucleus.
Supported by N.I.H. Grant NS 19483.

56.16 THE TIME COURSE OF FETAL AND POSTNATAL ACQUISITION OF TERMINALS ON CAT ENTOPEDUNCULAR NEURONS. C. Dvergsten*, A.M. Adinolfi, M. Levine, C. Hull, and N. Buchwald. Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

A stereological analysis of electron micrographs was used to define the temporal pattern of the acquisition of terminals on entopeduncular (ENTO) neurons. Approximately 1900-4700um of dendritic surface and 700-1600um of somatic surface were analyzed in 2 animals per age group. Parameters investigated were (1) the percent of the surface of the dendrites and somata contacted by axon terminals (columns A and D), (2) the number of terminals per 100um of membrane (columns B and E), and (3) the total number of terminal profiles per dendritic tree (i.e., number of terminals/um X the total dendritic length, (column C). All these parameters showed similar developmental trends except that since dendritic development was slightly in advance of the acquisition of terminals, the increase in the number of terminals/dendritic tree (column C) was slightly in arrears of the other parameters until postnatal day 25 (P25).

Initially, only a few terminals were found on the neurons. At E45, about 1%-12% of the adult number of terminals were present on both dendrites and somata. At day P25 81%-98% of the adult values had been attained. Approximately 55%-70% of the terminals were acquired after birth.

The similar temporal pattern of the postnatal acquisition of terminals and the transient spiny period of the dendrites suggests that dendritic appendages may aid in the acquisition of terminals. Spine-like processes did not develop into branches since the distribution of branches and the number of branches did not change postnatally. This pattern of terminal acquisition is consistent with reports indicating that the ability of ENTO neurons to respond to repetitive caudate stimulation increases from birth to 21 days. Further, although the neuropil is still immature at birth, ENTO neurons have a sufficient amount of connectivity to respond to caudate stimulation.

AGE	DENDRITE			SOMA	
GROUPS	A	B	C	D	E
E45	12	3.2	450	5	3
Birth	38	27.4	1507	15	10.5
P25	81	63	5040	40	18.9
Adult	90	62	5580	41	23.3

(Supported by HD Foundation Grant and USPHS HD 05958)

56.17 ONTOGENESIS OF CAUDATE NEURONAL RESPONSES TO AMINO ACID NEUROTRANSMITTERS IN THE CAT. J.H. Hannigan, C.D. Hull, M.S. Levine, and N.A. Buchwald. Mental Retardation Research Ctr., UCLA, Los Angeles, CA 90024.

The ontogeny of electrophysiological responsiveness of caudate neurons (CD) to glutamate (GLU) and GABA was investigated in adult cats and in kittens 5-30 days of age. Extracellular single unit recordings were obtained from anesthetized animals (N=15). Changes in spontaneous firing patterns and responses to stimulation of pericruciate neocortex (CX) and substantia nigra (SN) were assessed after micropressure-applied GLU (1 or 10mM, pH=8.0), GABA (1 or 10mM, pH=6.0) and saline vehicle (0.9%, pH=6.0 or 8.0). Solutions were applied through multibarrel glass micropipettes (tip diameter: 4-20u per barrel) attached to recording pipettes (distance between ejection and recording tips = 30-80u). Pressures ranged from 0.04 to 1.60 kg/cm^2 (duration = 0.5-30 sec). Control procedures eliminated possibilities that effects were artefacts of pressure, fluid or acidity.

GLU increased firing rates of 77% (33/44) of CD neurons in adults and decreased firing rates of 14% (6/44). In kittens there was an age-related increase in the proportion of neurons increasing firing rates after GLU (47% [9/19] at 1-10 days; 64% [7/11] at 11-20 days; 83% [10/12] at 21-30 days). GLU decreased firing rates in 21% (4/19) and 9% (1/11) of cells in the two youngest groups, respectively. No cells in 21-30 day kittens decreased firing rates after GLU (0/12).

GABA reduced firing rates in 74% (35/47) of adult CD neurons. No cells increased firing after GABA. In all kitten groups the percent of responsive cells was equivalent to, or even greater than in adults (80% [20/25] at 1-10 days; 83% [10/12] at 11-20 days; 73% [8/11] at 21-30 days). The percent of kitten CD cells that recovered from "GABA inhibition" was 40%, 80% and 86%, from the youngest to the oldest group, respectively, compared to 90% of adult CD cells. Younger animals required lower minimum pressures to induce responses. Responses to both CX or SN stimulation consisted of excitations followed by variable periods in which cells did not fire. After either CX or SN stimulation, GABA decreased evoked spikes in all age groups.

We conclude that CD neurons in even the youngest kittens tested (5 days) may be functionally sensitive to GLU and GABA; sensitivity to GLU increases postnatally; and cells in the younger kittens may be more sensitive to GABA than in the oldest kittens and cats.

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56.18 BRANCHED PROJECTIONS OF PALLIDAL NEURONS TO THE NEOCORTEX AND NEOSTRIATUM OF THE CAT. R.S. Fisher, M.K. Boylan*, M.S. Levine, C.D. Hull and N.A. Buchwald. Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

Earlier reports employing axonal uptake and retrograde transport of single marker substances such as horseradish peroxidase (HRP) have demonstrated 1) a peripallidal input to the neocortex (from substantia innominata, nucleus of the diagonal band, etc.) 2) a pallidal input to the neostriatum (from globus pallidus, the feline homolog of the primate external pallidal segment). Since these pallidal and peripallidal neurons are morphologically similar and lie in closely adjacent brain sites, we used double-labelling retrograde axonal transport methods (nuclear yellow-NY injected into caudate nucleus and wheatgerm lectin-bound HRP injected into the ipsilateral precruciate, cingulate and prorean gyri in 3 adult cats; reversed combinations in 2 cats; 3 controls with marker injected into subcortical white matter and/or lateral ventricles) to assess the existence of divergent, branched axonal fibers projecting from these brain sites to the ipsilateral neocortex and neostriatum.

Double-labelled large and medium-sized fusiform neurons were evident prominently at the level of the anterior commissure in all of the experimental animals. These cells were located in both the substantia innominata and the globus pallidus proper and were often located adjacent to more numerous single-labelled neurons. The larger branched projections neurons were morphologically similar to the cholinesterase- and choline acetyltransferase-positive cells previously described in these brain sites while the medium-sized neurons were similar to glutamic acid decarboxylase-positive cells in the basal forebrain of the cat. Additional double-labelled neurons were evident in 1) the contralateral frontal lobe neocortex (layers 3-5), 2) motoric and intralaminar thalamus, 3) ventral tegmental area, and 4) the mesencephalic raphe nuclei. Single-labelled cells projecting to the neocortex were also found in the locus coeruleus while many single-labelled cells coursing to the caudate were seen in both the ipsi- and contralateral neocortex. Such patterns of labelled neurons were not evident in control cats. The branched peripallidal and pallidal neurons suggest that the neocortex and neostriatum share small but potentially significant extrinsic cholinergic and GABAergic inputs.

56.19 BASAL GANGLIA, PARABRACHIAL, AND TRIGEMINAL INTERACTIONS IN THE CAT: AN ANATOMICAL AND FUNCTIONAL STUDY. J.S. Schneider, N.A. Buchwald, C.D. Hull, and M.S. Levine. Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

Interactions between the basal ganglia (BG), the pontine parabrachial region (PB), and the trigeminal system (V) have been investigated at structural and functional levels. In the present study, we have attempted to ascertain whether the PB area might, in part, function as a relay for V afferent information ascending to the BG and/or for BG influences descending to the brainstem. Previous research had demonstrated V sensory influences on BG neurons and BG influences on V motor activities. The PB was chosen for study because it is believed to be involved in both the relay of gustatory and possibly V afferent information to the forebrain and in the output control oral-lingual behavior. The degree of convergence of V and BG inputs to PB neurons was studied electrophysiologically. The major connections between the PB, BG, and V nuclei were examined by horseradish peroxidase (HRP) techniques. In neurophysiological studies, PB neurons were found to be responsive to ipsilateral sensory V (28%) and ipsilateral substantia nigra (SN) stimulation (35%), and less responsive to ipsilateral entopeduncular nucleus (ENTO) stimulation (10%). PB cells also received convergent sensory V and BG inputs (21%). In many PB cells responsive to sensory V stimulation, single pulse stimulation of the SN preceding sensory stimulation was effective in inhibiting the response to sensory stimulation. Wheat-germ lectin bound HRP injected into the PB at the level of the trigeminal nuclei resulted in labelled cell bodies in the ipsilateral SN pars reticulata (SNr), pars lateralis (SNl) and the spinal V nuclei. Less dense cell labelling was found in the ipsilateral ENTO and nucleus of the ansa lenticularis. Diffuse terminal field labelling was observed ipsilaterally in the ENTO, SN, amygdala, and VPM of thalamus. Retrograde double-labelling studies indicate an even greater complexity of this nigro-parabrachial system. Nuclear yellow injected into the centremedian/parafascicular area of thalamus and WG-HRP injected into the PB resulted in many double-labelled SN cells. Thus, many SN cells send axons both caudally deep into the pontine region and rostrally to the thalamus. These results demonstrate both functional and anatomical interactions between the BG, PB, and V systems and suggests the existence of complex ascending and descending control systems which may in some way modulate oral sensory-motor activities.

(Supported by USPHS Grant HD05958).

56.20 INTRACELLULAR RESPONSES OF CAUDATE NEURONS TO STIMULATION OF CORTICAL AND NIGRAL AFFERENTS IN BRAIN SLICE PREPARATIONS. T.C. Prentice, Jr*, M.S. Levine, C.D. Hull and N.A. Buchwald. Mental Retardation Research Center, UCLA Los Angeles, CA 90024.

Previous work has demonstrated the feasibility of studying intrastriatal electrophysiology in mammalian brain slices. We have been able to fashion slices (400um thick) from adult rat brain with electrophysiologically-demonstrated corticostriatal (CS) and nigrostriatal (NS) synaptic connections. Intact CS and NS connections were maintained in angled parasaggital slices in which the cortex, striatum, entopeduncular nucleus, and substantia nigra were all visible. To achieve this alignment, proper brain blocking was critical.

Intracellular responses were recorded in 30 caudate neurons, 15 responsive to cortical stimulation, 15 to nigral stimulation. Excitatory postsynaptic potentials, typically accompanied by action potentials, were the only synpatic responses observed. Inhibitory postsynaptic potentials did not occur. In addition, evoked action potentials without preceding synaptic potentials were observed. Responses could be divided into two classes on the basis of latency: short latency responses (CS .6 msec, mean for 8 cells; NS .5 msec, mean for 10 cells) and long latency responses (CS 4.0-4.7 msec, mean for 9 cells; NS 5.4-5.7 msec, mean for 7 cells). Some cells showed both short and long latency responses. We believe the short latency responses to CS activation may be evoked by direct current spread, whereas those to NS activation (in which stimulation and recording electrode distances are greater) are more likely due to antidromic activation. The longer latency responses to both CS and NS activation appear to be orthodromic, synaptically-mediated responses. To verify this, we sought to interrupt synaptic transmission by infusing a high Mg++ (8.1mM vs 1.3mM) bathing medium during recording. Long-latency CS responses in several cells tested to date were abolished, while spontaneous firing was not effected. When normal Mg++ (1.3mM) medium was reinfused, long-latency responses recurred.

These experiments demonstrate that striatal slices with intact afferents provide a useful model system for pharmacological assessment of synaptic inputs.

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57.1 DIFFERENTIAL DRUG SENSITIVITY OF VARIOUS FOREBRAIN FOCAL SEIZURES. J. A. Mace* and W. M. Burnham.(SPON: L. Grupp). Dept. of Pharmacology, University of Toronto, Toronto, Ontario. M5S 1A8.

It has recently been demonstrated that amygdala focal and cortical focal seizures differ in their response to anticonvulsant drugs (Albright and Burnham, 1980). Cortical focal seizures are easily suppressed by the clinically effective anti-tonic-clonic drugs, whereas amygdala focal seizures are extremely resistant to anticonvulsant action. This pattern corresponds to the situation in humans, in which cortical focal seizures are much more responsive to anticonvulsant therapy than complex partial seizures. The present study was designed to examine differential drug sensitivities in a wider variety of forebrain sites. Four limbic and two cortical foci were examined for their response to phenobarbital, a standard anticonvulsant. Royal Victoria Hooded rats were implanted with bipolar stimulating /recording electrodes aimed at the following limbic and cortical sites: amygdala, dorsal hippocampus,ventral hippocampus, septal area, motor cortex, and occipital cortex. Suppression of focal seizure activity was scored quantally. In agreement with previous work (Albright and Burnham,1980) a differential drug effect between the cortical and limbic sites was observed. Both occipital and motor cortical sites were highly responsive to phenobarbital whereas all limbic sites were much less responsive. Within the limbic system, the amygdala focus is the most drug resistant, with the septal area and the dorsal and ventral hippocampus all showing a similar pattern of drug response. Within the cortex, the occipital cortex was slightly more responsive to phenobarbital than the motor cortex. These results confirm and extend the observation that various focal sites have a differential drug response. A better understanding of the mechanism of differential drug sensitivity between the limbic system and cortex may further our understanding of the drug resistance seen in complex partial epilepsy.

(This work was supported by the MRC of Canada Grant # MT-5611. J.M. was supported by an MRC studentship.)

57.2 **ANTICONVULSANT SPECIFICITY AND ABILITY TO BLOCK SODIUM CHANNELS ARE DETERMINED BY 5-SUBSTITUTION OF HYDANTOINS AND α-SUBSTITUTION OF SUCCINIMIDES.** **S.K. Doster*, L.C. McKinney and J.A. Ferrendelli. Dept. of Pharmacology, Washington Univ. Sch. of Med., St. Louis, MO 63110.**

Many anticonvulsants which protect selectively against generalized tonic-clonic convulsions or petit mal absence seizures show the same specificity in protecting selectively against maximal electroshock seizures (MES) or against pentylenetetrazol (PTZ) induced seizures, respectively. It has been proposed that 5,5-diphenylhydantoin (phenytoin), which protects selectively against generalized tonic-clonic convulsions and MES, may act by blocking sodium (Na) channels in excitable membranes. To determine which structural aspects of hydantoins and succinimides are responsible for clinical specificity and Na channel blocking ability, we tested a series of these compounds substituted at the 5 or α position. Anticonvulsant specificity was tested in mice by injecting the drugs intraperitoneally (ip), followed by either 100 mg/kg PTZ injected ip or 2 msec pulses of 70 v delivered for 2 sec through ear clips (MES). Effects on Na channels were assayed by measuring tetrodotoxin sensitive ^{22}Na influx into sartorius muscles from Rana pipiens which were incubated with ouabain, veratridine and scorpion (Leiurus quinquestriatus) toxin.

COMPOUND	MES ED^{50}*	PTZ ED^{50}	Na Flux K_I
5,5-Diphenylhydantoin	10 mg/kg	No effect	60 μM
α,α-Diphenylsuccinimide	35 mg/kg	No effect	13 μM
5,5-Ethylmethylhydantoin	No effect	900 mg/kg	No effect
α,α-Ethylmethylsuccinimide	No effect	130 mg/kg	No effect

*Effective dose in 50% of animals tested

The data demonstrate that both diphenylhydantoin and diphenylsuccinimide block MES, but are ineffective against PTZ seizures and both drugs block Na influx at therapeutic concentrations. In contrast, alkyl substituted succinimide and hydantoin prevent PTZ seizures but have no effect on either MES or Na influx. This indicates that the substituents at the 5 and α positions, and not the ring structure of hydantoins and succinimides, determine the anticonvulsant specificity. Furthermore, we suggest that inhibition of Na influx may be a mechanism of action responsible for preventing MES or generalized tonic-clonic convulsions. **Supported in part by USPHS grant NS-14834 and the Muscular Dystrophy Association of America.**

57.3 BACLOFEN BLOCKS KAINIC ACID-INDUCED EPILEPTIFORM ACTIVITY. M. Gruenthal, B. Ault*, D. R. Armstrong and J. V. Nadler. Dept. Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Intracerebroventricular kainic acid (KA) produces limbic seizures in rats similar to seizures observed in temporal lobe epilepsy. KA-induced seizures are thought to originate in the hippocampal area and to depend, in part, on recurrent excitatory connections among CA3 pyramidal cells. The antispastic drug baclofen selectively inhibits transmission at synapses made by axons of CA3 pyramidal cells in the hippocampal slice and also depresses pyramidal cell excitability. We hypothesized therefore that baclofen would attenuate KA-induced epileptiform activity.

KA (940 pmol in 2.5 μl of artificial CSF) was infused at a rate of 0.2 μl/min into the lateral cerebral ventricle through an indwelling cannula. EEG activity was recorded during and after the infusion from electrodes placed bilaterally in the dorsal hippocampus and basolateral amygdala. Limbic seizures developed within 20 min after the end of the infusion. About 1 hr later seizures in the ipsilateral hippocampus became continuous, and this state persisted for several hours. A single dose of baclofen (5 mg/kg, i.p.) administered 1 hr after KA completely abolished seizure activity within 30 min. Preliminary findings suggest that baclofen also attenuated the cytopathology observed after administration of KA alone. Similar effects of baclofen were observed when the drug was given to KA-treated rats anesthetized with pentobarbital.

In studies on the rat hippocampal slice, superfusion with 50 nM KA induced bursts of population spikes in area CA3 at a frequency of 10-45/min. Baclofen abolished this burst firing at concentrations of 3 μM or less. IC_{50} values of 0.16, 0.7 and 0.9 μM were determined in 3 experiments. The potency of baclofen appeared to be inversely related to the rate of bursting, such that the drug was most potent against low frequency bursts.

These results suggest that baclofen may be useful in the treatment of limbic seizures with hippocampal foci. (Supported by NIH grant NS 17771.)

57.4 FREQUENCY-DEPENDENT SUPPRESSION OF NEUROMUSCULAR TRANSMISSION BY PHENYTOIN. Y. Yaari*, G. David*, E. Adler* and M. E. Selzer. Department of Physiology, Hebrew University, Hadassa Medical School, Jerusalem, Israel

Phenytoin (PT), the most widely used epileptic drug, exerts several depressant effects on neuronal function. However, the question remains as to how it prevents seizure activity without producing a general CNS depression. Since cortical neurons involved in epileptic discharges characteristically fire at high frequencies during normal activity, it is possible that PT's specific action is due to a frequency-dependent depression of neuronal and synaptic activity. We tested this hypothesis at the frog neuromuscular junction using conventional electrophysiological techniques.

Under conditions of low quantal content, tetanic nerve stimulation produces a gradual increase in EPP amplitude (tetanic potentiation; TP). PT (0.1 to 0.2 mM) markedly reduced TP without significantly altering EPP amplitude at lower rates of stimulation. The magnitude of this reduction was higher at higher stimulus frequencies. PT, also produced a use-dependent block of the EPP which appeared faster at higher stimulus frequencies.

Both actions of PT were accompanied by a use-dependent increase in EPP latency, suggesting a cummulative depressant action on the nerve action potential. Consistent with the latter finding, PT also produced a use- and frequency-dependent depression of the compound nerve action potential.

These findings suggest that the specific anti-epileptic actions of PT may be related to its preferential attenuation and/or block of the presynaptic action potential at high rates of activation.

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57.5 GLUTATHIONE AND LIPID PEROXIDATION IN SEIZURES CAUSED BY KAINIC ACID AND FOLINIC ACID P.A.Bradshaw, Z.H.Zhang* and S.R.Snodgrass. Neurology Res., Childrens Hospital of Los Angeles, Los Angeles, CA 90054

Glutathione(GSH) is an important protector against oxidant injury. To determine the role of GSH and lipid peroxidation (LP) in the seizures caused by kainic acid (KA) and folinic acid (FA), we depleted GSH levels with diethyl maleate (DEM) and observed the effect on seizures and chemical changes induced by KA or FA. We treated the following groups of rats: control, DEM, KA or FA, DEM+KA or DEM+FA, with normal saline or DEM 1.5 ml/kg intraperitoneally, and 15 min later injected stereotaxically into the right hippocampus 1.0 ul of phosphate buffer (pH7.4) or buffer containing either 15ug KA or 32ug FA. We observed the rats for seizures for 2 hrs before sacrifice. No seizures or other excitatory behavior were observed in control and DEM treated rats. Limbic and running seizures were observed in 25% of the KA rats, with a latency of 42±16 min; in 88% of the DEM+KA rats with a latency of 24±15 min; in 38% of the FA rats, with a latency of 35±14 min; and in 100% of DEM+FA rats, with a latency of 17±7 min. DEM significantly decreased GSH content in both cortices, both hippocampi and cerebellum, while neither KA nor FA affected GSH content. When DEM and KA (or FA) were given together, the content of GSH did not decrease further than that caused by DEM alone, although seizures were more severe and their latency decreased. Similar results were found in all regions examined for both the KA and FA study. When the extent of LP was measured with the thiobarbituric acid assay, we found that either DEM or FA significantly increased LP in all regions examined; while KA alone did not affect LP. When DEM and KA (or FA) were administered together, LP did not increase further beyond that caused by DEM alone. In other rats, some pretreated with DEM, we injected 0.01-0.6 mg sodium ascorbate (Asc), an agent known to produce LP, into the hippocampus and found increased lipid peroxidation in the injected hippocampus. Three features were noteworthy: low dose Asc was more effective in causing LP than high dose; effects of Asc and DEM were additive, and Asc did not cause seizures even though it produced as much LP as did FA. Two vitamins (FA and Asc) produced LP; only FA caused seizures which were exacerbated by DEM. These results suggest that GSH depletion enhances seizure activity but not via LP mechanism.

57.6 SEIZURE-INDUCED HIPPOCAMPAL DAMAGE IN RATS CAN BE REPRODUCED BY CENTRAL INJECTION OF GLUTAMATE OR ASPARTATE BUT NOT ACETYLCHOLINE OR GABA. R.S. Sloviter and D.W. Dempster* Neurology and Regional Bone Centers, Helen Hayes Hospital, West Haverstraw, NY 10993 and College of Physicians and Surgeons, Columbia University, New York, NY 10032

Hippocampal seizure activity, whether caused by convulsant drugs or electrical stimulation, is associated with a characteristic pattern of acute glial and dendritic swelling and damage to cell bodies. Olney's excitotoxic hypothesis(Exp. Br. Res.14:61,1971) suggests that seizure-related damage is caused by the release of excitatory transmitter(s) in amounts that lead to dendritic swelling and cell death. If so, it should be possible to reproduce acute seizure-related damage by central injection of putative hippocampal transmitters in doses capable of overcoming the processes of distribution, metabolism and neuronal and glial uptake. We have therefore compared the morphological effects of perforant path stimulation (Sloviter, Br. Res. Bull. 10: 675, 1983) with those caused by intracerebroventricular (icv) injection of glutamate, aspartate, Ach, GABA, NaCl or artificial CSF. Male Sprague-Dawley rats were anesthetized with chloral hydrate and implanted with icv cannulas immediately adjacent to the hippocampus. 3 days later, compounds (3umol) were injected in 5ul CSF every 5 min for 1 hr. Rats were perfused immediately for subsequent Rapid Golgi staining and routine light and electron microscopy.

Glutamate and aspartate produced acute glial and dendritic swelling as well as damage to cell bodies throughout stratum oriens and in the molecular layer of area dentata and these effects were virtually identical to those caused by perforant path stimulation, ie, swollen dendrites with normal presynaptic terminals and shrunken, darkly staining somata surrounded by glial swellings. GABA caused similar glial swelling but no dendritic swelling or cell body damage. Ach did not cause these local changes but did cause "distant" dendritic swellings where the perforant path terminates on the distal apical dendrites of pyramidal cells. These same swellings were also produced by perforant path stimulation. None of the above changes were seen after NaCl or CSF.

These results support the hypothesis that human epileptic brain damage and damage caused by kainic acid and other convulsants or electrical stimulation of the perforant path are the result of excessive activation of excitatory amino acid receptors.

This investigation was supported by a research grant from the Epilepsy Foundation of America.

57.7 NIFEDIPINE AND NIMODIPINE ANTAGONIZE SEIZURES AND INCREASED ATPASE ACTIVITY CAUSED BY 4-AMINOPYRIDINE Z.H.Zhang*, P.A.Bradshaw and S.R.Snodgrass. Neurology Res., Childrens Hospital of Los Angeles, Los Angeles, CA 90054

4-Aminopyridine(4AP) produces seizures by facilitating the calcium influx through the voltage-operated channel of the pre-synaptic membrane, inducing neurotransmitter release and enhancing neuronal firing (Rogawski,M.A. and Barker, J.L., Brain Res 280:180, 1983). Calcium channel blockers may reduce 4AP produced seizures by an effect on the metabolism of calcium. To determine the effect of nifedipine (NF), a calcium channel blocker used clinically, on the behavioral and chemical changes caused by 4AP, 3 groups of rats were injected stereotaxically in the right hippocampus with 1 µl of buffered saline with 8% ethanol containing 1mM NF, 20mM 4AP, or 20mM 4AP+1mM NF respectively. The rats were observed for 2 hours before sacrifice. NF alone caused no seizures or other obvious behavioral changes. 4AP induced generalized and running seizures in 75% of the treated rats, with a seizure latency of 68±10 min and duration of 40±22 min. 4AP+NF administered together caused only mild limbic seizures in 50% of the rats with a reduced latency of 15±4 min and a duration of less than 7 min. The activity of Na,K-ATPase in the right hippocampus of 4AP treated rats (0.96±0.04 µmole/mg prot/min) was higher than that of NF (0.83±0.03, $p<0.01$), or 4AP+NF (0.79±0.03, $p<0.01$). Similar results were found in the right and left cortex, which were not injected, while Na,K-ATPase did not change in cerebellum or left hippocampus. Mg-ATPase activity changed only in the right (injected) hippocampus. It was higher after 4AP treatment (0.18±0.01) than after NF (0.12±0.02, $p<0.05$) or 4AP+NF (0.14±0.01, $p<0.01$). ATPase activity in the brain regions for NF and 4AP+NF treated groups did not differ from enzyme activity in regions from rats given buffered saline only. In addition, no change in lipid peroxidation occurred as measured by the thiobarbituric acid assay. Additional studies employed the calcium channel blockers nimodipine (NM) and flunarizine (FL) each 40mg/Kg intraeritoneally prior to stereotaxic 4AP injection. No limbic or running seizures were seen in 4AP+NM treated rats. The activity of Na,K-ATPase in the right hippocampus of 4AP treated rats (0.97±0.03) was higher than that of NM (0.84±0.05, $p<0.05$), or 4AP+NM (0.86±0.07, $p<0.05$). The NM study also showed Na,K-ATPase results similar to NF in both cortices, cerebellum, and left hippocampus. FL had no effect on either seizures or ATPase activity.

57.8 CONVULSIVE ACTIVITY INDUCED BY AFFINITY PURIFIED ANTIBODY TO GM1 GANGLIOSIDE. F. Vilim*, S.P. Mahadik, M.M. Rapport & S.E. Karpiak (SPON: R. Gould). Div. of Neuroscience, NYS Psychiatric Inst. & the Depts. Psychiatry & Biochemistry, Coll. of Phys. & Surg., Columbia U., New York, N.Y. 10032.

An important goal in developing the immunological model of epilepsy is to induce motor convulsive activity. We have shown that antibodies to GM1 ganglioside (i.e. antiserum, Ig fractions, mono- & divalent fragments and affinity purified IgG & IgM) induce recurrent epileptiform activity after intracerebral injection but without convulsions [1-3]. We have now obtained convulsive activity using features of the kindling model.

Rats (250g Sprague/Dawley) were implanted with an array of cortical electrodes and a permanent cannula (used also as a recording electrode) unilaterally into the medial amygdala, and allowed to recover for 1 wk. Antibodies to GM1 ganglioside were purified by affinity column chromatography from both rabbit & goat antiganglioside sera. Rats (N=12) received 5 daily injections of 100µg of antibody protein in 10µl.This dose was selected since >95% of rats showed after-discharges within 3-10sec after a single amygdala injection. EEG was recorded in freemoving rats. Onset, duration & frequency of after-discharges were recorded. Convulsive activity was rated (Racine scale 1-5). On days 1-2 rats exhibited a "petit mal" reaction during after-discharges. After-discharge duration (median=20sec) and frequency (median=3) were maximal on Day 3 decreasing slightly by Day 5. After-discharges were followed by trains of epileptic spikes. On each successive day spike activity increased in frequency and amplitude. Maximal convulsive activity was seen on Day 3 (median rating=3). By Day 3 over 70% of all rats showed tonic-clonic motor seizures within 5 min after antibody injection (4 rats developed Stage 5 convulsive activity). Convulsions continued up to 30min after injection. In one experiment a 400µg intracortical injection caused repeated convulsive episodes (Stage 5) with continuous spiking for over 3 hr. Injections (100µg) of Ig fraction protein from either rabbit or goat preimmune sera produced no EEG alterations or convulsions in either untreated rats or in rats which had previously exhibited epileptic activity in response to antibody to GM1.

Supported in part by USPHS(NS-13762).

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57.9 50% CONVULSION DOSE OF LIDOCAINE ON FED AND FASTING MICE. S.K. Kim, Ball State University, Muncie, Indiana and K.C. Kim, M.D., Indiana University, Indianapolis, Indiana

It is well known that fasting can prevent epileptic seizures in patients.[1] In animal studies frequency of audiogenic seizures was progressively decreased as the length of fasting time was increased.[2] The purpose of this study is to determine the 50% convulsion dose of lidocaine(CD_{50}) on 24 hour-fasted mice.

White mice, CD-1 strain, were divided into two groups: fasting and fed. Fasting mice were given only water and no food for 24 hours. 10 mg/kg increments of 1% lidocaine were injected into the intraperitoneal space at the lower quadrant of the abdomen. Four difference doses of lidocaine were used for determining CD_{50}. Each group consisted of 10 mice. The four criteria used for determining convulsions were (1)loss of righting reflex,(2)clonic and tonic seizure, (3)episthotonus, and (4)curtailing of tail. Latency, duration of convulsion and recovery time were observed and data was analyzed by the method of Litchfield and Wilcox.

CD_{50} and 19/20 confidence limits of fed and fasting groups was 58 mg/kg, 53.2-63.2 mg/kg and 69 mg/kg, 62.1-76.6 mg/kg respectively. CD_{50} of fasting group was 20% greater than fed group and the difference was significant (P 0.05). Latency, duration of seizure and recovery time between fed and fasting groups were similar and the differences were not significant (P 0.05).

Seventy per cent of circulating lidocaine can be metabolized by liver and during fasting liver blood flow is significantly decreased.[3] However, fasting CD_{50} was 20% greater than fed group.

Ketone bodies in the blood related to brain energy metabolism appears to be responsible for any anticonvulsive effect.[4] This is not the result of water, electrolyte, acid-base or lipid fluctuations in the brain. During fasting ketone bodies in the blood are increased.

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57.10 ZINC ABSORPTION INCREASES ONLY DURING AMYGDALA KINDLING FOLLOWING DIETARY LOADING IN CATS. M.B.Sterman, M.N. Shouse* and M.D.Fairchild*. V.A.Med.Center, Sepulveda, CA and UCLA Sch.of Med. Los Angeles, CA 90024.

Zinc deprivation and toxicity both produce neurological symptoms in man. Studies in animals show that limbic structures contain relatively high levels of zinc. Moreover, zinc is a constitutent of many metaloenzymes, some of which are involved with neurotransmitter metabolism. Finally, direct injection of zinc into CSF produces seizure-like neural discharge.This literature suggested that nutritional manipulations of zinc could influence central nervous system excitability. In the first of a series of studies exploring this possibility we have examined the effects of increased dietary zinc on an experimental model of epilepsy in the cat.The model employed was amygdala kindling. Electrodes were placed in the basolateral amygdala and over sensorimotor cortex in ten adult cats. After recovery, venous blood samples were drawn at weekly intervals and analyzed for serum zinc, copper and iron concentrations. After one month of serum sampling with normal laboratory food and water (approximately 100 ppm zinc), both were supplemented with zinc gluconate to achieve a stable level of 700 ppm. Serum samples were again drawn at weekly intervals for one month. Amygdala kindling was then initiated in 5 cats. This procedure employs single, daily, incremented stimulation of the amygdala until focal afterdischarge (AD) threshold is obtained. Daily stimulation continues at AD threshold until a generalized convulsion is elicited. Serum samples were obtained throughout the kindling process and over a comparable interval in 5 unstimulated control cats. Results showed a differential serum level pattern for each mineral tested. Fe values were variable and unchanged across sampling. Cu values decreased significantly after zinc was increased in the diet. Zn values did not change until kindling was initiated, after which a marked and selective increase was observed. The decrease in serum Cu with dietary Zn increment could be explained by known interactions between these two minerals. The dramatic increase in serum Zn levels associated with amygdala kindling could result from unrelated metabolic dynamics but may reflect a neural response either to increased tissue excitability or to nonspecific stress. (Supported by the VA and a grant from the Wm. T. Thompson Co.)

57.11 THE INVOLVEMENT OF INHIBITORY AMINO ACIDS IN SEIZURES ELICITED FROM THE INFERIOR COLLICULUS. B. Givens*, T.J. McCown and G.R. Breese. Depts. of Pharmacology, Psychiatry and the Biological Sciences Research Center, UNC School of Medicine, Chapel Hill, NC 27514.

A low current, electrical stimulation applied to the dorsomedial aspect of the central nucleus of the inferior colliculus (IC) has recently been shown to induce a wild running seizure in the rat (McCown et al., Soc. Neurosci. Abstr., 1983). The seizure is characterized by frenzied running which continues 4-10 seconds after the termination of the electrical stimulation (120-200 μA, 30 Hz). It has also been shown that the wild running seizure coincides with afterdischarge in the IC, while no changes were noted in frontal cortex EEG activity. If the rats were stimulated chronically, the wild running episode was followed by myoclonic jerks or forelimb tonus, events marked by afterdischarge in the IC and frontal cortex. Thus, the seizure involves a well coordinated motor activity elicited from the brainstem, yet under certain conditions, the seizures can spread into the forebrain.

The purpose of the present study was to investigate the effects of amino acids on wild running seizures evoked from the IC in the rat. Initially, bipolar electrodes were implanted in the IC, and cannulae were placed either between the electrode tips or in the lateral ventricle. Five minutes after drugs were infused into the ventricle (5.0 μl) or into the electrode site (0.5 μl), the animals were tested. The threshold current was determined by stimulating with an initial current of 80 μA, followed by 20 μA increments every 10 seconds until wild running ensued. Immediately, the electrical stimulation was terminated, and post-stimulus running duration was measured.

Administration of the GABA agonist muscimol significantly increased the threshold current when injected either in the ventricle (100 or 300 ng) or in the IC (100 ng). Taurine (200 ng) and glycine (200 ng) increased the threshold current when administered at the electrode tips, but these increases did not reach significance. Ventricular administration of taurine (2.5 μg) or glycine (50 μg) did significantly increase threshold current. In contrast, ventricular administration of glutamate (100 μg) significantly increased wild running time, but did not alter current threshold. The results indicate an involvement of several inhibitory amino acids in the initiation of the wild running seizure and an excitatory amino acid, glutamate, in the maintenance of this seizure. (Supported by HD-03110)

57.12 AGE RELATED SUBSTANTIA NIGRA MEDIATED MODULATION OF SEIZURES: The Role of The Nigrostriatal Pathway. S.L. Moshé, L.L. Brown, B.J. Albala* and R. Okada* (Spon: R. Meibach) Dept. of Neurology, Albert Einstein College of Medicine, Bronx, New York 10461

Recent studies suggest that the increased susceptibility of the immature brain to the development of generalized seizures may be due to an immaturity or alteration of the functional activity of the substantia nigra, (SN) and its GABA sensitive efferent system (Moshe, Dev. Brain Res. 1984). In this report electrical stimulation of the SN was used with deoxyglucose (DG) autoradiography to explore the developmental differences of the nigral efferent systems.

Adult rats and 14 day old rat pups were implanted with electrodes in the left SN. After 1 week recuperative period for the adults and 2 days for the pups, the rats were stimulated with 60Hz constant current pulsed 20 seconds on followed by 20 seconds off. The current intensity was adjusted so that a motoric response consisting of the head turning towards the stimulated site and repetitive movements of the contralateral forelimb was obtained. DG was administered to the rats, IP, 5 min. before the beginning of the final stimulating session. After 45 minutes of stimulation, the rats were killed and autoradiograms were obtained using standard techniques.

Qualitative analysis of the autoradiograms revealed changes in optical densities in structures lying ipsilaterally to the stimulated SN. The structures in the nigrostriatal pathway (SN, globus pallidus, entopeduncular and subthalamic nuclei) exhibited the most pronounced increases in DG uptake in both age groups indicating that this pathway is functional or at least drivable in rat pups. However the ipsilateral midbrain reticular formation, ventrolateral thalamus, caudate and frontal cortex showed decreased optical densities only in the adults.

Since the SN effects on seizures differ with age, these data suggest that the SN modulatory influence can not be mediated by the nigrostriatal pathway.

57.13 VARIATION IN THRESHOLD AND PATTERN OF ELECTROSHOCK-INDUCED SEIZURES IN RATS DEPENDING ON SITE OF STIMULATION. R. A. Browning, and D. K. Nelson*. Southern Illinois Univ. Sch. of Med., Carbondale, IL 62901

Electroshock-induced seizures have been widely used as an experimental model of epilepsy and have been especially useful in the screening of antiepileptic drugs. Trans-corneal stimulation is more commonly employed as a technique for producing maximal electroshock seizures (MES), but trans-auricular stimulation is also used by many investigators. Although it is usually assumed that MES produced by ear-clip (EC) and corneal (C) electrodes are equivalent, this has not been systematically examined. In the present study we have compared the threshold and severity of electroshock seizures produced through C electrodes with those produced using EC electrodes. The threshold for the tonic seizure (i.e. forelimb extension) in male Sprague-Dawley rats (250-350 g) was found to be significantly lower using EC than using C electrodes. This was true using either a 60 Hz, 0.1 sec duration stimulus or a 60 Hz, 0.2 sec duration stimulus. Rats which responded to the standard MES stimulus (150 mA, 0.2 sec, 60 Hz AC) with full hindlimb extension (HLE) displayed a shorter latency and a longer duration of HLE when EC electrodes were used than when C electrodes were used. Moreover, the incidence of rats exhibiting HLE when subjected to the standard MES stimulus was found to be significantly higher with EC than with C electrodes. Thus tonic convulsions were more easily produced and more severe when the electroshock was delivered through EC than through C electrodes. On the other hand, the threshold for clonic seizures was slightly lower using C than EC stimulation. Moreover, the motor components of the clonus differed depending on whether it was induced by C or EC stimulation. The clonus produced by minimal electroshock stimulation (20-30 mA, 0.2 sec) through C electrodes was characterized by face and forelimb clonus similar to kindled convulsions, whereas the clonus resulting from EC stimulation was characterized by a running-bouncing clonus involving both forelimbs and hindlimbs similar to the clonus of minimal audiogenic seizures. On the basis of these findings we have hypothesized that tonic seizures are more easily triggered with trans-auricular stimulation because they originate in the brain stem, and because this brain region receives a higher density of stimulating current from EC than from C electrodes. Furthermore, it would appear that clonus can be triggered from either forebrain or brain stem and that the motor pattern observed depends on which neural substrates are involved.

57.14 ESTABLISHMENT OF STATUS EPILEPTICUS BY LIMBIC SYSTEM STIMULATION IN PREVIOUSLY UNSTIMULATED RATS N. W. Milgram, I. Green*, M. Liberman*. K. Riexinger* and T. L. Petit Life Science Division, Scarborough Campus, University of Toronto, Toronto, Ontario, Canada M1C 1A4.

Rats received 90 minutes of continuous sine wave stimulation at levels incrementally raised to 40μa through either hippocampal or amygdala electrodes. Following the offset of stimulation, 4 exhibited a syndrome of convulsive status epilepticus which was characterized by recurrent behavioral seizures, continuous EEG spiking, and which produced marked neuropathological changes. In 3 cases, the poststimulatory response was categorized by continuous seizure activity recorded from the EEG in the absence of tonic-clonic movements. The effect correlated with the response of the animal during the stimulation onset and was independent of electrode placement. Animals which exhibited repetitive convulsive seizures during the stimulation were most likely to continue convulsing following the offset of stimulation. The results indicate that persistent limbic system activation can produce a syndrome of recurrent seizures similar to that caused by either neurotoxic drugs or limbic system activation in kindled rats.

57.15 EFFECT OF OLFACTORY BULB KINDLING ON AVERAGED EVOKED POTENTIALS RECORDED FROM THE PYRIFORM CORTEX. R. D. Russell and J. S. Stripling. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Electrical stimulation of the lateral olfactory tract (LOT) elicits a field potential in the pyriform cortex (PC) consisting of an early surface negative wave (reflecting in part a monosynaptic EPSP produced at the synapses of the LOT terminals) and a later surface positive wave. Racine, Milgram, and Hafner (Brain Res. 260: 217-231, 1983) have reported that kindling the LOT produces a new late component in the pyriform cortex evoked potential. The present study examined the effect of olfactory bulb (OB) kindling on the PC averaged evoked potential (AEP).

Nine male Long-Evans rats were implanted with a stimulation electrode in the OB and a recording electrode in the PC. AEPs were computed from 10 PC evoked potentials produced by electrical stimulation of the OB (0.2 msec pulses at a rate of 1 pulse/sec). Two seizure afterdischarges (ADs) were elicited in all rats (one AD with threshold current and one AD with suprathreshold current) to determine the effect of localized ADs on the pyriform cortex AEP. Five of the rats were then kindled via daily stimulation of the OB (100 pulses at 50 pulses/sec). Four rats served as controls and received daily stimulation at a lower frequency (1 pulse/sec) which did not elicit ADs.

The localized AD produced increases in the amplitude of the initial surface negative wave (N1), a prolongation of the surface positive wave (P), and an increase in the amplitude of a late (approximately 28 msec latency) surface negative wave (N2). The effects on P and N2 were very consistent (e.g., P was lengthened and N2 was increased significantly for all rats at 5 and 15 min post-AD). These effects on the AEP dissipated by 24 hr post-AD (e.g., only 1 of 9 rats showed a statistically significant increase of N1 and only 2 rats showed a prolongation of P). Kindling extended the time course of the prolongation of P and increase in N2 (e.g., kindling produced a significant increase of N2 in 4 of 5 rats at 24 hr after reaching kindling criterion and in 3 of 5 rats at 72 hr). These results suggest that a single AD produces a short-lasting (less than 24 hr) increase in the efficacy of monosynaptic (N1 component) and polysynaptic (P and N2 components) transmission. The most striking effect of OB kindling found in this experiment was a long-lasting (longer than 72 hr) increase in amplitude of the N2 component, which appears to be polysynaptic in origin.

57.16 THE EFFECT OF SPINAL HEMISECTIONS ON GENERALIZED CONVULSIONS IN RATS. W.M. Burnham, E. Wee* and P. Hwang*. Department of Pharmacology, University of Toronto, Toronto, Ontario (W.M.B.) and Department of Neurology, Hospital for Sick Children, Toronto, Ontario (E.W., P.H.).

We have previously reported that spinal hemisections, placed at the cervical level in cortex-kindled rats, fail to suppress convulsive behaviour in the body. Apparently, epileptic discharge crosses the midline and activates both sides of the cord below the level of the cut. Since hemisections interrupt the direct descending pathways from the brain, these data suggest that a bilaterally distributed system within the cord itself plays an active role in supporting and spreading epileptic activity.

The present experiments were designed to extend these findings to two new seizure models - the amygdala-kindling model and the electroshock (ECS) model. Two groups of Royal Victoria hooded rats were prepared for testing. In one group, chronic electrodes were implanted in the right amygdala, and the animals were subjected to daily kindling stimulation (1 s, 60 Hz, 400 uA peak to peak) until they had exhibited six stage 5 convulsions. In the other group, the rats were simply pre-tested with the ECS stimulus (.2 s, 60 Hz, 150 mA via corneal electrodes) so that the configuration of their pre-surgical seizures could be recorded.

Subsequently both groups were anesthetized and complete hemisections were made in the right side of the cord at the C-3 level. Following a week's (minimum) recovery, the kindling or the ECS stimulus was re-applied. In both the kindled and the ECS subjects, vigorous clonus was seen bilaterally below the level of hemisection. In the ECS subjects, bilateral tonus was seen as well, although it was often somewhat weaker on the side of the cut.

These results confirm previous findings in the cortex-kindling model, and indicate that similar phenomena can be observed in the ECS preparation. It seems possible that bilaterally distributed systems within the cord itself play an important role in both types of convulsions.

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57.17 CORTICAL ACTIVITY DURING INTERICTAL AFTERDISCHARGE OF FOCAL PENICILLIN EPILEPSY. Birgit Albowitz* and Edgar L. Gasteiger. Department and Section of Physiology, N.Y.S. College of Vet. Med. and Div. of Biol., Cornell U., Ithaca, NY 14853

In a previous study of focal penicillin epilepsy in the precruciate cortex we characterized interictal afterdischarge (AD) as a 18-22/sec oscillation consisting of up to five cycles and following each interictal spike at a latency of 200-400 ms. The presence of AD was dependent on the ventrolateral thalamic nucleus (VL) and was correlated with bursting unit activity in this nucleus. Although this AD is clearly triggered via the thalamus it appears to be maintained by the cortex. Therefore, we investigated unit and field potential activity at a cortical level. Penicillin foci were initiated in the precruciate cortex of cats anesthetized with urethane. Recordings were made simultaneously from VL-Cortex and from intracortical sites with an extracellular microelectrode.

The AD invariably reversed its polarity with depth while the spike was less predictable in this regard. If reversal of the spike was present, its zero potential level was usually 100-300 μ above the reversal point for AD. Below the zero potential level the amplitude of AD increased as much as six fold. Except for polarity and amplitude, intracortical AD showed notable consistency with respect to its surface recording. In contrast, the spike frequently showed phase shifts and variability of waveform.

Extracellular recordings of cortical cells never revealed burst firing during AD as seen for thalamic cells. Cortical activity during AD was rare and if present it always occurred as a single spike. In view of these findings we consider AD, in contrast to the penicillin spike, to be a non-propagated graded potential.

57.18 SYNCHRONIZATION OF "NORMAL" AND EPILEPTIC HUMAN LIMBIC NEURONS. M. Isokawa-Akesson, T.L.Babb and C.L.Wilson. Brain Research Institute and Dept. of Neurology, School of Med., UCLA, Los Angeles, CA 90024.

Structured burst firing patterns have been reported as characteristic of some neurons in human epileptic cortex (Ward, 1969). Babb & Crandall (1976) however reported only one similar structured burst pattern in epileptic hippocampal neurons; and they reported that bursts were typical of non-epileptic hippocampal neurons. Based on these findings, we performed visual and quantitative analysis of burst firing by auto- and cross-correlation in 9 epileptic anterior pes hippocampi (Ammon's horn)(APH), 13 peri-epileptic amygdalae(AMYG), 7 non-epileptic (contralateral to focus) APH and 6 non-epileptic AMYG in 19 patients with unilateral hippocampal seizures. Special focus was on the temporal relationship (synchrony) of firing between neurons as a more likely mechanism for neuronal epileptogenesis.

Two patterns of burst activity were identified, i.e. short duration (<25 msec) and long lasting (100-300 msec) bursts. The former pattern was observed commonly in the non-epileptic neurons and the latter one was seen in the epileptic APH (100-300 msec) and AMYG (80-150 msec) neurons. In the non-epileptic APH, the neurons were found to fire rhythmically at the frequency of 220 c/sec, but infrequently synchronizing with other APH neurons at 1.26, 4.2 and 16.7 c/sec. In the non-epileptic AMYG neurons, rhythmicity in firings showed more variation than the APH neurons and those were 3.3, 62.5 and 166 c/sec. They also synchronized infrequently with other AMYG neurons at 4.25 c/sec. In summary, non-epileptic neurons have quite high firing rates individually but synchronize with other neurons with very low frequency of occurrence. By contrast, in the epileptic APH, neurons fired at 3.3 c/sec, showing synchronized activity with other APH neurons at a similar frequency of 3.34 c/sec. Furthermore, epileptic AMYG neurons showed rhythmical firings at 55.2 c/sec, synchronizing with other AMYG neurons at a similar frequency of 67 c/sec. Hence, although epileptic neurons fire at relatively low frequencies individually when compared with non-epileptic neurons, these "epileptic" firings are time-locked among neurons within a given structure. These firing characteristics of epileptic neurons may form a series of mass-synchrony of neuronal activity and produce a volley strong enough to evoke "epileptic" discharges that propagate into seizure activity. Supported by NIH Grant NS 02808.

57.19 NEUROTRANSMITTER RECEPTOR ALTERATIONS IN HUMAN EPILEPTIC TISSUE. R.A.E. Bakay* and A.B. Harris. Div. of Neurosurgery, Emory Univ.,Atlanta,GA 30322 and Dept. of Neurological Surgery, Univ. of Washington, Seattle, WA 98195.

Tissue samples were obtained from 18 patients who underwent cortical resection of an epileptic focus. Intraoperative electrocorticography (ECoG) was performed to determine the degree of electrical abnormality present in the tissue samples. From all but one of the patients two or more samples were obtained which differed significantly in the ECoG with at least one sample exhibiting a markedly abnormal electrical activity and another sample demonstrating minimal or no abnormalities.

Tissue samples were immediately assayed for Na^+-dependent GABA receptor binding using ^3H-GABA in 6 patients. The remaining tissues were frozen and stored at -70° C until assayed. The GABAergic receptor assay was performed with ^3H-Muscimol and muscarinic cholinergic receptor binding was obtained using ^3H-QNB. Liquid scintillation spectrometry was performed on duplicate or triplicate samples depending on amount of tissue available.

When the abnormal electrically active tissue was pair matched using the Wilcoxon test against the electrically more normal tissue a significant difference was obtained both for the Na^+-dependent ($p<.05$) and the Na^+-independent GABA receptors ($p< .01$). The degree of receptor loss for the Na^+-independent GABA receptor binding varied from 12% to 63%. Similar pair matching using the QNB binding also demonstrated a significant difference in the cholinergic receptors ($p< .01$). The degree of receptor loss varied from 8% to 35% and there were 3 samples in which the binding was greater in the tissue which was electrically abnormal. Thus while the GABAergic and cholinergic receptor losses appeared to parallel each other, the loss of GABAergic receptors appears to be more severe. This finding parallels studies obtained from chronic epileptic monkeys previously published (Bakay and Harris, Brain Research,1981, 206, 387). (Supported by NIH Grants NS 09678 and NS07144)

57.20 PERSISTENT FREEZE-LESION DAMAGE TO NEOCORTEX: A LIGHT AND ELECTRON MICROSCOPIC STUDY. D. A. Kristt, J. Lighthall and D.A. Prince. Depts. of Neuropath. and Neurology Stanford Univ. School of Med., Stanford, CA 94025.

Although freeze-lesions of cerebral cortex have been used for many years by workers in several disciplines, the progressive development of the pathological alterations in chronic lesions, both temporally and spatially, have not been well characterized. The purpose of this qualitative ultrastructural study was to provide that information. Dry ice/alcohol-cold probes (2 mm dia.) were briefly applied to the dura overlying motor cortex (area 4) of adult guinea pigs. Animals were perfused fixed at 1 day and 1, 2, 3, and 4 weeks post-lesion (wpl). At 1 day post-lesion, a zone ca. 1-2 mm wide was seen throughout the depth of cortex in which the neuropil was highly vacuolated and extensive neuronal necrosis and dropout were seen. However, at longer survival intervals, the area of the lesion consisted of a funnel-shaped zone with its base along the pia and its apex towards the cingulum. The aneuronal region appeared as a much narrower zone than in acute material, generally less than several hundred micra wide. Vacuolation of the neuropil in layer I persisted and extended for several mm from the center of the lesion. Ultrastructurally, the most dramatic changes at all survival intervals were in the molecular layer which showed a substantial increase in extracellular space and dilatation of many of the remaining processes. In comparison to non-lesioned animals, a dramatic loss of all but small sized dendritic branches was observed. The loss was seen laterally, even 1-2 mm from the lesion center. This finding was associated with a substantial reduction in synapses, extending for at least 1-2 mm from the lesion center, which was qualitatively evident in layers II and III. Also, despite the extensive damage, proliferation of astrocytic processes was minimal, although moderate glial (astro- & micro-) hyperplasia was noted at the lesion center. Evidence of perikaryal and neuropil degeneration was seen at the earliest period, but was also in evidence at 4 weeks. We conclude that rapid and substantial alteration in the neuronal, dendritic and connectivity organization of motor cortex occurs rapidly in response to a freeze lesion. Damage and degeneration are seen for weeks afterwards. Since a large proportion of pyramidal neurons normally have apical arbors terminating in layer I, the present findings suggest that a substantial number of these cells exhibit evidence of persistent injury to their apical dendrites. This pathological situation would be compatable with persistently altered physiology of this cortical region after freeze lesion. Support: NIH 2P50 NS 12151-09.

57.21 BEHAVIOURAL AND PHYSIOLOGICAL CONSEQUENCES OF AN EXPERIMENTAL EPILEPTIFORM SYNDROME. *
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A small dose, (4 mouse LD50), of tetanus toxin injected bilaterally into the rat hippocampus causes an epileptic syndrome characterised by overt spontaneous, myoclonic seizures lasting about three to four weeks before recovery (Mellanby et al. J.Neurol.Neurosurg.Psychiat. 40, 404-414 1977). This model resembles human temporal lobe epilepsy in that the animals become hyperactive and show learning and memory deficits (Mellanby et al., Exp. Neurol. 75, 690-699 1982; George and Mellanby, Exp.neurol. 75, 690-689, 1982).

We have reconsidered the learning deficit using a circular platform task (Barnes J.Comp.Physiol. Psychol. 93, 74-104 1979) which is thought to be more dependent on hippocampal function than the tasks used in the existing literature. The animals had to locate a safe dark box beneath one of eighteen holes evenly spaced round the circumference of a brightly lit circular platform. Once all the animals had learnt the location of the box, its position was rotated through 120° and the rate of relearning was investigated. The post-epileptic animals learnt both stages of the task more slowly than their peers. They took 12 trials to reach a criterion of an average of less than 2 errors while the control group took 7. On reversal the control group was back to criterion after 6 trials at which point the post epileptic animals were still making, on average, five errors.

Preliminary physiological studies suggest that commissural postsynaptic responses in CA3 were depressed in a subset of the post-epileptic rats. However long term potentiation, over periods of 6 hours, was similar in both groups of rats.

Supported by the Medical Research Council and the Thorn Fund.

AUDITORY SENSORY ORGANS

58.1 FREQUENCY CODING IN THE AUDITORY PERIPHERY OF THE OYSTER TOADFISH, OPSANUS TAU. Stephen M. Echteler. Neurobiol. Unit, Scripps Instit. of Oceanog. and Dept. Neurosci., U.C.S.D., La Jolla, CA. 92093.

Toadfish produce two types of sounds used in social interactions. The boatwhistle, a mating call produced only by males, is a long duration tone with a fundamental frequency which may vary from 150 to over 250 Hz. The grunt, produced by both sexes, is a short duration wide-band sound with a fundamental at 90-100 Hz emitted in aggressive displays or when animals are stressed. A recent study of auditory neurons in the saccular nerve of the toadfish reported that most units (83%) were maximally sensitive to frequencies of 25-40 Hz, suggesting an apparent mismatch between sound production and hearing (M.L. Fine, in Hearing and Sound Communication in Fishes, W.N. Tavolga, A.N. Popper, R.R. Fay, Eds. Springer-Verlag, New York, 1981, pp. 257-263.).

To test this conclusion, the frequency selectivity of the toadfish auditory periphery was assessed through microphonic recordings obtained from the sacculus and single unit recordings from the saccular nerve. Auditory stimuli consisted of airborne tonebursts which ranged in frequency from 30-500 Hz. Sound pressure and particle velocity were monitored with hydrophones for all acoustic stimuli. Animals were completely submerged and lightly curarized, but alert, during all recording sessions.

In contrast to the previous report, frequency threshold curves constructed from saccular microphonic responses revealed peaks of sensitivity at 90-120 Hz, within the range of the grunt, and at 250 Hz, a fundamental frequency of the boatwhistle. These peaks were especially prominent when thresholds were measured to particle velocity rather than sound pressure. Interspike interval histograms compiled from saccular nerve units, in the absence of imposed stimuli, revealed four classes of activity patterns: nonspontaneous, regular, irregular and bursting. Best tested frequencies of these units ranged from 40-300 Hz. Many tuning curves were bimodal with one peak of sensitivity at 90-100 Hz and another either at 40-70 Hz or at 150-300 Hz. Occasionally trimodal tuning curves were observed with peaks within each of these frequency ranges. Unit thresholds varied from -8 dB to + 20 dB (re 1 μbar for sound pressure; re 1 μvar for particle velocity). (Supported by a Grass Fellowship to S.M. Echteler and NIH and NSF grants to T.H. Bullock.)

58.2 COMPARISON OF FILTER FUNCTIONS OBTAINED WITH NARROWBAND AND BROADBAND STIMULI. R. Dunia* and P.M. Narins. (SPON: C.D. Clemente). Interdepartmental Neuroscience Program and Dept. of Biology, UCLA, Los Angeles, CA 90024.

Intracellular recordings of individual anuran (Rana pipiens) auditory nerve fibers indicate that the filter functions obtained in response to tones embedded in broadband noise do not differ significantly from traditional frequency threshold curves (FTCs). Using a ventral approach, we recorded from adult immobilized frogs (20-25 g) using glass microelectrodes with tip impedances of 10-50 megohms. After determining the tone-derived FTC, we presented the frog with a broadband stimulus consisting of the variable intensity characteristic frequency tone burst mixed with continuous, fixed-intensity cosinusoidally modulated noise (comb-filtered noise, CFN). The tone level was adjusted until it produced a just distinguishable neural response in the noise. We then inverted the noise spectrum, and redetermined the tone threshold, obtaining a threshold difference. By changing the modulation frequency of the CFN, we obtained a series of threshold differences, from which we calculated a filter function based on the Pick paradigm (J. Acoust. Soc. Am. 68: 1087-1095, 1980).

The resulting filter functions show no systematic signs of narrowing, supporting the notion that two-tone suppression plays a small role in sharpening the FTC. Thus, the system appears to act as a linear filter. However, the shape of the filter is dependent on the noise level. There appears to be a noise level which produces the sharpest filter function; increasing or decreasing the noise level from this optimum broadens the function. The broadening with increasing noise level is consistent with Moller's findings (Acta Physiol. Scand. 104: 24-32, 1978) in which he compared FTCs with filter functions obtained by cross-correlating the synchronous activity evoked by white noise. In low noise levels the threshold difference spectrum loses its characteristic peaks and valleys. We therefore suggest that the broadening with decreasing noise level is due to the inability of the ear to detect the changes occurring in the noise. Work supported by DRF grant No. AC831215 and NIH grant No. NS19725-01 to PMN.

58.3 FACTORS AFFECTING DETECTION OF AMPLITUDE FLUCTUATIONS BY THE GOLDFISH AUDITORY SYSTEM. S. Coombs and R. Fay. Parmly Hearing Institute, Loyola University of Chicago, Chicago, Il, 60626

Measurements of respiration suppression were used to determine the ability of goldfish to detect the occurence of intensity fluctuations in a variety of signals. Preliminary measurements of the goldfish's ability to detect sinusoidal amplitude modulations (AM) impressed on a 570 Hz bursting signal revealed that the just-detectable depth of modulation decreased from 0.25 to 0.012 dB as modulation frequencies were increased from 10 to 200 Hz. The hi-pass characteristic of this function is qualitatively similar to that obtained from the goldfish by Fay (J. Neurophys.,44:312,1980) using a continuous 800 Hz signal, but quantitatively different in that overall sensitivity is from 10 to 30 dB less and that growth of sensitivity occurs more rapidly (between 5-10 dB/octave compared to 3 dB/octave). To account for these differences, we examined the effects of signal frequency, burst duration, and overall signal level. Randomly varying overall intensity for the bursting case resulted in a decrease in AM sensitivity that was clearly dependent upon the degree of variation. Since the temporal pattern of the modulation envelope is independent of overall level, these results suggest that the nervous system integrates spike rate over time and compares total spike counts prior to and during an AM trial, rather than comparing temporal patterns of spike activity.

Doubling the burst duration and varying the signal frequency resulted in small frequency-specific and temporal summation effects, but these were not sufficient to account for the large difference between modulation thresholds obtained in the continuous and bursting case. Since the adaptation state of the nervous system also differed for the two conditions, a third possibility is that AM sensitivity is dependent on the level of adaptation. The response of eighth nerve units to similar stimulus conditions will be presented and discussed relative to these findings.

58.4 EFFERENT INHIBITION OF PRIMARY AUDITORY AFFERENTS EVOKED BY MAUTHNER CELL IMPULSES. J.-W. Lin, and D.S. Faber, Div.of Neurobiology, Dept. of Physiology, SUNY/Buffalo, Buffalo, NY 14214 .

The sound-evoked tail flip mediated by the goldfish Mauthner (M-)cell in turn stimulates stato-acoustic sense organs. It has been shown that M-cell activation depresses saccular nerve auditory responses, and we report here that this inhibition is associated with an all-or-none hyperpolarization of saccular fibers. Simultaneous intracellular recordings were obtained from individual fibers at their point of entry to the medulla and from the M-cell axon, soma or lateral dendrite, using KCl-filled electrodes. The M-cell was activated antidromically by spinal stimulation or directly with intra-axonal current injections; the consequent hyperpolarization of saccular fibers had an amplitude in the range of 0.2-4.0 mV, a mean latency of 6.23 msec (SD=0.62 msec,n=12),and a half-decay time of 6-12 msec. Its inhibitory effect was demonstrated as a delay or block of spike initiation by direct stimulation of the saccular fibers.

Possible origins of this afferent hyperpolarization include: 1) the collateral IPSP in the M-cell, transmitted electrotonically to the axon terminals; 2) presynaptic inhibition of the axon terminals; 3) efferent inhibitory input to the afferent dendrites; or 4) reduction of a tonic hair cell input. The first possibility is unlikely because the inhibition is recorded in afferents not coupled to the M-cell and has a longer latency than the collateral IPSP. Since sound-evoked EPSPs were greatly attenuated when paired with the hyperpolarization, but coupling potentials arising in the M-cell were not affected, this potential is most likely produced by a dendritic conductance increase. Although efferent inhibition of the hair cells themselves cannot be ruled out totally, the hyperpolarization was still observed after surgical removal of the sensory epithelium.

Some of the saccular fibers exhibiting this hyperpolarization give rise to club endings on the M-cell's lateral dendrite while others do not and presumably project to second order auditory neurons. The M-cell collateral network, which is known to reduce this neuron's sensitivity to afferent input through recurrent inhibition, therefore also acts peripherally to depress that input both to the M-cell and to other neurons whose impulse activity might otherwise counteract or modify the startle response.
Supported in part by NIH Grant No. NS15335.

58.5 TWO POPULATIONS OF HAIR CELLS IN THE TURTLE AUDITORY RECEPTOR. M. Sneary* (SPON E. Glazer). Dept. of Anatomy, University of California, San Francisco, CA 94143.

The turtle auditory receptor contains two distinct populations of sensory cells. Approximately 60% of the total 900 cells are in that portion of the basilar papilla which sits atop the basilar membrane (Miller, 1978). The remaining 40% of the hair cells rest on the limbus at either end of the papilla. Each of these regions can be distinguished by differences in cell structure, density, innervation and extracellular elements.

Hair cells on the membrane are covered by a massive tectorial membrane into which short stereociliary groups project. These cells are functionally organized in a tonotopic fashion (Crawford and Fettiplace, 1980). The density of hair cells increases as much as two-fold from the high frequency (saccular) to the low frequency (lagenar) end. As a result, the larger population of cells in the low frequency region may be functionally coupled in their response. Afferent nerve fibers which innervate cells on the membrane show no evidence of branching and do not appear to contact more than one cell. Furthermore, most, if not all, cells synapse with efferent fibers. Efferent endings are also seen on afferent fibers as they leave the receptor. As a result, innervation in this region appears to be discrete and displays a prominent central control over peripheral input.

Limbic cells show different characteristics. These cells are smaller, more densely packed (sometimes contiguous) and have longer stereociliary groups. They are covered by an irregular network of filaments. In addition, while cells on the membrane have a strict unidirectional orientation, these cells are variably oriented. Nerve fibers in limbic areas branch among the hair cells. Individual afferent fibers may synapse with more than one cell. This indicates a convergence of sensory information. Finally, the relative absence of efferent endings on these cells indicates a lack of central control over peripheral input.
Supported in part by USPHS Grant No. 2 RO 1 NS 11836.

58.6 DIRECTIONAL PROPERTIES OF THE EAR OF THE BARN OWL, TYTO ALBA Roger B. Coles and Anna Guppy*. Acoustic Lab., Dept. of Behavioural Biology, Research School of Biological Sciences, Australian National University, Canberra ACT, Australia 2601

The acoustic properties of the external ear of the barn owl were investigated by measuring the sound pressure transformation in and at the entrance to each ear canal. Significant acoustic gain in the ear canal occurs above 1kHz and amplification of 20dB is seen for frequencies between 3-10kHz. Above 10kHz acoustic gain decreases sharply. Removal of the feathers of the facial ruff causes a considerable loss of sound pressure in the ear canal, up to 15dB in the region 5-7kHz. Ear canal gain measurements without the facial ruff reveal resonance due to sound transmission through the owls large interaural canal. With the ruff intact, sound pressure measurements at the entrance to the ear canal yield a gain curve which closely resembles that of a finite conical horn equivalent to the physical dimensions of the ruff. The directional properties of the ear canal show an acoustic axis for frequencies above 3kHz. Contour plots indicate that the directivity patterns are elongated in the horizontal plane. Above 5.5kHz the major lobe containing the acoustic axis is surrounded by crescent-shaped troughs of low pressure (nulls). In elevation, the acoustic axes of each ear are displaced above (right) and below (left) the horizon by ±14°, due to the asymmetry of the ear canal openings. There is a systematic shift in the position of the acoustic axis towards the midline as frequency increases. The ear canal directionality is explained by sound diffraction at the open face of the ruff.

The directional sensitivity of the cochlear microphonic (CM) was compared to that of the ear canal and substantial differences exist above 3kHz. Up to 10kHz, the CM has considerably enhanced directionality compared to the ear canal. This is due to the major lobe of the CM directivity pattern forming a narrow diagonal band of high sensitivity closely bordered by null regions. The CM directivity patterns systematically shift their positions with frequency, but unlike the acoustic axis, the CM axis crosses the midline at 7kHz (both ears) and is located about 20° contralateral at 9kHz. Furthermore, CM directionality is abruptly lost above 10kHz.

The contrast between ear canal and CM directionality provides direct evidence that the ear of the barn owl does not function purely as a pressure receiver. It is suggested that pressure-gradients are operating due to effective transmission of sound through the owls interaural cavity.

58.7 NEURON-SPECIFIC ENOLASE-LIKE IMMUNOREACTIVITY IS IN INNER BUT NOT OUTER HAIR CELLS IN THE GUINEA PIG COCHLEA. R. A. Altschuler, K. A. Reeks*, P. J. Marangos and J. Fex. Laboratory of Neuro-otolaryngology, NINCDS, National Institutes of Health and National Institute of Mental Health, Bethesda, MD 20205.

Neuron-specific enolase (NSE) is the neuronal isoenzyme of the glycolytic enzyme enolase. NSE has been localized only in neurons and cells with characteristics of neurons, such as paraneurons or neuroendocrine cells. We examined the immunocytochemical localization of NSE in the guinea pig cochlea to determine if hair cells, which have many neuronal characteristics, would show NSE-like immunoreactive labeling.

Guinea pigs were perfused with 4% paraformaldehyde in 0.1M sodium cacodylate buffer, cochleae were fixed locally and then rinsed in phosphate buffered saline. Next, bony shells were removed; several cochleae were cryostat sectioned and indirect immunofluorescence techniques followed while with other cochleae immunoperoxidase techniques followed on the whole cochlear spiral. In either case the primary incubation was in a commercially available (Polysciences) polyclonal rabbit antiserum to NSE, diluted 1:1000 - 1:4000 in phosphate buffered saline with 0.3% triton X-100.

NSE-like immunoreactivity was seen in inner hair cells but not outer hair cells. NSE-like immunoreactivity was also seen in efferent fibers and terminals and in both type I and type II spiral ganglion cells. The finding of NSE-like immunoreactivity in inner but not outer hair cells adds to the number of differences found between them and may be related to differences in function and action.

58.8 OTOTOXICITY PRODUCED BY CHLORAMPHENICOL-PALMITATE AND SHORT DURATION, HIGH INTENSITY BROAD-BAND NOISE: A SCANNING EM STUDY. J.E Penny, C.M. Henley*, R.D. Brown, K.A. Purdy*. Depts. of Pharmacology and Anatomy, LSU Med. Sch., Shreveport, LA 71130.

Previous studies of chloramphenicol (CAP) and noise have revealed ototoxic interactions of either a permanent or temporary nature. Results have been dependent on the presence or absence of otitis media (OM) or the short duration of noise exposure (90 sec/d once per wk for 3 wks; total 4.5 min.) This study was to determine whether an ototoxic interaction exists between CAP and noise in the absence of OM using a longer noise duration. Fifty adult female rats were grouped: Group (G)-C (n=10), no treatment; G-1 (n=20), broad band noise (124 dB SPL peak intensity) 90 sec/d for 5 days - total 7.5 min; G-2 (n=10), noise (G-1 protocol) then CAP 100 mg/kg/d for 10 days beginning on the last d of noise; G-3 (n=10), noise (G-1 protocol) plus CAP 100 mg/kg/d for 10 days beginning on the 1st day of noise exposure. Chloramphenicol alone has not caused any cochlear effects in our studies; therefore a CAP only group was not included. Immediately following recordings of cochlear potentials on day 26 (21 days post noise exposure) all animals were perfused with 3% buffered glutaraldehyde. The temporal bones were removed; the cochleas dissected and prepared for SEM. G-2 exhibited the most cochlear damage followed by G-3, then G-1. This trend paralleled electrophysiological findings. Stereocilia damage of the inner hair cells (IHC's) was more prevalent than that of the outer hair cells (OHC 's) in G-1, 2, and 3. G-2 IHC stereocilia were in disarray, were swollen, broken, and/or completely missing primarily in the middle and basal turns. Sporadic OHC loss or stereocilia clumping occurred in conjunction with an erosion of the phalangeal surface of the Deiters' cells. Similar changes occurred in G-3 and G-1 but to a much lesser degree. That more extensive damage occurred in G-2 (NOISE then CAP) than in G-3 (NOISE plus CAP CONCURRENTLY) emphasizes that time of CAP dosing relative to noise exposure is significant in this noise - drug interaction. In addition, erosion of the phalangeal surface of the Deiters' cells may be most important relative to diminished cochlear function.

58.9 OTOTOXICITY PRODUCED BY CHLORAMPHENICOL-PALMITATE AND SHORT DURATION, HIGH INTENSITY BROAD-BAND NOISE: AN ELECTROPHYSIOLOGICAL STUDY. C.M. Henley*, R.D. Brown, J.E. Penny, K.A. Purdy*. Depts. of Pharmacology and Anatomy, LSU Med. Sch., Shreveport, LA 71130.

Previous studies of chloramphenicol (CAP) and noise have revealed ototoxic interactions of either a permanent or temporary nature (Henley, C.M. et al., Neuropharm. 23(2A): 197, 1984). Results have been confounded by the presence of otitis media (OM) and a relatively short duration noise exposure (90 sec/day once per week for 3 weeks; total duration 4.5 minutes). The purpose of this study was to determine whether an ototoxic interaction exists between CAP and noise in the absence of OM using a different noise paradigm of longer duration. Fifty adult female rats were grouped as follows: Group (G)-C (n=10), no treatment; G-1 (n=20), broad band noise exposure (124 dB SPL broad band noise) 90 sec/day for 5 days - total duration 7.5 minutes; G-2 (n=10), noise (G-1 protocol) and CAP 100 mg/kg/day (orally, divided into 3 equal daily doses) for 10 days beginning on the last day of noise exposure; G-3 (n=10), noise (G-1 protocol) and CAP 100 mg/kg/day for 10 days beginning on the 1st day of noise exposure. Chloramphenicol alone has not caused any effects in cochlear electrophysiological function or on cochlear anatomy in any of our studies; therefore a CAP only group was not included in this study. Round window recordings of ac cochlear potentials (ACCP's) at 4, 12, and 20 kHz and N_1 (8th nerve action potential) recorded on day 26 (21 days post noise exposure) revealed significant depressions (unpaired students' t-test; $\alpha = 0.05$) in ACCP's at 12 kHz and N_1 in G-2 (noise then CAP) only. There was a trend for G-2 ACCP's at 4 kHz to be lower than the other 3 groups. G-1 (noise only) and G-3 (noise plus CAP simultaneously) responses were not different from G-C. CAP administered for 10 days immediately following noise exposure (G-2) resulted in significant cochlear deficits whereas concurrent administration of CAP-noise (G-3) did not. This same phenomenon has been observed in earlier, unpublished studies. Thus the time of CAP dosing in relation to noise exposure appears to be of utmost importance in this interaction. Scanning electron microscopic findings paralleled these functional changes (see Penny et al., also, this meeting).

58.10 HISTOPATHOLOGICAL AND OTOACOUSTIC OBSERVATIONS FROM CHINCHILLAS HAVING SPONTANEOUS OTOACOUSTIC EMISSIONS. D.O. Kim[1], W.W. Clark*[3] and B.A. Bohne*[2]. 1. Dept. Physiol. & Biophys., 2. Dept. Otolaryngol., Washington Univ. Sch. Med., 3. Central Inst. Deaf., St. Louis, MO 63110.

Two cases of spontaneous otoacoustic emissions (SOAE) have been found among a sample of 28 chinchilla ears after noise exposure, and no cases of SOAE have been found among 28 unexposed ears. In order to determine whether or not pathological changes in the organ of Corti (OC) are correlated with SOAE, cytocochleograms were obtained from both ears of chinchilla No. 5176 which had an SOAE only in its right ear (Zurek and Clark, 1981). In the emitting ear, there was a punctate 0.3 mm loss of OC located from 69.4 to 70.9% from the apex and a diffuse 12% loss of outer hair cells (OHC) in the apical 15-35% region. The characteristic place of this ear's SOAE 4650 Hz is estimated at 70.9% and closely corresponds to the OC loss. The nonemitting ear was similar: there was a punctate 0.28 mm loss in the 68.2-69.6% region and a diffuse 12% OHC loss in the 15-35% region. In the emitting ear, the basilar membrane in the middle of the OC loss was covered with a layer of squamous epithelial cells having clear cytoplasm similar to the Claudius' and inner-sulcus cells. At the apical edge of the OC loss, there was an abrupt transition in the height of the tissue which decreased from approximately 57 μm to 17 μm within 60 μm. At the basal edge of the lesion, there were residual Deiters' and Hensen's cells distinguishable by their dense granular cytoplasm. The transition in height was more gradual decreasing from 57 μm to 19 μm in 143 μm. In the nonemitting ear, there were residual Deiters' and Hensen's cells covering almost all of the OC loss, and the two edges of the OC loss gradually changed in height. There was significant difference between the two OC losses regarding height, cytoplasmic density and number of the epithelial cells in the OC loss. In the other emitting ear, No. 3476-R, there were two punctate OC losses lcoated at 54.7-55.3% and 80.1-80.5%. The latter corresponds reasonably well with 77.4% expected from this ear's SOAE 6470 Hz. In contrast to ear No. 5176-R, the OC losses in this ear had a nearly full complement of Deiters' and Hensen's cells. From these data we suggest that the SOAE occurs as a result of a disruption in a localized region of the OC. However, a punctate OC loss does not always produce SOAE. We believe that the SOAE provides important indirect information about active and nonlinear mechanisms of cochlear biomechanics.

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58.11 TRAVEL TIME ESTIMATES IN THE ANURAN AMPHIBIAN PAPILLA. C.M. Hillery* and P.M. Narins. Dept. of Biology, UCLA, Los Angeles, CA 90024.

Recently we presented neurophysiological evidence to support the notion of a traveling wave in the amphibian papilla (a.p.) of a neotropical treefrog, Eleutherodactylus coqui. This evidence comes from phase-locked responses of VIIIth N. fibers which show a preferred firing phase angle, PA, which is linearly related to stimulus frequency. From $T=d\theta/2\pi df$, where T=time (s), θ=PA (radians), and f=stimulus frequency (Hz) we estimated the time delays (corrected for neural delay) which occur in the a.p. We find that they are inversely related to characteristic frequency (CF) ranging from 1.6 to 7.3 ms for neurons with CFs from 1.3 to 0.1 kHz respectively. Coupled with the known tonotopy of this organ, these CF-dependent delays are consistent with a low-velocity mechanical disturbance (traveling wave) on the tectorial membrane (TM) of the a.p.

Two assumptions are made to arrive at these travel time estimates: 1) the best-fit linear regression of the phase/frequency data is an accurate descriptor of the system's delay, and 2) the phase responses of a.p. neurons are linear (show no level-dependent effects). Frequency-dependent phase behavior was examined by subtracting the best-fit regression line (equivalent to subtracting a constant delay) from the phase-frequency data. Units with CFs < 0.3 kHz showed the greatest variation ($\pm$ 0.8 π) in their phase response; phase accumulated more rapidly for stimulus frequencies < 0.4 kHz. Since low-CF neurons derive from hair cells near the rostral end of the papilla, beneath the more massive end of the TM, they may be subject to greater mechanical non-linearities (edge effects and reflections).

The relation between PA and stimulus intensity was ambiguous. For 50% of the neurons, PA showed no systematic relation to intensity. However, for about 40% of the neurons sampled, PA showed a 30-90° increase with intensity (60-110 dB SPL). Tests using an electrical circuit which mimicked neural response (artificial neuron) predicted a slight decrease ($\pi/4$) in PA with intensity. Thus a positive monotonic relation of PA with intensity was unexpected and may imply either the involvement of middle ear mechanics or hair cell micromechanics in the generation of these non-linearities. Although the a.p. time delays suggest a traveling wave, the system non-linearities imply a complex interaction between the TM and the receptors. Supported by NIH grants Nos. NS07005-02 to CMH and NS19725-01 to PMN.

58.12 TRAVELING-WAVE MECHANICS IN THE BULLFROG AMPHIBIAN PAPILLA. E.R. Lewis and S. Yeh*. Electronics Research Lab., Univ. of California, Berkeley, CA 94720.

During evolution of the anurans, the amphibian papilla (an auditory endorgan of the inner ear) underwent striking elongation (Lewis, E.R., Neurosci. Lett., 21:131, 1981), perhaps reflecting derivation of a traveling-wave tuning structure. Evidence for such a structure in a modern frog (Rana catesbeiana) includes the presence of extremely steep high-frequency rolloff in neural tuning, combined with the absence of the sort of cycle-by-cycle energy accumulation associated with high-Q resonance (Lewis, E.R. & Leverenz, E.L., Scan. Electr. Microsc., 1983:189, 1983). One way to achieve intense high-frequency rolloff without high-Q resonance is with a traveling-wave structure, as in the mammalian cochlea. If such a structure is tapered, then the corner frequency for rolloff can vary from locale to locale. In modern anurans, the amphibian papilla appears to be tapered, along its long axis, with respect to both mass and stiffness. In Rana catesbeiana, this taper is congruent with the observed tonotopy-- the mass increasing toward the low-frequency end of the papilla, the stiffness increasing toward the high-frequency end.

As further test of the possibility of traveling-wave operation in the amphibian papilla of Rana catesbeiana, we examined the frequency dependence of phase shift of single-axon response relative to the input acoustic sinusoid under steady-state conditions. Among more than twenty papillar afferent axons examined to date, only one exhibited near-saturation of phase lag. From that unit, we determined that the maximum contribution to phase lag from structures outside the papilla (e.g., stimulus and recording apparatuses, the middle ear, and the afferent axon) was approximately one cycle of phase lag per kHz (i.e., 1.0-ms time lag). All other units exhibited unrelenting phase accumulation as the stimulus frequency was increased, with rates of phase accumulation typically ranging from 5 to 10 cycles of phase lag per kHz, and some units showing phase-lag accumulations of more than three full cycles at the higher ends of their frequency-response ranges. This implies separate and independent storage of the energies of at least six half cycles of the stimulus sinusoid, and is compelling evidence for the presence of a traveling-wave structure.

Thus the auditory endorgans of the frogs and toads and the mammals seem to have converged on analogous tuning mechanisms.

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58.13 HAIR CELL INNERVATION DIFFERENCES BETWEEN UNIDIRECTIONALLY AND BIDIRECTIONALLY ORIENTED HAIR CELL SEGMENTS IN A LIZARD AUDITORY PAPILLA. P. Teresi*(SPON: W. Mehler). Department of Anatomy, University of California, San Francisco, California 94143.

The auditory organ of the western fence lizard, Sceloporus occidentalis, is an epithelial ridge that is homologous to the mammalian organ of Corti. This epithelial ridge (also known as the papilla basilaris) consists of apical and basal bidirectionally oriented hair cell segments, separated by a central unidirectionally oriented hair cell segment. Hair cells in the central segment resemble those found in the mammalian organ of Corti in that they are all abneurally oriented and are covered by a tectorial cap. The bidirectionally oriented segments, which in this lizard consist of but two opposingly oriented rows of hair cells are not covered by a tectorial membrane and thus have free standing stereocilia.

In the present study, hair cell innervation patterns in the papilla basilaris of this lizard were examined. This serial-section, electron microscopic analysis revealed significant differences between unidirectionally oriented and bidirectionally oriented hair cell segments. Hair cells from the unidirectionally oriented segment ("unidirectional hair cells") synapsed with 4 to 17 afferent nerve fibers (mean of 10) and with 0 to 5 efferent nerve endings (mean of 2.7). Hair cells from the bidirectionally oriented segment ("bidirectional hair cells") synapsed with 5 to 13 afferent nerve fibers (mean of 10), but did not receive any efferent innervation. While these two hair cell types were innervated by a similar number of afferent nerve fibers, the "unidirectional hair cells" had twice as many synaptic contacts per afferent nerve fiber than did the "bidirectional hair cell". In particular, a "unidirectional hair cell" made 3.6 synapses (range of 1 to 10) with a single afferent nerve fiber, while a "bidirectional hair cell" had 1.8 synapses (range of 1 to 5) per afferent nerve fiber. Although hair cells had multiple synaptic contacts with the same afferent nerve fiber, a single afferent nerve fiber innervated only one hair cell whether it was a "unidirectional" or a "bidirectional" one. Supported in part by USPHS GRANT NUMBER 2 RO 1 NS 11838.

58.14 THREE-DIMENSIONAL RECONSTRUCTION OF THE SACCULAR MACULA IN THE GOLDFISH EAR PREDICTS TWO MAJOR VECTORS FOR NEURONAL DIRECTIONAL SENSITIVITY. C. Platt. Dept. Anatomy, Georgetown Univ. Sch. Med./Dent., Washington DC 20007.

The saccular macula of the inner ear is considered to be the major auditory receptor in goldfish. Sensory hair cells of the macula form a dorsal and a ventral population with opposite orientations and directional sensitivity. However, the in vivo orientations are more complex than seen using a simple flat projection. The three-dimensional layout of the saccular macula in its normal position was determined from photographs of whole ears during dissections, from whole maculae while still hydrated, from scanning EM of dried maculae, from sections of maculae imbedded in plastic, and from both transverse and longitudinal sections of fixed decalcified whole heads of goldfish. A model of the macular surface incorporated the features consistently found.

The elongate macula has a bend near the middle, and a longitudinal twist between rostral and caudal regions. The bend is roughly 20°, flexing the lateral surface which faces the otolith. In the horizontal plane the rostral region diverges more than 20° from the midline of the fish, while the caudal region lies within 5° of the midline. In the vertical plane the macula slopes upward toward the front. The rostral region slopes roughly 20° more than the caudal region, which itself slopes upward roughly 10° from the horizontal. The transverse twist along the macula is estimated at 50°. The curved surface of the most rostral region has a tangent plane angled nearly 10° off the vertical, facing laterally downward, while the caudal region faces laterally upward at nearly 45°.

The bend and twist produce a marked difference in spatial orientations of the hair cells between the rostral and caudal regions. Vector addition of these data predict maximal sensitivities to displacement. Rostral sensory neurons should be most sensitive to a stimulus vector oriented a few degrees medially and roughly 30° posterior to the vertical; caudally innervating neurons should be most sensitive to a vector oriented roughly 40° medial and 10° posterior to the vertical. The slight curvature of the macula means that some hair cells could contribute more extreme angles of sensitivity. Since acoustic stimuli drive the saccule via a linkage from the gasbladder, the different orientations suggest that macular stimulation might differ with different acoustic stimuli.

(Supported by ONR contract N-00014-82-K0345)

58.15 THE IONIC BASIS OF THE OSCILLATORY RECEPTOR POTENTIAL OF TUBEROUS ELECTRORECEPTORS IN STERNOPYGUS. H.H. Zakon, Dept. of Zoology, The University of Texas, Austin, TX 78712

Weakly electric fish generate an electric organ discharge (EOD) from an electric organ in the tail, and detect these electric fields with specialized receptor cells, termed electroreceptors, thought to be derived from hair cells. In most species these receptors are tuned to a particular frequency range such that the frequency sensitivity of an individual's receptors is well matched to the power spectrum of the individual's EOD. However, these cells possess no cilia or mechanical accessory structures for tuning. Rather, stimulus filtering occurs at the level of the cell membrane. In order to better understand the tuning process of the receptor cell membrane, the ionic basis of the receptor potential was studied.

A patch of receptor-rich skin from Sternopygus (EOD freq. about 50-200 Hz) was clamped between 2 chambers and 1 msec constant current pulses were passed across it to induce oscillatory "ringing" of the receptors. This receptor oscillation (RO) is an extracellularly-recorded compound receptor potential analogous to a cochlear microphonic. There was a good correlation between the EOD frequency of a fish and its RO (r=0.96). Different pharmacological agents were applied to the inside surface of the skin, allowing only access to the basal surface of the receptor cells, and their effects on the impulse-induced RO were studied. 1) 3 μM TTX had no effect 2) treatments that interfere with Ca^{2+} currents (250 μM Co^{2+}, 5 mM Mg^{2+}, 0 external Ca^{2+}) decreased RO amplitude and frequency, higher concentrations of divalent cations abolishing it completely. Increased extracellular Ca^{2+} (2-5X) increased RO amplitude and caused regenerative-like undamped oscillations upon stimulation. 3) treatments that interfere with K^+ currents (1mM TEA or 4-AP) or with Ca^{2+}-activated K^+ currents specifically (250 μM Ba^{2+}) also decreased RO amplitude and frequency. Thus, as in hair cells of the sacculus (Lewis and Hudspeth, 1984), these receptor oscillations depend on Ca^{2+} and K^+, but not Na^+, currents, and it is likely that at least part of the K^+ current is a Ca^{2+}-activated K^+ current.

58.16 TUNING OF NEWLY GENERATED ELECTRORECEPTORS. D. Yialamas* and H.H. Zakon (SPON: J.L. Larimer). Dept. of Zoology, The University of Texas, Austin, TX 78712

The weakly electric gymnotid fish, Sternopygus macrurus emits an electric organ discharge (EOD) the frequency of which varies between individual fish. This EOD is detected by a class of receptors termed tuberous electroreceptors, presumably derived from hair cells. These electroreceptors are tuned to the EOD frequency of each individual by an undetermined process. We have begun to study the reappearance of these receptors and the emergence of tuning in regenerated skin.

Studies were done with fish all of approximately 30cm snout-to-tail length. Patches of cheek skin ($1cm^2$) were surgically removed and regeneration of the skin was allowed to occur. The newly generated patches were then either sectioned and stained with cresyl violet or stained with the Winkelmann-Schmit silver stain for visualization of nerve fibers and whole mounted. Skin from normal fish was also taken and processed for baseline measurements.

The tuberous organs (TOs) normally appear as capsules which lie at the base of pores on the skin's surface. Within the lumen of each capsule there are approximately 30 receptor cells. Each receptor cell is innervated at its basal surface by an afferent fiber from the anterior lateral line nerve. In the regenerated epidermis, at one month after removal of the skin, there is evidence of incipient TOs that appear as aggregates of densely staining cells at the base of pores. With time, a capsule forms and a lumen develops filled with distinct receptor cells. By 3 - 4 months, the capsules appear totally normal with the proper number of receptor cells and each cell has become normally innervated. The tuning of these newly generated receptors was determined by measuring impulse-induced receptor oscillations (ROs) in regenerated skin in vitro (see Zakon these meetings). The RO frequency recorded from an individual's skin is in close agreement with the EOD of that individual.

These results indicate that the tuberous electroreceptors regenerate in 3 - 4 months in S. macrurus and become tuned to the EOD frequency of the individual fish.

58.PO COCHLEAR DEGENERATION IN RATS WITH PROLONGED LIFESPANS. Martin L. Feldman and Christopher D. West*. Dept. of Anatomy, Boston Univ. School of Medicine, Boston, MA 02118.

Dietary restriction was used to extend the life spans of Charles River albino rats (free-feeding median lifespan about 26 months). Cochleas of restricted (R) rats were examined in plastic section at 26 and at 45+ months of age; tissue was compered with that from tandem-reared ad lib. fed controls (C) at 26 months and also with that from normal (N) animals at 2-32 months (Keithley & Feldman, J.C.N. '79 and Hearing Res. '82).

While the cochleas of 26 month R and C rats were comparable, cochleas of the 45+ month R rats exhibited degenerative changes more severe than those seen in C or aged N rats.

In each of the 4 very old R rats, the sensory cells of the organ of Corti were completely absent in the basal region of the cochlea. In 3 animals, tectorial membrane attachment to the spiral limbus appeared defective, with intra-limbus cellular degeneration present in all very old rats. Other changes in these rats included: strial atrophy; accelerated loss of spiral ganglion neurons; an increased incidence of axonal degeneration in ganglion cell processes, often coupled with hypermyelination; and persistance of peripheral ganglion cell processes even in cases of obliteration of the sensory cells. In many profiles of the spiral ganglion, 100% of the Type I cells exhibited large lipofuscin deposits, but interestingly, Type IIs remained largely unaffected (Feldman et al., Am. Aging Assn., '81).

The observations indicate that if steps are taken to extend the lifespan, one must be prepared to encounter an auditory system in a severely degenerated state. Also, the severe changes seem to occur in advanced old age - there is not a general slowing down of the aging process. Finally, the changes seen approach in severity those reported in old humans, suggesting that the absolute length of lifespan is an important factor in animal models of aging.

(Supported by NIH grants AG03574 and AG00001).

59.1 CALCIUM CURRENTS IN THE PRESYNAPTIC TERMINAL OF THE BARNACLE PHOTORECEPTOR Jon H. Hayashi and Ann E. Stuart . Univ. of N. Carolina, Chapel Hill, NC ,27514

The input/output (I/O) relation of the graded synapse between the median photoreceptor cell (PR) and the second-order I-cell of the giant barnacle (Balanus nubilus) has been previously reported (Hayashi, Moore and Stuart, Soc. Neurosci. Abstr., 8: 522, 1982). A continous release of transmitter occurs both dark and light adapted preparations. In addition, the I/O relation shifts with presynaptic holding potential, such that the operating range of the synapse is extended. As a first step in understanding these synaptic phenomena, we voltage clamped the presynaptic terminal regions. PR axons were drawn into a current-passing suction electrode until the only the terminal branches and a short segment of axon were left exposed. A microelectrode which penetrated this axon segment monitored membrane potential. All experiments were done in 20 mM TEA. A voltage sensitive K+ current observed in 0 mM Ca++, 20 mM TEA, and 16 mM Co++ was measured and subtracted from the records.

At a holding potential of -80 mV, depolarizing voltage steps of 500 msec duration produced inward, then outward currents. The outward current had a prolonged tail that reversed at about -80 mV in normal 8 mM K+. The amplitude of the inward current decreased as the external Ca++ concentration was lowered and was abolished in either Ca++ free or Co++ superfusate. We conclude that the inward current is carried predominantly by Ca++. The outward current vanished along with the inward current; this, along with its timecourse and reversal potential, suggests that it is a Ca++ activated K+ current.

When Ca++ was substituted by Ba++, the inward current, but not the outward current was observed. The currents observed in Ba++ were maintained for the duration of the 500 msec pulse, indicating that the channel does not inactivate within this time frame.

When either Ca++ or Ba++ carried the inward current, depolarizing pulses of a few mV, delivered from holding potentials in the range of dark- and light-adapted values (-60 to -40 mV), elicited inward currents. Equally small hyperpolarizing pulses elicited a net outward current. Because both currents are absent in Co++ superfusing salines, we interpret this outward current as a decrease, at least in part, in an ongoing inward divalent current present at the holding potential. A continuous Ca++ current would be expected to underly the ongoing transmitter release in light or dark adapted preparations. Supported by NIH EY03347.

59.2 CALCIUM-DEPENDENT INACTIVATION OF PRESYNAPTIC CALCIUM CHANNELS. George J. Augustine and Roger Eckert. Department of Biological Sciences, University of Southern California and Department of Biology and Ahmanson Laboratory of Neurobiology, UCLA.

Previous studies suggest that Ca channels in presynaptic terminals undergo inactivation. We have voltage clamped the presynaptic terminal of the squid giant synapse to examine the kinetics and mechanism of presynaptic Ca channel inactivation. Experiments were performed with the 3-electrode technique on the most distal giant terminal of both Loligo pealei and L. opalescens. To block Na and K currents, TEA was iontophoretically injected inside the presynaptic terminal and preparations were bathed in a solution containing TTX (1 μM), TEA (200 mM) and 3,4-diaminopyridine (2 mM). To avoid contamination by residual K current we measured Ca tail currents following repolarization to potentials near E_K. Inactivation was measured as a decrease in the amplitude of the tail currents elicited by test depolarizations to -10 mV. Presentation of a depolarizing prepulse to 0 mV caused inactivation of the Ca tail currents produced by test pulses. Development of inactivation, which was measured by varying prepulse duration, can be described by a slow exponential ($\tau \approx 1.5$ s; n=12) summed with a non-inactivating component (Chad et al., J. Physiol. 347:279). Recovery from inactivation, measured by varying the prepulse-test-pulse interval, occurred over many seconds ($\tau \approx 70$ s; n=6). Three lines of evidence suggest that inactivation depends upon Ca entry. i) Inactivation has a U-shaped dependence upon prepulse potential, similar to the presynaptic Ca current. ii) Inactivation depends upon the permeant divalent cation, presynaptic Ba currents inactivating much more slowly than Ca currents. iii) Rate of inactivation depends on $[Ca]_o$. Raising external Ca from 11 to 50 mM causes more rapid inactivation. We conclude that presynaptic Ca currents at the squid synapse can be inactivated by an intracellular action of Ca ions on presynaptic Ca channels. This Ca-dependent inactivation is similar to that reported for molluscan nerve cell bodies and other tissues. Identification of this effect demonstrates that Ca ions have presynaptic actions in addition to triggering transmitter release, and also indicates that presynaptic Ca influx depends upon $[Ca]_i$ and upon conditions which influence $[Ca]_i$. Supported by an NRSA fellowship and NSF BNS 83-16417.

59.3 RESIDUAL FREE CALCIUM HYPOTHESES MAY NOT ACCOUNT FOR FACILITATION OF TRANSMITTER RELEASE IN THE SQUID GIANT SYNAPSE. S. M. Simon and R. Llinás. Dept. Physiol. Biophys., New York Univ. Med Ctr., 550 First Ave., New York, NY 10016.

In most chemical synapses the presence of a depolarizing prepulse at the presynaptic terminal can facilitate the release of transmitter by a subsequent depolarizing pulse. This facilitation has been attributed to a residual calcium concentration increase in the pre-terminal. This increase is assumed to follow the initial depolarization and add to, and thereby potentiate, the effects of a subsequent calcium influx (I_{Ca}) by a second depolarizing pulse. The 'residual free calcium' hypothesis requires a high stochiometry of calcium to transmitter release (3 to 4) (c.f. Parnas et al., Pfluegers Arch. 393:1-14, 1982). This latter assumption was tested here by using diffusion equations to model the changes of submembrane $[Ca^{++}]_i$ resulting from the opening of discrete calcium channels and relating the $[Ca^{++}]_i$ to the rate of transmitter release. The greatest changes of $[Ca^{++}]_i$ were predicted for the smallest depolarizations from rest where the flux per open channel was greatest (Simon, Sugimori & Llinás, Biophys. J. 45:264a, 1984). The peak $[Ca^{++}]_i$ decreased with increasing depolarizations from rest and resulting decreased flux per open channel. If it is assumed that the stochiometry for submembrane $[Ca^{++}]_i$ to transmitter release was greater than one, then the greatest transmitter release/I_{Ca} is predicted for the smallest depolarizations from rest. This is contrary to all published results from this preparation. Thus the assumption of a stochiometry greater than one may not be applicable to the giant synapse of the squid. This suggests the need for new models to explain facilitation. The general applicability of this assumption will be discussed in the light of our results and those obtained from other preparations. Two alternative hypothesis will be presented for biochemical and biophysical changes which modulate either the transmitter release apparatus and/or the vesicular pool that is readily available for release. They are both consistent with the experimentally observed physiology of presynaptic calcium movements and transmitter release. Supported by grant NS14014 from NINCDS.

59.4 **A MOLECULAR MODEL OF THE FACILITATION OF EVOKED NEUROTRANSMITTER RELEASE** **J. A. Borden* (SPON: L. A. Raskin). Neuroscience Program, Amherst College, Amherst, MA 01002.**

An artificial intelligence based computer language was developed for Semantic Network Event Simulation. This language was used to simulate chemical reaction networks.

Based upon measurements of calcium (Ca++) influx associated with nerve depolarization (Llinás and Heuser, Neur. Prog. Res. Bull., 15:557,1977), it was found that the step change in [Ca++] for a simple two-compartment model of calcium influx is too small to explain the speed at which release is initiated (200μsec). A three-compartment model with a transient high-[Ca++] immediately inside the synaptic membrane during calcium influx, was found to explain the time-course of release. Immediately after the cessation of influx, free Ca++ diffuses into the intracellular space causing a rapid drop in [Ca++] at the inner membrane.

Several lines of evidence indicate that calmodulin (CaM) or a calmodulin-like protein activates release: CaM binds four Ca++ at two high- and two low-Kd sites (Huang et al. PNAS 78:371,1981); Phenothiazines bind CaM and inhibit catecholamine release (Bradford et al. J. Neurochem., 41:1684,1983); CaM activates phospholipase A_2 (PLA_2) and prostaglandin E_2 (PGE_2) inhibits PLA_2; PGE_2 inhibits catecholamine release and PLA_2 causes vesicle fusion (Moskowitz et al. J. Neurochem., 41:1576,1983). This evidence lends support to the idea that CaM.Ca^{++}_4 activates PLA_2 which causes vesicle fusion and release.

During transient [Ca++] increase at the membrane four Ca++ molecules bind to CaM within 100μsec. After [Ca++] drops, thermodynamic equilibrium favors release of Ca++ from CaM. By examination of the curves which describe release of Ca++ from the four sites of CaM, we see that CaM.Ca^{++}_4 becomes deactivated within 1 msec, a time compatible with termination of release. [CaM.Ca^{++}_4] rises to relatively high levels and remains so for several msec, which corresponds to the fast-component of facilitation. Given this model, it it evident that if there are already two Ca++ molecules bound to CaM, more CaM.Ca^{++}_4 will be formed in the next impulse and release will be facilitated.

59.5 BLOCKADE OF K^+ CHANNELS IN APLYSIA SENSORY NEURONS REDUCES PRESYNAPTIC FACILITATION INDUCED BY SEROTONIN. B. Hochner*, M. Klein*, and E.R. Kandel. H. Hughes Medical Institute for Molecular Neurobiology & Behavior, Columbia Univ., P & S, and NYS Psychiatric Institute, N.Y., N.Y. 10032.

Sensitization of the gill and siphon withdrawal reflexes in Aplysia involves cAMP-dependent presynaptic facilitation at the synapses between mechanoreceptor sensory neurons and gill and siphon motor neurons in the abdominal ganglion. Since facilitation is accompanied by a reduction in a specific K^+ current in the sensory neurons (the S current), it was proposed that the increase in duration of the sensory neuron action potential caused by reduction of the current might contribute to facilitation by prolonging Ca^{++} influx into the presynaptic terminals. Consistent with this idea we find that transmitter release is significantly enhanced by small changes in duration of the presynaptic depolarization. We have now also examined the relation between the reduction in S current and facilitation by observing the effects of different degrees of blockade of the S channel on the facilitation induced by serotonin, an agonist of the naturally occurring facilitating transmitter.

Abdominal ganglia were voltage clamped in a solution containing 410 mM tetraethylammonium chloride (purified according to the method of Zucker, Brain Res., 208:473, 1981) and 10 mM 4-aminopyridine with a corresponding reduction in NaCl to maintain physiological osmolarity. The effects of serotonin on both the presynaptic current and the postsynaptic potential were substantially smaller than in controls that lacked the blocking agents. Moreover, the degree to which facilitation was blocked was correlated with the degree to which the K^+ current was blocked; in some cases both effects were completely blocked. We never observed facilitation without a concomitant reduction in outward current.

To insure that K^+ channel blockers do not interfere with the serotonin receptor or with the generation of cAMP in the sensory neurons, we assayed cAMP levels (with the help of T. Abrams and V. Castellucci), expressed as percent of ATP, in control and blocking solutions in the presence and absence of 10^{-4} M serotonin. We found that cAMP levels approximately doubled in response to serotonin in both control and blocking solutions, consistent with the hypothesis that blockade of K^+ channels was responsible for the reduction in serotonin-induced facilitation.

These results support the suggestion that reduction in the S current by facilitating stimuli is causally related to presynaptic facilitation. Our findings are not as yet sufficiently quantitative, however, to exclude the possibility of an additional contribution to presynaptic facilitation by an alteration in Ca^{++} handling (see Boyle et al., this volume) or by some other process.

59.6 5-HT INCREASES INTRACELLULAR CALCIUM TRANSIENTS DURING DEPOLARIZATION OF VOLTAGE-CLAMPED MECHANOSENSORY CELLS OF APLYSIA CALIFORNICA. M.B. Boyle, M. Klein*, S.J. Smith*, and E.R. Kandel. Depts. of Pharmacol & Physiol., Yale Univ. Sch. Med., New Haven, CT 06510; H. Hughes Med. Instit. for Molec. Neurobiol. & Behav., Columbia Univ., P & S, and NYS Psychiat. Instit., N.Y., NY 10032.

Behavioral sensitization of the gill- and siphon-withdrawal reflexes in Aplysia californica after tail shock has been shown to result from heterosynaptic facilitation of transmitter release from central mechanosensory neurons in the abdominal ganglion. The heterosynaptic facilitation has been attributed to suppression of a K^+ current, the S current, and the consequent broadening of the spike leading to enhanced accumulation of intracellular Ca^{2+}. We now report a component of enhanced Ca^{2+} accumulation in presynaptic facilitation which is independent of changes in spike shape. We have injected the dye arsenazo III to measure Ca^{2+} transients in the cell body during depolarization of voltage-clamped sensory neurons. During steps of 50 mV for 500 ms, the Ca^{2+} transients approximately doubled in amplitude when 5-HT, which mimics the natural transmitter, was applied either iontophoretically or in the bath. The percent increase in dye-Ca^{2+} signals by 5-HT was similar at all voltages. No consistent changes in the time course of the individual Ca^{2+} transients were observed. The onset and recovery of the 5-HT-induced increase in the dye-Ca^{2+} signals had a time course roughly similar to that for the suppression of the S current.

To study the effect of 5-HT on intracellular Ca^{2+} more closely, we developed a procedure for high-resolution optical recording from different areas of the sensory cell. After treatment with 1% protease (Sigma, Type IX) for 75 min at 34°C, the ganglion was desheathed and a sensory cell impaled with one microelectrode. Using the micromanipulator, the cell body was then pulled from the cluster without any apparent damage. We recorded from discrete areas of the cell body with a 20-um light pipe and obtained a resolution of 10 um or better. These experiments indicate that the increase in Ca^{2+} transients is occurring in the cell body proper; the enhancement is not due to unclamped regions outside the soma.

This increase in the Ca^{2+} transients within the cell body is therefore not secondary to changes in outward current but must reflect an additional action of 5-HT, a direct effect on Ca^{2+} handling by the cell. It will be important to determine whether this increase in Ca^{2+} accumulation results from an increase in Ca^{2+} influx, from a reduction in intracellular Ca^{2+} uptake, or from intracellular release of Ca^{2+}. However, independent of its detailed mechanism, this increase in Ca^{2+} availability may act synergistically with the reduction in K^+ current in the terminal to produce short-term facilitation of transmitter release.

59.7 EFFECTS OF TRYPTAMINE AND ETHANOL ON SYNAPTIC PLASTICITY AT A CRAYFISH NEUROMUSCULAR JUNCTION. R.N. Friedman* and G.D. Bittner. Depart. of Zoology, Univ. of Texas, Austin, TX 78712.

Short-term facilitation was induced by stimulating the nerve to the opener muscle in the propodite of the crayfish (Procambarus simulans and clarkii) with superthreshold pulse trains having frequencies of 15 to 40 Hz. Control measurements of excitatory junction potential (EJP) amplitudes observed with the muscle fibers bathed in van Harreveld's solution were compared with EJP amplitudes evoked in the presence of tryptamine (TA) or ethanol (EtOH) at concentrations of 0.001 to 4.0mM and 25 to 500mM, respectively.

Lower concentrations of TA reversibly enhanced facilitation while higher concentrations depressed or blocked facilitation. This result is consistent with earlier findings at lobster and frog NMJs (R.N. Friedman, et al., Brain Res. 214: 101-111, 1981). Double firing was occasionally observed with higher TA concentrations.

EtOH-induced increases in EJP amplitude were observed at a 50mM concentration. Concentrations at and above 100mM usually depressed EJPs. 500mM EtOH depressed EJPs, produced instability of the membrane potential and altered the shape of the evoked EJPs. These effects were reversible.

Preliminary results obtained by intracellular recording of nerve terminal potentials at Y-branches within 0.25 - 0.5 λ of the sites of transmitter release (D.A. Baxter and G.D. Bittner. Brain Res. 223: 422-428, 1982) show that TA at concentrations that affected EJP amplitude (2.0 to 4.0mM) does not cause depolarizations. This contrasts with a 7mV depolarization observed with 2.0mM serotonin (5-Hydroxytryptamine), suggesting that the mechanism of TA's action on transmitter release may differ from that of serotonin.

(Supported by NIH grant #RR 07187 to RNF and NIH grant NS-19764 to GDB.)

59.8 PRESYNAPTIC LONG-TERM POTENTIATION OF NICOTINIC TRANSMISSION IN THE RAT SUPERIOR CERVICAL GANGLION. R.E. McCaman, C.A. Briggs and D.A. McAfee. Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Intracellular studies have shown that long-term potentiation (LTP) in the superior cervical ganglion is an hours-long potentiation of nicotinic synaptic transmission that does not correlate with measurable changes in postsynaptic cell potential or input resistance. LTP is elicited by brief repetitive synaptic stimulation, but not by repetitive non-synaptic stimulation of the postsynaptic neuron. Pharmacological studies (Briggs, McKenna and McAfee, this volume) indicated that the induction of LTP was dependent on Ca^{2+} influx but independent of the activation of cholinergic or adrenergic receptors. From this and other data, we hypothesize that LTP is dependent upon presynaptic processes. We tested this idea by using a radioenzymatic assay to measure the release of acetylcholine (ACh) from the ganglion into the bathing medium. Ganglia were maintained in vitro at 21-23°C in oxygenated Locke's solution containing 20 uM eserine to inhibit cholinesterases, 2 uM atropine to block muscarinic receptors, and 100 uM curare or 200 uM hexamethonium to partially inhibit ganglionic transmission so that the postganglionic response to preganglionic stimulation was sensitive to changes in the strength of nicotinic transmission (Brown and McAfee, Science 215:1411, 1982). ACh release was evoked by stimulation of the preganglionic nerve (0.2 Hz) and, at the same time, ganglionic transmission was measured by recording the postganglionic compound action potential. It was important to allow 25 minutes for stabilization of ACh release before beginning the measurements. Tetanic preganglionic stimulation (20 Hz for 20 sec) caused a marked release of ACh which was washed out within 12 minutes. Afterwards, the evoked release (0.2 Hz) of ACh was potentiated by 30% for at least an hour, and this correlated with LTP of ganglionic transmission. Spontaneous (non-stimulated) ACh release was not potentiated. The content of ACh in other ganglia was unchanged 5 minutes after similar tetanic stimulation. Thus, LTP in the rat superior cervical ganglion is, at least in part, a potentiation of the stimulation of ACh release. This effect, which lasts for hours, is apparently triggered by Ca^{2+} accumulating in the cholinergic nerve terminal during a few seconds of repetitive stimulation. Supported by NIH Grants NS18857 and NS18966, NSF Grant BNS 81-12414 and American Heart Association Fellowship #766-F1.

59.9 NEUROTRANSMITTER REGULATION OF THE PHOSPHORYLATION STATE OF PROTEIN IIIa AND PROTEIN IIIb, TWO SYNAPTIC VESICLE-ASSOCIATED PHOSPHOPROTEINS. P. Mobley*, M.D. Browning, and P. Greengard. (Spon: M. Biedenbach). Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, N.Y. 10021.

Protein IIIa (M_r 74,000) and Protein IIIb (M_r 55,000) together with Synapsins Ia and Ib constitute a family of synaptic vesicle-associated phosphoproteins. These four proteins have been shown to be phosphorylated in intact nerve cells by electrical stimulation, by depolarization, and by 8-bromo cAMP. In addition these proteins are co-localized in the presynaptic terminal in association with synaptic vesicles. This localization suggests a role for these proteins in regulation of synaptic vesicle function. Previous work in our laboratory indicated that neurotransmitters, acting on presynaptic receptors, regulated the state of phosphorylation of Synapsins Ia and Ib. Therefore, it was of interest to determine if neurotransmitters could also regulate the phosphorylation state of Protein IIIa and Protein IIIb.

Incubation of frontal cortex slices in the presence of (-)norepinephrine (NE) led to a dose-dependent increase in the phosphorylation state of Protein IIIa and Protein IIIb. The concentration of NE that produced the half-maximal effect on the phosphorylation state of Protein IIIb was 2 μM. In the presence of maximally effective concentrations of NE, the amount of dephospho-Protein IIIb converted to the phospho-form was 15-20% in the absence and 25-30% in the presence of 5μM isobutylmethylxanthine (IBMX). IBMX alone had only a slight effect. Exposure of brain slices to NE elicited a similar conversion of dephospho-Protein IIIa to the phospho-form, although this response was more variable.

The conversion of the dephospho-forms of Proteins IIIa and IIIB to their phospho-forms was also observed in frontal cortex slices incubated in the presence of either (-)adrenaline or the selective β-agonist (-)isoproterenol. No effects were observed with the α_1 agonist phenylephrine or the α_2 agonist clonidine. Little if any effect on the phosphorylation states of Proteins IIIa and IIIb was observed in slices incubated in the presence of dopamine, histamine, or serotonin.

The effects of NE on the state of phosphorylation of Proteins IIIa and IIIb suggest one possible mechanism by which NE may modulate synaptic function.

59.10 CHOLINERGIC REGULATION OF PROTEIN III PHOSPHORYLATION IN ISOLATED CHROMAFFIN CELLS. J.W. Haycock, M.D. Browning and P. Greengard. Lab. Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10021.

A family of synaptic vesicle-associated phosphoproteins (Synapsin Ia and Ib, Protein IIIa and IIIb) exists in central and peripheral neurons. Suspension cultures of adrenal medullary chromaffin cells do not have detectable levels of Synapsin Ia and Ib but do contain both Protein IIIa and Protein IIIb (referred to collectively as Protein III). As measured both by immunolabeling of SDS-PAGE slab gels and by a detergent-based RIA, the levels of Protein III are 5-10% of those found in brain.

Treatments which activate stimulus-secretion coupling processes increase the phosphorylation state of both Synapsin I and Protein III in several tissue preparations (brain slices, posterior pituitary explants, and superior cervical ganglion explants). In the present studies we have investigated the regulation by acetylcholine (ACh) of Protein III phosphorylation in chromaffin cells isolated from bovine adrenal medulla.

Incubation of chromaffin cell monolayers with $^{32}PO_4$ led to a time-dependent ^{32}P-incorporation into several protein bands revealed by SDS-PAGE. Addition of polyvalent serum anti-Protein III antibodies to SDS extracts of the chromaffin cells immunoprecipitated both an Mr=74,000 (Protein IIIa) and an Mr=58,000 (Protein IIIb) phosphoprotein band. Partial proteolysis of phosphorylated Protein IIIa and IIIb, cut from the SDS-PAGE gels, with S. aureus V8 produced an Mr=18,000 phosphopeptide fragment (characteristic of Protein III from brain) from each band. The incorporation of ^{32}P into Protein IIIa and IIIb was time-dependent, and addition of ACh produced a rapid, 2-4 fold increase in ^{32}P incorporation into each of these bands. This increase was maintained for several minutes and was completely dependent upon extracellular calcium. Parallel experiments investigating the ACh-induced release of previously accumulated ^3H-norepinephrine yielded similar results.

The identities of the second messenger(s) and protein kinase(s) responsible for the ACh-induced phosphorylation of Protein III and the role which this phosphorylation may play in the ACh-induced release of ^3H-norepinephrine are under investigation.

59.11 A FAMILY OF SYNAPTIC VESICLE-ASSOCIATED PHOSPHOPROTEINS: SYNAPSIN Ia, SYNAPSIN Ib, PROTEIN IIIa, AND PROTEIN IIIb. M.D. Browning and P. Greengard. Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, N.Y. 10021.

Previous studies from our laboratory have shown that there are four prominent phosphoproteins present in acid extracts from all regions of the central nervous system (Synapsin Ia, M_r 85,000; Synapsin Ib, M_r 80,000; Protein IIIa, M_r 74,000; and Protein IIIb, M_r 55,000). All four of these proteins are phosphorylated in intact nerve cells by electrical stimulation in the presence of calcium, by depolarization in the presence of calcium, and by 8-bromo cAMP. We now report that these four phosphoproteins appear quite homologous. When the proteins were phosphorylated by cAMP-dependent protein kinase and then subjected to limit digestion by trypsin and chymotrypsin, only a single phosphopeptide was obtained from each protein; and these four phosphopeptides very nearly comigrate on 2-dimensional peptide maps. A number of monoclonal and serum antibodies raised against Protein III exhibit significant cross-reactivity toward Synapsin I. Quantitative immunolabeling in SDS-polyacrylamide gels was used to determine the concentration of these proteins in a variety of tissues, brain regions and subcellular fractions. The proteins were, with one exception, found only in nervous tissue; and, in the central nervous system, all four proteins exhibited a distribution that paralleled the relative density of nerve terminals in the region studied. The single exception to the exclusively neuronal distribution of these proteins is that Protein III has been found in the adrenal medulla. The subcellular distribution of the four proteins in brain tissue was also essentially identical since all four proteins were substantially enriched only in the synaptic vesicle fraction

These data indicate that four prominent phosphoproteins which are phosphorylated in parallel in intact nerve cells possess significant structural homology and are co-localized within the presynaptic terminal in association with synaptic vesicles. These data suggest that these proteins constitute a family of phosphoproteins that have similar roles in brain presumably mediating or modulating some aspect of synaptic vesicle function.

59.12 NEUROBLASTOMA X GLIOMA HYBRID CELLS DIFFERENTIATED IN CULTURE: A MODEL FOR STUDYING THE FUNCTION OF NEURONAL PHOSPHOPROTEINS. T.B. Davis*, E. Kornecki*, R.H. Lenox, and Y.H. Ehrlich. Neuroscience Res. Unit, Dept's. of Psychiatry and Biochemistry. Univ. of Vermont, Burlington, VT 05405.

The great heterogeneity of cell types present in the brain may account for the sparsity of information on the role of specific phosphoproteins in the regulation of various neuronal functions. This difficulty can be overcome by studying a model system consisting of a homogenous population of cells grown in culture. Neuroblastoma X Glioma (NG) hybrid cells represent an appropriate model system for this purpose, since after differentiation in culture they exhibit many properties characteristic of mature neurons, including the ability to form functional synapses.

In the present studies, cells of the clonal line NG108-15 were induced to differentiate in-culture by treatment for 4 days with 1mM dibutyryl cyclic-AMP. Intracellular ATP pools were labeled by incubating the cells with ^{32}Pi. Endogenous phosphorylation assays were carried out by incubating cell homogenates and subcellular fractions with gamma-^{32}P labeled ATP and GTP. The major proteins phosphorylated in intact cells co-migrated in slab-SDS polyacrylamide gels with the proteins phosphorylated in-vitro in homogenates incubated with $AT^{32}P$. The main changes seen in phosphorylative activity following differentiation were a several fold increase in phosphorylation of a protein with an apparant MW of 80Kd, and a comparable decrease in phosphorylation of a 100 Kd protein. A protein with an apparant MW of 54Kd was phosphorylated by Mn-$GT^{32}P$. This GTP utilizing system, shown previously in synaptic plasma membranes from rat brain, was present in a highly enriched plasma membranes fraction prepared from differentiated NG cells. The plasma membranes also showed a multiplicity of phosphorylated proteins in the MW range of 70-120Kd. Two of these proteins appear to be specific substrates for an ecto-protein kinase. This was shown in experiments where intact cells grown in chemically defined (serum-free) medium were incubated with extracellular $AT^{32}P$. The localization of these proteins on the outer surface of the cell membrane is investigated by labeling the surface proteins of intact cells with ^{125}I. The identification of protein phosporylation systems characteristic of differentiated NG cells provides the data-base needed for investigating the role of specific phosphoproteins in the responses of mature neurons to hormonal, pharmacological and environmental stimulations. Supported by grants MH35735 and NSF/BNS 82-09265.

59.13 Regulation of the synaptosomal vesicle Ca^{++} pump by a Ca^{++}/calmodulin-dependent protein kinase. David S.Lester, Shew Y.Chan, Mark K.Bennett[1], Mary B.Kennedy[1] and Stanley M.Goldin. Harvard Medical School, Department of Pharmacology, Boston, MA., 02115.
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The ATP-dependent Ca^{++} pump of bovine brain synaptosomal vesicles was reconstituted into liposomes and substantially purified by "transport-specific fractionation" as previously described (Chan, S.Y., et al., J.Neurosci., June 1984). Type II Ca^{++}/calmodulin-dependent protein kinase, isolated from rat brain (Bennett, M.K. et al., J.Biol.Chem. 20, 12,735, 1983) stimulated ATP-dependent Ca^{++} uptake by this purified, reconstituted Ca^{++} pump. Both the initial rate and steady state levels of Ca^{++} accumulation at 30°C increased 1.7-2.4 fold. At a concentration of reconstituted protein of 15 µg/ml, half maximal kinase-dependent activation of the Ca^{++} pump occurs at a kinase concentration of 0.3 µg/ml. Kinase-dependent activation was dependent on the presence of 0.5 µM calmodulin.

In parallel and under identical conditions, kinase-dependent incorporation of [γ]-^{32}P from ATP was determined by protein precipitation and scintillation counting, and by SDS-gel electrophoresis. In the absence of the reconstituted Ca^{++} pump the kinase autophosphorylates, incorporating 5.5 mol ^{32}P per 110,000 daltons of kinase. In the presence of the reconstituted pump, ^{32}P incorporation into TCA-precipitatable protein increased ~3-fold. Autoradiographic analysis of ^{32}P phosphoproteins on SDS-Laemmli gels revealed that addition of the reconstituted Ca^{++} pump stimulated autophosphorylation of the kinase, but phosphorylation of the Ca^{++} pump was not resolved. This stimulation of autophosphorylation was not mimicked by protein-free liposomes or by the kinase substrate, synapsin I. The autophosphyloration and the increase in TCA-precipitable ^{32}P incorporation are both Ca^{++}- and calmodulin-dependent. An unresolved question is whether the increase in autophosphorylation is involved in the mechanism of kinase activation of the Ca^{++} pump or is an independent effect.

59.14 CALCIUM-DEPENDENT PROTEIN PHOSPHORYLATION IN SYNAPTOSOMES. P.R. Dunkley* and P.J. Robinson* (Spon:P. Jeffrey). The Neuroscience Group, Faculty of Medicine, University of Newcastle, N.S.W., Australia. 2308

Depolarisation of synaptosomes causes a calcium-dependent increase in the phosphorylation of specific phosphoproteins. In this project the changes were studied after incubation of synaptosomes for 45 min with ^{32}P-inorganic phosphate in buffers containing various concentrations of calcium (Robinson, P.J. & Dunkley, P.R. Neurosci.Letts 43, 85-90,1983). After 5 sec depolarisation, maximal increases were observed with 0.1mM calcium, with higher concentrations up to 2.5mM being progressively less effective. Decreasing the concentration of calcium also increased the time at which maximum phosphorylation changes were observed on depolarisation, thus with 2.5mM calcium the peak of labelling occurred at 5s, while with 1.2mM and 0.1mM the peaks occurred at 10s and 15s respectively. Prolonging the period of depolarisation leads to an extensive dephosphorylation of proteins which again is dependent on the buffer concentration of calcium. The highest rate of dephosphorylation was observed at 2.5mM calcium, where a rapid and then a slower phase occurred leading after 5 min to a level of phospholylation of only 80% of control. With 0.1mM calcium, only the slower phase was observed and the level of phosphorylation after 5 min was still 115% of control. Analysis of ATP levels were also undertaken with samples incubated under the same conditions as those used for protein phosphorylation. Overall, the results are consistent with depolarisation of synaptosomes causing an influx of calcium which activates protein kinases. As the calcium influx is increased a marked dephosphorylation occurs and although the mechanism is unknown it must involve either calcium stimulated inactivation of protein kinases or activation of protein phosphatases.

59.15 DEPOLARIZATION-INDUCED INCREASE IN MEMBRANE Ca AND Ca UPTAKE IN RAT BRAIN SYNAPTOSOMES. W. Hoss, B. Labkovsky* and M. Formaniak*. Ctr. for Brain Research, Univ. of Rochester Med. Ctr., Rochester, NY 14642.

Depolarization-induced Ca uptake is essential for transmitter release from nerve endings. Electrophysiologic measurements of this process are difficult, owing to the small size of most presynaptic endings and the low level of current carried by Ca compared with Na and K. The fate of presynaptic membrane Ca during depolarization is unknown. These experiments compare the effects of depolarization on membrane Ca using chlorotetracycline as a fluorescent probe and Ca uptake using ^{45}Ca. Suspensions of synaptosomes, which were isolated by density gradient centrifugation, were depolarized by abruptly increasing the K concentration at 30°C. Changes in membrane Ca were monitored by fluorescence intensity at 520 nm and Ca uptake was measured by depolarizing in the presence of ^{45}Ca, quenching uptake by 10-fold dilution in cold buffer containing excess ^{40}Ca and subsequently filtering through glass fiber filters and washing.

Depolarization of nerve endings results in potential-dependent increases in both membrane Ca and Ca uptake. The response of membrane Ca to depolarization is slower than that observed for Ca uptake. Depolarization-induced increase in membrane Ca is primarily associated with the internal surface of the plasma membrane. Using methanol to mimic the low dielectric of the membrane environment, the net increase in membrane Ca after depolarization with 70 mM K is estimated to be 0.5 µM at the internal surface of the plasma membrane. Loss of membrane Ca from preloaded synaptosomes has a time course similar to Ca efflux. Inactivation can be observed for Ca uptake but not for the increase in membrane Ca.

Inactivation of Ca uptake depends on external Ca concentration, but not onmembrane potential or amount of Ca taken up. Drugs that effectively block voltage-sensitive Ca channels in heart and smooth muscle are only weak blockers of Ca uptake in nerve endings and do not affect efflux or inactivation. Supported in part by DA 01851.

59.16 **EFFECTS OF TRIFLUOPERAZINE ON K^+ STIMULATED $^{45}CA^{++}$ INFLUX AND [^{3}H] ACh RELEASE IN SYNAPTOSOMES** **M. Murawsky* and J.B. Suszkiw, Dept. of Physiology and Biophysics, Univ. of Cincinnati College of Medicine, Cinti., OH 45267-0576**

The effects of trifluoperazine (TFP) on K^+-stimulated influx of $^{45}Ca^{++}$ and release of [^{3}H] acetylcholine ([^{3}H] ACh), were examined in rat cerebrocortical synaptosomes. Ten min preincubations of synaptosomes with 2.5-50 µM TFP inhibited K^+-stimulated Ca^{++} influx and [^{3}H] ACh release in a dose dependent manner. 50 µM TFP reduced $^{45}Ca^{++}$ influx by approximately 80% and caused a similar reduction in [^{3}H] ACh release. The effect of 50 µM TFP on $^{45}Ca^{++}$ influx measured during 5 sec depolarizations by high (52.5 mM) K^+ was rapid. Inclusion of 50 µM TFP only during the 5 sec depolarization resulted in 50% inhibition of $^{45}Ca^{++}$ influx and reached its full effect (80% inhibition) in less than 1 min of exposure to synaptosomes to the drug prior to K^+-depolarization.

Contrary to the report by Schweitzer and Kelly (1982, Soc. Neurosci. Abstr. 8:493) but in agreement with Baba et al. (1983, J. Neurochem. 40:1758) the present results suggest that TFP reduces transmitter release by inhibiting the voltage-dependent influx of Ca^{++} into synaptosomes. The relatively rapid onset of inhibition of K^+-stimulated $^{45}Ca^{++}$ influx suggests that in this case TFP acts at synaptosomal membrane sites rather than on intraterminal targets, e.g. calmodulin.

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59.17 DETECTION OF PRESYNAPTIC CALCIUM-BINDING PROTEINS
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Calcium binding proteins are considered to be a class of proteins involved in such significant processes as exocytosis, endocytosis, muscle contraction, chromosome movement, and cell motility. Thus, their possible involvement in neuronal function is of great interest. Probably the most well-studied calcium-binding protein involved in neuronal functions is calmodulin. Another important brain calcium-binding protein is S-100. The presence of other possibly important regulatory calcium-binding proteins has not been investigated. Using our recently developed method to detect $^{45}Ca^{2+}$-binding proteins in polyacrylamide gels, we have discovered several calcium-binding proteins in brain with the majority present in presynaptic cytoplasm. One- and two-dimensional gel electrophoresis of these proteins revealed calcium-binding proteins varying in molecular weight and isoelectric point. Because an influx of calcium into the nerve terminal is essential for neurotransmitter release, we suggest that these synaptoplasmic calcium-binding proteins may represent triggers for neurotransmitter release.

59.18 PRESYNAPTIC CALCIUM ENTRY AND TRANSMITTER RELEASE AT THE SQUID GIANT SYNAPSE.
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We have re-evaluated the relationship between Ca^{++} entry and transmitter release in the squid giant synapse using a focal source of extracellular Ca to restrict both calcium current ($I\ Ca^{++}$) and transmitter release to the same relatively isopotential tip region of the presynaptic terminal.

The 3-microelectrode voltage clamp method was used to measure presynaptic I Ca produced by brief (6 msec or less) depolarizations. External TEA (20 mM), 3,4-diaminopyridine (2 mM) and TTX (1 uM) and internal TEA were used to block K and Na currents. Remaining Ca-independent currents were measured in the absence of Ca application, and were subtracted from currents obtained in the presence of extracellular Ca ions to yield net I Ca. To provide an idependent measure of presynaptic Ca entry, intracellular Ca transients in voltage-clamped presynaptic terminals were detected with the Ca indicator dye Arsenazo III. Dye signals rose rapidly during presynaptic depolarizations, and decayed over seconds following repolarization. The voltage dependence of intracellular Ca transients was similar to that of presynaptic I Ca. Over the entire range of presynaptic potentials examined, Arsenazo signals were closely correlated with I Ca integrals which suggests that both methods reliably measure presynaptic Ca entry. Transmitter release was measured under similar experimental conditions, using voltage clamp measurements of transmitter-induced postsynaptic currents (PSCs) as an assay for release. PSCs had a U-shaped dependence upon presynaptic potential, being maximal at -10 to 0 mV and suppressed at potentials above +50 mV. Measurements of presynaptic I Ca and PSCs were correlated to determine the synaptic transfer relationship. This relationship was highly nonlinear, having a limiting slope of approximately 3 on log-log coordinates (range = 2.4-3.5 n=18). This value is higher than that previously reported for voltage clamp experiments upon this preparation, but is consistent with earlier studies which found that release is proportional to the 3rd power of extracellular Ca concentration. This re-establishes the possibility that release reactions require the cooperative action of several Ca ions.

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PRESYNAPTIC MECHANISMS II

60.1 SOLUBILIZATION AND PARTIAL PURIFICATION OF THE RECEPTOR FOR THE PRESYNAPTIC NEUROTOXIN ß-BUNGAROTOXIN.
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ß-Bungarotoxin (ß-Btx) is a presynaptic snake venom neurotoxin of MW 21,000 with a phospholipase A_2 activity (Abe, T. et al., Eur. J. Biochem., 80:1, 1977). The toxin blocks neurotransmission at the neuromuscular junction and is cytotoxic for some classes of central neurons (Rehm et al., Brain Res., 250:309, 1982).

A high affinity protein binding site for ^{125}I-B-Btx was identified in crude synaptic membrane fractions of chick brain (Rehm, H. and Betz, H., J. Biol. Chem., 257:10015, 1982). This ß-Btx binding protein was characterized by photoaffinity crosslinking of ^{125}I-ß-Btx to chick brain membranes (Rehm, H. and Betz, H., EMBO J., 7:1119, 1983). A MW 95,000 peptide was labelled on SDS gels run with these crosslinked membranes. No labelled band was seen with crosslinked liver membranes or with crosslinked chick brain membranes which had ^{125}I-ß-Btx bound in the presence of an excess of unlabelled toxin, or EDTA.

Recently, we solubilized the ß-Btx binding protein from chick brain membranes with Triton X-100. K^+ was necessary to keep the solubilized protein in a native conformation. The partial specific volume, the MW and the Stokes radius of the ß-Btx binding protein were determined by H_2O/D_2O sucrose centrifugation and Sepharose 6B chromatography to be 0.784 ml/g, 431,000 daltons and 8.6 nm, respectively (Rehm, H. and Betz, H., J. Biol. Chem., in press, 1984). The protein was partially purified through affinity chromatography on ß-Btx agarose.

Evidence is provided that the toxin-receptor complex is rapidly internalized by coated pits. This internalization however seems not to be necessary for the cytotoxicity of ß-Btx as the cytotoxicity can be blocked at any time after toxin addition through anti-ß-Btx antibodies. Therefore the mechanism of action of this presynaptic toxin seems restricted to the occupation of toxin receptors on the outer plasma membrane.

This work was supported by grants from the Deutsche Forschungsgemeinschaft and the Bundesministerium für Forschung and Technologie.

60.2 PEPTIDIC NEUROTOXINS FROM THE MEXICAN SCORPION CENTRUROIDES NOXIUS MODIFY TRANSMITTER RELEASE THROUGH SELECTIVE CHANGES IN THE PRESYNAPTIC Na^+ AND K^+ PERMEABILITIES.
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The venom of the scorpion C. noxius contains at least eight toxic fractions (Possani et al, Carls. Res. Commun. 46,207,1981 and 47,285,1982). Carbone et al (Nature 296,90,1982) described the actions of some of these toxins on the squid axon. Here, we present a study of the effects of noxiustoxin (II-11) and of the two homologous toxins II-9.2.2. and II-10 on ^{3}H-GABA release from mouse brain synaptosomes. Noxiustoxin (0.1 µM) added to the perfusion Ringer increases the release of ^{3}H-GABA and this effect is additive to that produced by 15mM K^+. Substitution of choline for Na^+ or the presence of tetrodotoxin (TTX) do not inhibit these effects which, in contrast, are blocked by Ca^{2+} channel antagonists. These actions of noxiustoxin are those expected from a blocker of K^+ permeability. Noxiustoxin also acts on the squid axon as a selective blocker of K^+ conductance (Carbone et al, ibid). Toxins II-9.2.2. and II-10 in the µM range, also increase ^{3}H-GABA release; however their effects are blocked by TTX or by the replacement of external Na^+ for choline. It is noteworthy that, in contrast with the action of veratrine on ^{3}H-GABA release, the effect of these toxins is dependent on external Ca^{2+} and blocked by Ca^{2+} channel antagonists. Thus, these two toxins appear to increase Na^+ permeability depolarizing the terminals to evoke transmitter release. Nevertheless, toxin II-10 acts on the squid axon by decreasing the peak Na^+ permeability and toxin II-9 is devoid of effect in this preparation (Carbone et al , J. Physiol. Paris, in press). These differences could be explained by the presence of different Na^+ channels in the axon and in the synaptic terminals. Supported by Grants from CONACyT and Fundación R. Zebada (BCH) México. M.S. is an Associate of Research at the Inst. Mexicano de Psiquiatría.

60.3 **MONOCLONAL ANTIBODIES AGAINST BINDING AND NONBINDING FRAGMENTS OF TETANUS TOXIN CAN PREVENT CONVULSANT ACTION OF TOXIN ON DISSOCIATED SPINAL CORD NEURONS IN CULTURE**

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The effects of tetanus toxin on dissociated fetal mouse spinal cord neurons in culture have recently been characterized (Bergey, et al., J. Neurosci. 3:2310, 1983). This system offers advantages for the study of the cellular action of tetanus toxin by allowing direct application of known concentrations of toxin without requirements of axoplasmic transport or diffusion through neuropil. After a dose-dependent latent period tetanus toxin produces paroxysmal depolarizing events (PDE) characterized by abrupt depolarizing shifts in membrane potential with resultant triggered action potentials. The tetanus-PDE results from a relative reduction in presynaptic release of inhibitory transmitters.

Recently monoclonal antibodies have been prepared that are specific for identified regions of the tetanus toxin molecule (Kenimer, Habig and Hardegree, Infect. Immun. 42:942, 1983). Selected antibodies from this group have been used here in investigations of the action of tetanus toxin on dissociated spinal cord in culture.

Spinal cord neurons were exposed to tetanus toxin (100 ng/ml) for 90 minutes at 35° C. Intracellular recordings performed during the following 90 minute periods uniformly revealed typical PDE and reduced synaptic inhibition. Preincubation of tetanus toxin with a monoclonal antibody that was specific for the C fragment and reduced toxin binding prevented the subsequent appearance of PDE. Polyclonal antibody preparations also prevented the convulsant action of tetanus toxin. Another monoclonal antibody specific for fragment C but which did not reduce toxin binding did not prevent PDE. An additional monoclonal antibody directed against the nonbinding heavy chain portion of the B fragment of toxin was found to prevent the onset of PDE during the assay period.

The ability of a monoclonal antibody directed against the B fragment of tetanus toxin to neutralize the convulsant action, independent of effects on binding, suggests that this nonbinding subunit of the toxin is important for membrane or subcellular interactions.

60.4 PASSIVE TRANSFER OF THE LAMBERT-EATON MYASTHENIC SYNDROME TO MICE USING WHOLE PLASMA AND IMMUNOGLOBULIN FRACTIONS.

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Lambert-Eaton myasthenic syndrome (LES) is recognized as a deficient evoked release of ACh from the motor nerve terminal (MNT). Recent findings (Lancet ii:224, 1981; PNAS 80:7636, 1983) suggest that autoantibodies may contribute to the presynaptic impairment. We have further characterized the electrophysiology of murine passively transferred LES.

The mean quantum content of the end-plate potentials (m) in diaphragm muscles of Swiss Webster mice was estimated by a direct method under conditions of high Mg^{++} and low Ca^{++}. Mice receiving daily injections (1.5 ml IP) of plasma from a patient without neoplasm had m values reduced to 50% by day 12 and to a plateau by day 27 which from 41 to 59 days remained at about 30% of m in controls injected with normal plasma. Crude immunoglobulins (Ig's) from the same patient's plasma (isolated by ammonium sulfate precipitation, and containing 10 mg IgG) after 20 and more injections reduced m to 21% of values in mice receiving control Ig. Similar injections of Ig from two LES patients with carcinoma (oat cell and renal cell) reduced m to 42% and 61% of control, respectively. Indirect variance measurement of quantal content in curarized diaphragm in recipients of Ig from the latter patient gave comparable reductions. Postsynaptic changes in LES recipients were absent. Miniature end-plate potential (MEPP) amplitude, time course, and resting membrane potential were normal. With 5 mM K^+, the rate of the spontaneous transmitter release was normal, but its increase in 17.5 mM K^+ was significantly less (29%-68% of control values) in LES recipients. Nerve conduction and muscle fiber action potentials were normal. The mean size of MNTs, studied by zinc iodide-osmium impregnation, was normal or slightly greater with absence of significant terminal sprouting. The ratio of postsynaptic/presynaptic membrane length was also slightly greater in the LES treated mice. These results are consistent with minimal structural changes in MNTs of LES recipients.

Our findings confirm that LES with and without carcinoma can be passively transferred by injection of Ig. Study of passively transferred LES will allow more detailed analysis of the presynaptic blocking mechanism of human LES. (Supported by NIH grants NS-18607, NS-07199, NS-17699 and CA-37343).

60.5 RAPID CHANGES IN LASER LIGHT SCATTERING ACCOMPANY SECRETION BY NERVE TERMINALS IN THE INTACT MAMMALIAN NEUROHYPOPHYSIS.

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We have measured rapid changes in laser light scattering from the unstained neurohypophysis of the mouse (CD-1), during and immediately following the occurrence of action potentials in the neurosecretory terminals. These intrinsic optical signals are related to the opacity changes reported earlier (Salzberg, Obaid, Orkand, and Gainer. Biophys. J. 45, 314a, 1984) and are easily recorded without averaging, using a Helium-Neon laser, amplitude stabilized to 0.001 % (0.1 Hz - 1 kHz). The fractional changes in scattered intensity depend upon frequency of stimulation and the $[Ca^{++}]_o$, as well as the scattering angle. Some components of the light scattering change are blocked by Ca^{++} antagonists and are enhanced by known secretagogues; these optical signals appear to monitor an early event in the neurosecretory process. A different component appears to reflect the arrival of the impulse in the terminals.

The angular dependence of the light scattering changes may provide important information about the identity of the scatterers, and optical heterodyning may permit the detection of organelle movement with millisecond time resolution. Supported by USPHS grant NS 16824.

60.6 PRESYNAPTIC ACTION OF 4-AMINOPYRIDINE.

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At several different synapses 4-aminopyridine (4-AP) markedly potentiates transmitter release by increasing the quantal content. Since 4-AP is also a blocker of the delayed potassium conductance it has been proposed that 4-AP enhances transmitter release by prolonging the time course of the action potential. On the other hand since the presynaptic effects of 4-AP on transmitter release at frog neuromuscular junctions or the squid giant synapse require only micromolar concentration, whereas channel blockade by 4-AP in squid, lobster and cockroach giant axons requires millimolar concentrations it has been suggested that 4-AP enhances transmitter release and increases Ca influx by affecting the voltage dependent Ca permeability change during depolarization of the nerve terminal.

If 4-AP increases the quantal content by blocking the delayed potassium conductance it should also cause an increased dispersion of synaptic latencies - which is an indicator of the time course of transmitter release (Katz & Miledi, Proc. Roy. Soc. B, 1965, 161, 483-495) - comparable to the increased dispersion produced by tetraethylammonium (TEA) that causes similar enhancement of transmitter release.

The present experiments were performed in vitro on a frog (Rana Pipiens) "cut" cutaneous pectoris preparation (kept at a low temperature 9-15°C). The nerve was stimulated every 2-4s by supramaximal pulses of 0.1 to 0.2 msec duration via a suction electrode. Intracellular recording was done with microelectrodes filled with 3M KCl. When focal recordings were made to measure the synaptic latencies microelectrodes were filled with 0.5 Na Cl.

4-aminopyridine (4-AP) markedly potentiated transmitter release by increasing quantal content (2 to 9 times), measured as a ratio of mean EPP's and MEPP's even when applied at low concentrations (5-20 μM). This enhancement of the transmitter release was not associated with an increased dispersion of the latencies of nerve evoked responses. This is in contrast to the effect of tetraethylammonium (TEA) in which case a similar enhancement of transmitter release resulted in marked increase in the dispersion of synaptic latencies. This difference in action between 4-AP and TEA indicates that at low concentrations 4-AP probably does not produce an enhancement of transmitter output by prolonging its time course of release. Its mode of action is therefore more likely to be through a direct enhancement of nerve terminal calcium permeability. (Supported by MRC and MDA (Canada)).

60.7 UNEQUAL OCCURRENCE OF SPONTANEOUS RELEASE ALONG THE FROG NEUROMUSCULAR JUNCTION. G. Grenon*, R. Robitaille*, J. P. Tremblay (SPON: C. Radouco-Thomas). Lab. of Neurobiology, Laval Univ., Dept. of Anatomy, Québec, Canada, G1J 1Z4.

Using extracellular electrodes, Bennet and Lavidis (Devel. Br. Res. 5 (1982) 1-9) have found that the average quantal content declines with increasing distance from the last myelin segment along individual terminal branches of amphibian neuromuscular junctions (nmj). Their result was confirmed by Zefirov (Neirofiz. 15 (1983) 362-9) but not by Alonzo and Grinnell (Soc. Neurosc. Abstr. vol 8, p. 493, 1982). This abstract describes a new method of studying the distribution of spontaneous release along the frog nmj. Simultaneous intracellular recordings of MEPPs were done with two electrodes at the nmj of small Rana pipiens (1-1½ inch, body length). The electrode were positionned at the distal ends of the nmj using Nomarski optic. The MEPP amplitude recorded simultaneously by the two electrodes (A1 in elect. 1 and A2 in elect. 2) is not the same and the amplitude ratio (A1/A2) varies from one MEPP to the following. Assuming that the MEPP is due to release of neurotransmitter at one site along the n.m.j. and that the MEPP amplitude decays exponentially along the muscle fiber, it is possible to evaluate the space constant for that muscle fiber. The closest the release occurs from electrode #1, the highest the amplitude ratio (A1/A2). At the limit, the highest ratio for 1000 MEPPs represents release occurring at or very close from electrode 1. The space constant can therefore be obtained from the distance D between the electrodes and the highest amplitude ratio: = D/ln (A1/A2). Knowing the space constant for this muscle fiber, it is then possible to calculate the distance (X) between the release site and electrode #1 for every MEPPs X = (D- ln (A1/A2))/2. The distance between electrode #1 and the release site was calculated for 1000 MEPPs in each preparation. A frequency distribution of the release site distances was then made. It shows that the occurrence of spontaneous release is not equally distributed along the nmj. The release is less frequent in the distal portions of the nmj. The release frequency is distributed polymodally along the whole nerve terminal. We are currently applying this method to study the distribution of the occurrence of release along the nmj during unitary endplate potentials.

60.8 MEPP AMPLITUDE CORRECTED FOR SPATIAL DECAY ARE POLYMODALLY DISTRIBUTED. J.P. Tremblay, G. Grenon* and R. Robitaille*. Lab. of Neurobiology, Laval Univ., Dept. of Anatomy, Québec, Canada, G1J 1Z4.

MEPPs were recorded simultaneously by two intracellular electrodes placed at the distal ends of a frog neuromuscular junction (nmj). The position of the electrodes was determined by observing the junction with Nomarski optic. The relative amplitudes (A1 and A2) of the MEPPs recorded by both electrodes vary due to spatial attenuation. The frequency distribution of the amplitude of 1000 MEPPs recorded in each electrode is polymodally distributed confirming the results of Kriebel and Gross (J. Gen. Physiol. 64 (1974) 85-103), Wernig and Stirner (Nature 269 (1977) 820-822), Vautrin and Mambrini (J. Physiol. (Paris) 77 (1981) 999-1010) and Zefirov (Fiziol. Zh. SSSR, 69 (1983) 1015-1022). Zefirov has suggested that the secretion of quanta of transmitter from spatially separate areas of the nerve ending may lead to the appearance of a population of low-amplitude MEPPs and of polymodality in the distribution of MEPP amplitudes. This would contradict the subquantum hypothesis of transmitter release formulated by Wernig and Stirner. To verify whether spatial decay is responsible for the polymodality of the MEPP amplitudes observed following recording by both electrodes, we have corrected our MEPP amplitudes for spatial decay. Knowing the distance (D) between the 2 electrodes and the highest MEPP amplitude ratio, it is possible to calculate the space constant = (D/ln (A1/A2)). Using this space constant, it is then possible to evaluate the distance (X) between the release site of each MEPP and electrode #1 (see abstract by Grenon et al.). It is also possible to calculate the amplitude (Ar) of each MEPP at its release site before any exponential space decay: Ar = A1/exp (-X/)(where A1 is the MEPP amplitude in electrode #1). The amplitude at the release site was calculated for 1000 MEPPs for each preparation, and a frequency distribution of these corrected MEPP amplitudes was made. In each preparation the distribution of corrected MEPP amplitudes is significantly different from a normal distribution and is polymodal. This result indicates that the polymodal frequency distribution of MEPP amplitudes is not due to variation of the spatial attenuation of groups of MEPPs occurring more or less close from the recording electrode.

60.9 HYPEROSMOTIC SOLUTION CAN CHANGE THE MEPP AMPLITUDE DISTRIBUTION OF THE FROG NEUROMUSCULAR JUNCTION TO s-MEPPS WITH LITTLE CHANGE IN SYNAPTIC VESICLE NUMBERS. M.E. Kriebel and G.D. Pappas. Dept. of Physiology, Upstate Medical Center, S.U.N.Y., Syracuse, NY, 13210 and Dept. of Anatomy, College of Medicine, University of Illinois, Chicago, IL 60680.

Miniature endplate potentials (MEPPs) recorded from the frog neuromuscular junction show 2 classes in the adult, a small class (s-MEPPs) of 1/7 to 1/10th the size of the classical MEPP composes about 2% of the MEPPs (Gross and Kriebel, 1973, J. Gen. Physiol., 62:658a). However, the percentage of s-MEPPs is larger in the tadpole leg muscle (Kriebel and Gross, 1974, J. Gen. Physiol. 64:85-103) and can be increased by nerve stimulation until only s-MEPPs remain (Rose, Pappas and Kriebel, 1978, Brain Res., 144:213; Kriebel, 1978, Brain Res., 148:381). By studying identified junctions, Rose, et al. (1978) showed that with prolonged stimulation which reduced the evoked response to mainly failures and changed the MEPP distribution to mainly s-MEPPs, there was no vesicle depletion. Since there was considerable variation in electrophysiological conditions with nerve stimulation it is imparative to determine the ultrastructure of a given neuromuscular junction. The frog sartorius is ideal for studies of identified fibers because the edge muscles are readily located in cross sections after fixation, embedding and sectioning. After recording enough MEPPs to establish the s-MEPP mode we added sucrose to the bath to double the osmolarity. Either the edge muscle fiber was recorded from for the duration of the experiment or the edge 3 or 4 fibers were periodically sampled. The MEPP frequency greatly increased (600 fold) and stayed relatively high for 10-30 minutes. After an hour, the MEPP frequency was reduced and in some cases only s-MEPPs remained although many of the endings do not show a decrease in number of synaptic vesicles. Even though 10^5 MEPPs were generated, the time characteristic of the remaining bell-MEPPs as well as s-MEPPs did not change. However, the gaussian bell-MEPP amplitude profile was changed to a uniform distribution and the percentage of s-MEPPs was greatly increased. The variance of the s-MEPP distribution did not change. We interpret the small variance of the s-MEPP to reflect a constant subunit size and the changes in the bell-MEPP distribution and different overall distributions to reflect variations in the number of subunits (s-MEPPs) that compose the classical bell-MEPP.

60.10 STIMULUS-INDUCED ANTIDROMIC ACTIVITY AFTER NEOSTIGMINE IS PREVENTED BY NEUROMUSCULAR BLOCKADE WITH BOTULINUM TOXIN. E.Aizenman, E.F.Stanley, and G.G.Bierkamper*. Depts. of Env. Health and Neurology, The Johns Hopkins Univ., Baltimore, MD 21205, and Dept. of Pharmacology, Univ. of Nevada Sch. of Medicine, Reno, NV 89557.

Motor nerve antidromic activity produced by acetylcholinesterase inhibitors (a-AChE) was described over 40 years ago but there is as yet no conclusive evidence to show whether this activity is due to the evoked release of acetylcholine or to direct action of the a-AChE on the motoneuron terminal. In order to differentiate between these two mechanisms we have explored backfiring after blocking transmission with botulinum toxin (BOT).

Left hemi-diaphragms from female Swiss mice (23-39g) were cannulated and perfused via the central diaphragmatic vein (ca. Bierkamper, et al.,1978 J.Electrophys.Tech. 6:40) with an oxygen-saturated, HEPES-buffered Ringer solution. Stimulus induced antidromic activity (sADA) in the left phrenic nerve was elicited with a pair of wire stimulating electrodes and recorded with two monopolar silver electrodes placed distally to the stimulating site. In the normal controls sADA was observed within a minute after neostigmine (NEO,2uM) infusion;ranging from 15 to over 70 spikes and lasting for up to 50 msec after stimulation. The number of antidromic action potentials remained at a constant level for at least 40 min (10 min NEO, 30 min wash), and could be reversibly blocked with decamethonium (.8uM) or curare (.1uM). Treatment with a lethal dose of BOT (100ug I.P.) 40 min prior to dissection resulted in a virtually complete blockade of neuromuscular transmission (Stanley,et al.1983 Brain.Res.261:172). Backfiring could not be induced in these preparations after NEO infusion, even though it could be confirmed that the conditioning action potential invaded the nerve terminal. Thus, these results suggest that the occurrence of sADA requires the release of acetylcholine and that NEO cannot by itself produce backfiring.

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60.11 DECREASED QUANTAL CONTENT ASSOCIATED WITH DITHIOBIURET-INDUCED PARALYSIS IN THE RAT. William D. Atchison. Dept. of Pharmacol./Toxicol. and Center for Environ. Toxicol., Michigan State University, East Lansing, MI 48824.

Daily treatment of rats with dithiobiuret (DTB, 1 mg/kg/day, ip) produces a flaccid, ascending neuromuscular weakness after 5-6 days of treatment. This condition is characterized by diminished contractile strength in pancuronium-paralyzed preparations following single shock and tetanic stimulation of the sciatic nerve, but no effect on contractions elicited by direct muscle stimulation (Atchison et al., Neurotox. 2: 329-346, 1981), indicating an apparent impairment of motor axon conduction, junctional transmission or both. The purpose of the present study was to characterize further the potential neuromuscular effects produced by DTB using conventional intracellular microelectrode recording techniques. All experiments were conducted using the extensor digitorus longus muscle isolated from male rats treated for 6-7 days with 1 mg/kg/day DTB (ip) or with 0.9% NaCl (1 ml/kg/day) as control. End-plate potentials (EPPs) and miniature end-plate potentials (MEPPs) were recorded from single junctions of DTB-poisoned or NaCl-treated paired controls using conventional techniques. Amplitude of EPPs recorded in d-tubocurarine-paralyzed preparations was decreased in DTB-treated rats. The depression of EPP amplitude was more pronounced when stimulus frequency was increased from 0.5 to 2 and 5 Hz. Decreased EPP amplitude was associated with a decrease in mean quantal content. End-plate resting membrane potential was not affected. In some preparations in which transmission was blocked with elevated Mg^{2+} and lowered Ca^{2+} concentrations (4 mM and 1 mM, respectively), stimulation of the motor nerve from DTB-poisoned rats was associated with a complete failure to evoke an EPP, while use of a similar paradigm in control preparations was associated with the normal pattern of fluctuating EPP amplitude. Finally, in 3 DTB-poisoned animals, subthreshold EPPs were recorded from preparations in which neuromuscular transmission was not blocked with either tubocurarine or Mg^{2+}, an effect not observed in controls. Mean MEPP frequency was similar for DTB-treated and control groups. Mean MEPP amplitude was increased in the DTB-treated group and DTB-paralyzed preparations were characterized frequently by the presence of very large MEPPs with prolonged decay times. Thus, paralysis induced by chronic DTB treatment appears to be associated with an impairment of presynaptic processes resulting in a diminished number of quanta liberated in response to motor nerve stimulation. (Supported by a starter grant from the Pharmaceutical Manufacturers Association Foundation and by NIH grants ES03299 and ES00560.)

60.12 THE RELEASE OF GABA FROM GOLDFISH HORIZONTAL CELLS. George S. Ayoub and Dominic Man-Kit Lam, Program in Neurosci and Cullen Eye Inst, Baylor Coll of Med, Houston, TX 77030.

In the goldfish retina, H1 horizontal cells, which receive input from red-sensitive cone photoreceptors, possess a high-affinity uptake mechanism for GABA. The accumulation of GABA by these cells is enhanced by light stimulation of the retina, which hyperpolarizes the H1 cells. In contrast, GABA may be released by incubation in darkness. The cellular mechanisms regulating these stimulation-dependent processes were examined by dissociating fish retinas into single cells and obtaining a fraction enriched with horizontal cells containing few synaptic endings. The release of recently accumulated GABA was examined by preloading the cells with 1uM, 1uCi/ml ^{3}H-GABA, blocking further uptake with 100uM nipecotic acid, and examining the effects of various agents in eliciting release. The accumulated GABA could be released in a quantitative manner by K-stimulation. In addition, micromolar concentrations of either L-glutamic acid or L-aspartic acid, the leading transmitter candidates for cone photoreceptors, were effective in eliciting release, with glutamate being more effective at saturating concentrations. Confirmation of the isolated cell findings were made on intact, radiolabeled retinas, in which, by adjusting the lighting and incubation procedures, preferential labelings of horizontal or amacrine cells with ^{3}H-GABA were made. Release experiments on these preparations reveal that while GABA release from amacrine cells is Ca-dependent, the release of preloaded GABA from H1 cells is primarily independent of extracellular Ca.

To correlate the ^{3}H-GABA release findings with the release of endogenous GABA, the amino acid contents of aliquots of isolated horizontal cells were determined with high performance liquid chromatography (HPLC). Isolated cells were incubated in K-substituted media in the presence of external Ca, or in Ca-free solutions containing 20mM Mg. The amino acids were extracted by homogenization in 1N acetic acid and this homogenate analyzed on reverse phase HPLC. Endogenous GABA can be released from isolated cells by depolarization with external K, and this release mimics the form observed with preloaded GABA. Endogenous GABA release has a significant Ca-dependent component, which predominates under small depolarizations. Of interest, only glutamate, and not aspartate, was effective in eliciting release of the endogenous GABA.

60.13 ANION PERMEABILITY OF MOUSE MOTOR NERVE TERMINALS. J. G. McLarnon*, D. M. J. Quastel and D. A. Saint*. Department of Pharmacology & Therapeutics, The University of British Columbia, Vancouver, B. C., V6T 1W5, Canada.

In mouse diaphragm, in vitro, the frequency of miniature end-plate potentials (F_{mepp}) is sensitive to alteration of nerve terminal membrane potential by change of external K^+ or extrinsic polarization. We now find that in the presence of 15 mM K^+, F_{mepp} is increased by partial substitution of Cl^- by other anions. The amplitude of the response is in the order $Br^- < NO_3^- < I^- \cong$ acetate. With NO_3^-, Br^- and acetate, responses are transient; F_{mepp} rises to a peak of up to 10 times control (with acetate) within about 1 min and then gradually subsides to near control with a time constant of the order of 5 min. After equilibration, switching back to Cl^- solution results in a transient reduction of F_{mepp}, with a return to control with much the same time constant. In 5 mM K^+ (where F_{mepp} is insensitive to moderate depolarization of the nerve terminal) substitution of Cl^- by other anions results in little change of F_{mepp}. However, threshold for nerve terminal action potential generation (elicited by focal current pulses) is transiently lowered by substitution of Cl^- by NO_3^-, keeping constant the conductivity of the solution. Both the change in threshold and increase of F_{mepp} (in raised K^+) with NO_3^- were consistent with a transient depolarization of about 8 mV, as would be expected if the Cl^- permeability of the nerve terminal membrane is not very different from its K^+ permeability. The apparent permeability sequence for anions - $Cl^- > Br^- > NO_3^- > I^- \cong$ acetate - is the same as that reported for mammalian and amphibian skeletal muscle; the absolute permeability per membrane area, estimated from the time course of equilibration, is much less than that of skeletal muscle membrane.

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60.14 MITOCHONDRIAL AND NON-MITOCHONDRIAL CALCIUM UPTAKE DURING HYPOXIA AND 3,4-DIAMINOPYRIDINE TREATMENT. C. Peterson and G. Gibson. Cornell Univ. Medical Coll., Burke Rehabilitation Center, White Plains, New York.

The transport and intracellular compartmentation of calcium in the nerve ending are important for neurotransmitter release. Low oxygen decreases synaptosomal calcium uptake and the calcium-dependent release of acetylcholine in parallel. To determine whether these deficits are due to altered mitochondrial or non-mitochondrial calcium homeostasis, synaptosomes were incubated for varying times under 100% or 2.5% oxygen in the presence of high potassium and calcium-45. Total calcium uptake was terminated by EGTA/ruthenium red treatment and rapid centrifugation through oil. Calcium uptake by mitochondria within the synaptosomes was determined after digitonin and shear force disruption of the synaptosomes in the presence of EGTA/ruthenium red and rapid centrifugation through oil. The non-mitochondrial compartment was the calculated difference between total and synaptosomal mitochondrial calcium uptake. A decline in the oxygen tension from 100 to 2.5% reduced total calcium uptake from 0.80±0.02 (nmol/mg protein/min) to 0.53±0.02 and mitochondrial calcium uptake from 0.70±0.01 to 0.38±0.01. Thus, the calculated non-mitochondrial uptake increased 50% from 0.10±0.01 to 0.15±0.01. 3,4-Diaminopyridine (10 nM) increased total uptake to 0.85±0.02 or 0.88±0.01 under 100% or 2.5% oxygen, respectively. 3,4-Diaminopyridine elevated mitochondrial calcium uptake to 0.77±0.01 or 0.72±0.01 under 100% or 2.5% oxygen, respectively. However, the calculated non-mitochondrial compartment was unaltered by 3,4-diaminopyridine. These findings suggest that the reversible deficits due to low oxygen may be due to diminished calcium accumulation of calcium by the mitochondria. The non-reversible increase in the non-mitochondrial compartment may eventually lead to pathological damage.

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60.15 WITHDRAWN

61.1 NICOTINIC RECEPTOR BLOCKADE BY Ca^{++}-CHANNEL ANTAGONISTS IN FROG SKELETAL MUSCLE. L. Adam* and E.G. Henderson. Dept. of Pharmacology, University of Connecticut Health Center, Farmington, CT 06032.

We have previously demonstrated that Ca^{++}-channel antagonists block [^{3}H]-PCP binding to Torpedo membranes while not affecting the kinetics of endplate currents in cutaneous pectoris muscle (Epstein et al., Soc. for Neurosci. 9:735, 1983). In the current study, carbomylcholine (CARB 10^{-5}M) induced contractures in frog sartorius muscle were blocked by methoxyverapamil, nicardipine, nitrendipine and bepridil in a concentration and time dependent manner. Indirectly elicited and direct muscle stimulated twitch were unaffected by any of these drugs. The block of the CARB-induced contracture by nicardipine (10^{-7}M) had a half-time of 30 minutes, while that for (10^{-6}M) was about 10 minutes. Bepridil was equipotent with nicaripine while nitrendipine and methoxyverapamil were about 10-fold less potent. Unexpectedly, the Ca^{++} channel agonist, Bay K 8644 (10^{-6}M), also antagonized the CARB-induced contracture with a time course similar to nitrendipine. Acetylcholine (100 μM) induced contractures were similarly blocked by these agents. The block was independent of $[Ca^{++}]_o$ (0-10 mM) or pH (6.3 - 8.1). Tetanizing contractures caused by nerve stimulation (50 Hz for one sec every minute) were unaffected by the antagonists. Verapamil (10 x 10^{-6}M) and methoxyverapamil (10^{-6}M) also blocked CARB (5 x 10^{-5}M) induced fluxes of ^{42}K, ^{22}Na and ^{45}Ca in denervated muscle bathed in Na^+-free (sucrose substituted) Ringer solution. The mechanism by which these agents block the response to exogenous nicotinic agonist while not affecting endogenous agonist remains to be determined.

61.2 CHARACTERISTICS OF FAST SYNAPTIC CURRENTS ARE ALTERED BY A REDUCTION IN EXTERNAL SODIUM. R.L. Parsons, D.S. Neel* and E.A. Connor. Dept. of Anatomy and Neurobiology, Univ. of Vermont, Burl., VT 05405 and Dept of Neurobiology, Stanford Univ., Stanford, CA 94305.

The influence of a reduction in extracellular sodium concentration on characteristics of nicotinic fast excitatory postsynaptic currents (EPSCs) has been studied in voltage-clamped sympathetic ganglion B cells of the bullfrog. Lithium substitution for sodium influenced both the EPSC size and decay timecourse. In either a 50% or 100% lithium-substituted solution, the EPSC decay was faster than that of control EPSCs. With a 50% replacement of lithium for sodium, the EPSC size at -50mV was similar to control values. However, with a 100% substitution, the EPSC size was significantly reduced below control values although the voltage dependence of the decay τ, the shape of the peak EPSC-voltage relationship, or the EPSC reversal potential was not changed by replacing lithium for sodium. The change in EPSC size and decay τ in the lithium solution was due to the presence of lithium and not simply the consequence of a reduction in the external sodium concentration. For instance, with a 50% substitution of sucrose or mannitol for sodium chloride the EPSC decay was slowed. EPSC size at -50mV and the voltage dependence of τ was similar to control values when 50% of the sodium was replaced by sucrose or mannitol. The peak EPSC-voltage relationship was linear in cells exposed to either the control or the 50% sucrose-substituted solution, although the EPSC reversal potential was shifted to a more negative voltage with 50% sucrose substitution. Facilitation of phasic transmitter release, estimated using paired pulses, was depressed below control levels in those cells exposed either to a 100% lithium-substituted solution or a 50% sucrose-substituted solution. This suggested that phasic transmitter release is enhanced in both experimental conditions. Consequently, the decrease in EPSC size observed in lithium-treated cells resulted from a decrease in postsynaptic effectiveness rather than a decrease in EPSC quantal content. We conclude that the kinetics of receptor-channel gating of the ganglionic fast EPSC are sensitive to an alteration in the concentration of the external sodium concentration. Further, the influence of lithium substitution on ganglionic EPSC decay is opposite that reported for muscle MEPC decay. This suggests that the nicotinic channels in postganglionic cells and at the motor end-plate may have different pharmacological properties. Supported by NSF and MDA Grants.

61.3 KINETIC PROPERTIES OF TWO CLASSES OF ACh RECEPTOR CHANNELS IN CULTURED XENOPUS MUSCLE CELLS. Y. Igusa* and Y. Kidokoro. The Salk Institute, San Diego, CA 92138.

A gradual shortening of the mean open time of the acetylcholine (ACh) receptor channels has been demonstrated during early developmental stages in rat muscle cells with innervation (Sakmann and Brenner, 1978) and in cultured Xenopus muscle cells without innervation (Brehm et al.,1982). In single channel current recording, two distinct classes of events were observed, namely, events with a long open time and a low-amplitude and ones with a short mean open time and a high-amplitude. It was interpreted to mean that there are two classes of ACh receptor channels and that shortening of overall channel open time is due to a shift of channel population to ones with a short mean open time. The purpose of the present study is to further investigate the properties of two classs of events and particularly to determine whether these two types are in fact different channels or represent two different states of one type of channels. Dissociated myotomal muscle cultures were prepared from embryos of Xenopus laevis. Single ACh receptor channel currents were recorded on muscle cells 2 to 8 days after dissociation using the giga-seal technique with the cell attached configuration. The concentration of AChCl contained in internal solution of the patch electrode was varied between 0.2 and 100μM. At 0.2μM ACh, the two classes of events were observed as reported previously. However, when the burst events appeared at higher concentrations of ACh (10-100μM), only one class of events was observed during a given bursting period. This result indicates that one class of channels does not convert into the other class during the bursting period of up to a few seconds. While the mean apparent open time during burst ranged between 1.0 and 2.9 msec at 100μM ACh in high-conductance events and 2.3-3.3 msec in the low-conductance events, these apparent mean open times were similar to those at 0.2μM ACh. The mean close time was 0.2-0.5 msec for both high- and low-conductance events at 100μM ACh. In high concentrations of ACh, the current fluctuations during the open state of high-conductance events was more prominant than those in low-conductance events. Our observations are consistent with the hypothesis that two classes of channel events correspond to two classes of channel molecules.

Sakmann, B. and Brenner, H.R. (1978), Nature (Lond.) 276:401-402. Brehm, P., et al. (1982), Develop. Biol. 91:93-102.

61.4 ABNORMAL T-TUBULE SYSTEM AND DECREASE IN VOLTAGE-DEPENDENT Ca^{++} CHANNEL BLOCKER SITES IN EMBRYONIC MUSCULAR DYSGENESIS (mdg/mdg) SKELETAL MUSCLE. M. Pinçon-Raymond, F. Rieger, M. Fosset and M. Lazdunski. INSERM U 153 - 17 rue du Fer à Moulin 75005 PARIS and Institut CNRS Parc Valrose 06034 NICE - FRANCE.

Muscular dysgenesis (mdg) in the mouse is a spontaneous, recessive lethal mutation expressed in the mouse embryo as a total lack of muscle contractile activity, (excitation - contraction uncoupling). The internal structure of the mdg/mdg myofiber is disorganized and the transverse tubule system is immature. An ultrastructural study of the embryonic diaphragm shows dilatation of the sarcoplasmic reticulum (SR) and fragmented and disorganized myofibrils as early as days 13-14 of gestation. Peripheral couplings of sarcoplasmic reticulum and plasma membrane similarly occur in normal or mdg/mdg developing myotubes. However, structural coupling of sarcolemma and SR (diads, triads) is not observed or is extremely immature in mdg/mdg myotubes, although these structures show considerable development in normal myotubes. It has been previously demonstrated that voltage-dependent Ca^{++} channels, characterized by high affinity ^{3}H-nitrendipine binding, are preferentially located in purified transverse tubule membranes from rabbit skeletal muscle (FOSSET et al 1983, J. Biol. Chem., 258, 6086-6092). We found in mdg/mdg diaphragm, limb or tongue muscles an important decrease of the specific ^{3}H-nitrendipine binding per mg of protein (about 50 % of control (+/mdg ?) values). Thus, the lack of a differentiated T-tubule system (diads-triads) in mdg/mdg muscle is correlated to an important, although not total, deficit in voltage-dependent Ca^{++} channels. These observations may have two important implications for muscular dysgenesis : 1) The lack of a well-differentiated physical coupling between sarcolemma and SR in mdg/mdg muscle may explain that membrane depolarization is not efficiently transduced to the SR. 2) The decrease of voltage-dependent Ca^{++} channels may represent the molecular alteration of the excitation-contraction coupling which is related to abnormal SR Ca^{++}-permeation and/or accumulation (supported by MRI).

61.5 INTRACELLULAR Ca^{2+} ACTIVITY INCREASES DURING SYNAPTIC POTENTIALS IN HELIX. G.R.J. Christoffersen* and L. Simonsen* (Spon: Lee Miller). Zoophysiological Lab., Univ. of Copenhagen, Denmark.

In cell RPal of Helix pomatia the right pallial nerve evokes three consecutive inhibitory synaptic potentials: ILD-I (loo msec range), ILD (seconds) and LLH (minutes). (ILD = inhibition of long duration; LLH = long lasting hyperpolarization). The LLH arises primarily as the result of potassium permeability increase caused by a transient enhancement of intracellular Ca^{2+} activity ($a_{i,Ca}$) from a steady state value of 30 nM to 75 nM, presumably due to release from intracellular stores (Christoffersen & Simonsen, Comp. Biochem. Physiol. 76C:351, 1983).

Presently, ILDs were observed while $a_{i,Ca}$ was monitored with a Ca-selective neutral carrier type micro-electrode. During ILD the electrode potential increased by 1 mV corresponding to an enhancement of $a_{i,Ca}$ at the electrode tip by a factor of 1.2. The signal to noise ratio was approx. lo and the respons time for the electrode was about lo times shorter than the time constant for activation of ILD. In 4 experiments, ILDs were elicited soon after electrode penetration during the period when $a_{i,Ca}$ slowly decreased towards the steady level. When $a_{i,Ca}$ was still above 100 nM the ILD-Ca signal had a negative polarity, typically -3mV. Later, at 100 nM the signal disappeared , and below loo nM positive signals of 1-2 mV occured. The existence of a 'reversal activity' for the direction of change in $a_{i,Ca}$ was tested by raising $a_{i,Ca}$ from the steady level up to loo nM through increase of the extracellular Ca^{2+} activity. Again no Ca-signal appeared during ILD. The polarity of ILD itself was always negative regardless of the level of $a_{i,Ca}$. Dopamine (lo^{-6} M) mimicked and cis-flupenthixol (lo^{-6} M) inhibited ILD and ILD-Ca signals.

It is tentatively suggested that during ILD, dopamine induced, $a_{i,Ca}$-dependent affinity changes for Ca^{2+} occur in a protein having a dissociation constant for Ca^{2+} near loo nM.

61.6 GABA MEDIATES A CALCIUM-DEPENDENT POTASSIUM CONDUCTANCE IN HIPPOCAMPAL CA1 PYRAMIDAL CELLS. T.J. Blaxter*, M.F. Davies, P.L. Carlen and N. Gurevich (SPON R. Blair).Playfair Neuroscience Unit, Toronto Western Hospital; Addiction Research Foundation Clinical Institute; Depts. of Medicine and Physiology, University of Toronto, Ontario, Canada.

GABA is known to hyperpolarize hippocampal pyramidal cell bodies and to depolarize their dendrites. These responses appear to be largely chloride dependent. With agonists other than GABA, such as THIP and ethylenediamine, a prominent hyperpolarization of the dendrites is seen which is resistant to picrotoxin and bicuculline.

In rat hippocampal slices, we have blocked the depolarizing response of CA1 dendrites to GABA with picrotoxin, revealing an underlying hyperpolarizing response to GABA. This response is not chloride dependent (Blaxter and Cottrell, 1982, J. Physiol. 330 46P). Instead, it appeared to be potassium dependent, since perfusion of the slice with low $[K^+]$ solution increased the size of the hyperpolarizing response despite the small tonic hyperpolarization produced by low $[K^+]$. In addition, perfusion of the slice with zero $[Ca^{2+}]$ solution reversibly blocked the hyperpolarizing response to GABA. This saline also reversibly blocked synaptic transmission and the long-lasting part of the post-spike afterhyperpolarization which is due to a Ca^{2+}-dependent K^+ conductance.

We conclude that this hyperpolarizing response of the dendrites to GABA is a Ca^{2+}-dependent K^+ conductance and may be part of the physiological response to synaptically released GABA.

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61.7 HISTAMINE AND NOREPINEPHRINE DECREASE CALCIUM CURRENT IN HIPPOCAMPAL PYRAMIDAL CELLS. T. C. Pellmar. Physiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814.

Work by others (Haas & Konnerth, Nature, 302:432, 1983; Madison & Nicoll, Nature, 299:636, 1982) suggested that both norepinephrine (NE) and histamine (HA) block the calcium-mediated potassium current (C-current) in hippocampal pyramidal cells. Using the single-electrode voltage clamp technique, I reexamined the mechanism of action of NE and HA on CA1 pyramidal cells in the guinea pig hippocampal slice preparation. In all experiments tetrodotoxin was present to block sodium spikes. As expected from the previously hypothesized mechanism, HA (1 uM) and NE (10 uM) decreased the outward current in the depolarized region of the current-voltage (I-V) relationship where C-current predominates. When manganese was present in the bathing solution, the transmitters were without effect. M-current (which could be blocked by 20 uM muscarine) and Q-current were unaffected by 10 uM HA.

A decrease in C-current could result from a direct action of HA or NE on calcium inward current. This possibility was tested by blocking potassium currents with 15 mM tetraethylammonium or with barium replacement for calcium. Under these conditions, the I-V relationship revealed a negative slope region due to regenerative inward calcium current. HA (1 uM) and NE (10 uM) reduced this inward current.

These results suggest that both HA and NE directly decrease calcium current and thereby decrease C-current in CA1 pyramidal cells of the hippocampus.

61.8 SMALL EPSPS IN HIPPOCAMPAL CA1 PYRAMIDAL CELLS-COMPARISON OF CABLE MODEL PREDICTIONS AND SYNAPTIC MICROPHYSIOLOGY. D.A. Turner. VAMC, Univ. Minnesota, Minneapolis, MN 55417.

Factors influencing the shape and efficacy of small EPSPs (<1-2mV) include synaptic location, dendritic spines, passive cable transfer, IPSPs and active currents. I have evaluated passive geometry and spines in CA1 pyramids with a detailed cable model (Turner, Biophys. J., July, 1984). This model predicts that small proximal and distal EPSPs should behave classically, with clear differences in EPSP rise time and halfwidth. However, large EPSPs (> 5mV) in these cells do not demonstrate such differences (Andersen et al, J. Physiol., 1980). I have analyzed small averaged EPSPs to evaluate the discrepancy between model predictions and previous reports.

Somatic EPSPs were recorded in response to proximal and distal afferent stimulation (apical electrode separation of 350-400 μm). Averaging of 50-100 postsynaptic responses revealed small EPSPs in the range of 150-500 μV. A threshold could usually be defined between the absence or presence of an averaged response, though individual failures could not be discriminated. All CA1 pyramidal cells (n=14) showed a clear difference in rise times between small proximal (2.6-4.6 msec) and distal (3.8-7.5 msec) EPSPs. These EPSPs were not, however, as well separated as in the model predictions. The model suggested a proximal/distal rise time ratio of 1/4, whereas the measured values were only 1/1.5-1/2. These values are consistent with an electrotonic separation of 0.3-0.4 λ within an apical dendrite of 0.6-0.7 λ.

Progressively larger stimulation revealed two major changes in the EPSP waveforms. In some cells (n=4) a small IPSP (400-600 μV) could be recruited at a sharp threshold. This IPSP had an onset within 2-4 msec after the stimulus, and truncated most of the rising phase of the EPSP. This finding suggested that recruitment of feed-forward IPSPs may alter EPSP rise time as well as halfwidth. The other change occurring with larger stimulation was recruitment of successively more proximal (and potent) EPSPs, presumably due to fanning of the afferents.

Thus, small proximal and distal EPSPs generally followed the model predictions, though with less electrotonic separation than assumed. The transition to larger EPSPs suggested that the most potent factor in adjusting EPSP waveform shape and efficacy may be early, feed-forward IPSPs. Both the addition of IPSPs and afferent fanning in an electrically compact dendritic tree may contribute to the near-identity of larger proximal and distal EPSPs in these neurons.

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61.9 EXTRACELLULAR VOLTAGE GRADIENTS AND EPHAPTIC INTERACTIONS IN THE HIPPOCAMPAL FORMATION.
TL Richardson, RW Turner and JJ Miller, Dept. Physiol, UBC, Vancouver, BC, Canada V6T 1W5

Recent studies indicate an important role for field effects (ephaptic interactions) in the recruitment and synchronization of hippocampal neuronal activity. Since field effects are thought to be the result of passive current flow along the dendro-somatic axis, laminar profiles of extracellular fields were examined in order to measure voltage gradients in CA1 and dentate regions of the hippocampal slice.

Stimulating electrodes were placed in the stratum radiatum or perforant path for orthodromic activation of CA1 pyramidal cells or dentate granule cells, respectively. Averaged extracellular records were obtained at 15-20 locations, each displaced 25u along the dendro-somatic axis. Voltage gradients were constructed by taking the absolute voltage at a constant latency for each field potential within the profile and plotting these values against distance. Voltage measurements were taken at latencies corresponding to the peak of the extracellular EPSP (P1) or the population spike (N1) at the cell layer.

In CA1, voltage profiles at the N1 latency exhibited a discrete negativity corresponding to the cell layer. The steepest gradient occurred between the basal dendrites and soma as would be required for passive current flow leading to ephaptic depolarization of pyramidal cells. The absence of a sharp dendro-somatic gradient on the N1 profile for the dentate suggests that ephaptic depolarization may be less potent in this region. Analysis of the profile for the P1 latency indicates a sharp soma-dendritic gradient in the dentate but a relatively shallow gradient in CA1. The presence of this steep gradient in the dentate indicates that the extracellular voltage present during P1 may result in a greater depression of the somatic transmembrane potential in this region. The observed differences in the voltage gradients of the N1 and P1 profiles recorded in CA1 and dentate suggests that the ephaptic interactions in these regions may be fundamentally different.

61.10 FOLIC ACID IS A GAMMA-AMINOBUTYRIC ACID ANTAGONIST, NOT A KAINIC ACID AGONIST, IN THE RAT HIPPOCAMPAL-SLICE PREPARATION. L.C. Otis*, D.V. Madison, and R.A. Nicoll (SPON: A.J. Hudspeth). Departments of Pharmacology and Physiology; and Graduate Program in Neuroscience, University of California, San Francisco, CA 94143.

Folic acid, a dicarboxylic acid, has long been known to produce epileptiform activity when applied to the brain. Recently, a controversy has arisen as to whether folic acid produces this excitation by binding to the receptors that also bind kainic acid or by acting postsynaptically as a gamma-aminobutyric acid (GABA) antagonist, as suggested in earlier studies. We have studied the effects of folic acid (100-500 uM) on rat hippocampal CA1 pyramidal cells in an in vitro slice preparation, using intracellular recording to determine the mechanism of folate-mediated excitation. Application of folic acid caused the appearance of multiple population spikes in the extracellularly recorded field potential. Intracellular recordings, however, revealed that folic acid, unlike kainic acid, caused no depolarization. Instead, folic acid greatly reduced the rapid, Cl^--mediated phase of the inhibitory postsynaptic potential (IPSP), while prolonging the slower, K^+-mediated phase. Furthermore, application of folic acid abolished spontaneous IPSPs. All of these effects were reversible on washing with drug-free medium. Finally, bath-applied folic acid reduced the hyperpolarization produced by iontophoretically applied GABA while producing little or no change in membrane potential. Based on these results, we conclude that folic acid produces its excitatory effects on hippocampal pyramidal cells by a disinhibitory action. We propose that folic acid acts in the hippocampus as a GABA antagonist, not as a kainic acid agonist.

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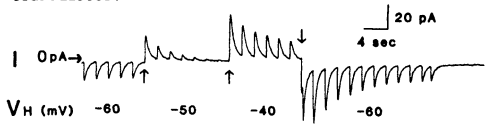
61.11 REPETITIVE GABA APPLICATION LEADS TO SHIFTS IN GABA REVERSAL POTENTIAL: A VOLTAGE CLAMP STUDY IN ACUTELY ISOLATED HIPPOCAMPAL CELLS. J.R. Huguenard and B. E. Alger, Dept. Physiol., Univ. Md. Sch. Med., Baltimore, MD 21201.

Studies on use-dependent modifications of GABAergic ipsps in rat hippocampus (McCarren and Alger, Neurosci. Abst. 9:603 1983) suggested that shifts in E_{ipsp} can occur quickly in response to repetitive activation of the ipsps. However, studies in vivo or in the in vitro slice preparation are complicated by overlapping epsps and changes in extracellular ion concentrations. Direct application of GABA can lead to desensitization and to depolarizing responses due to activation of an extrasynaptic GABA receptor.

Whole-cell voltage clamping of acutely isolated hippocampal cells (Numann, et. al., Neurosci. Abst. 8:413 1982) may simplify the study of GABA responses. We find that these cells (at 24°C) have membrane potentials $\simeq$-55 mV, overshooting action potentials, input resistances $\gtrsim$1.0 gigaohm, and time constants $\simeq$30 ms. Using this preparation we have been studying the rate and voltage dependence of shifts in E_{GABA} caused by 5-30 ms pressure pulses of GABA (1 mM), which produce conductance increases of 4-10 nS. When GABA pulses are given at 0.5 Hz, a 20 mV change in holding potential (V_H) can cause a 10 mV shift in E_{GABA} which occurs with a time constant of 7.2 $\pm$ 2.8 s (n=21). The time constant is dependent on the rate of GABA application. Although with rapid rates of GABA application successive responses clearly overlap (see figure) shifts in E_{GABA} occur even with pulses given at 20 s intervals, when responses do not overlap. Shifts in E_{GABA} occur in the absence of changes in GABA-activated conductance. Thus E_{GABA} shifts quickly in response to repetitive elicitation of small GABA responses. This phenonemon may be physiologically relevant since GABA is a major inhibitory transmitter.



The current responses above were elicited by repetitively pulsing GABA onto a cell every 2 s (the inward currents at V_H of -60 mV were due to slightly elevated [Cl] in the recording pipette). V_H was stepped from -60 to -50 (↑) to -40 (↑) and to -60 mV (↓). The decreases in currents following each step are apparently not due to desensitization.

61.12 DOPAMINE ENHANCEMENT OF A SPIKE-EVOKED CURRENT IN THE APLYSIA BURSTING NEURON R15. W.W. Anderson, W.A. Wilson*, and D.V. Lewis. Epilepsy Center, V.A. Hospital and Dept. of Pediatrics, Duke Univ. Med. Center, Durham, NC 27705.

The ability of 50-500 uM dopamine (DA) to inhibit bursting and the inward current which produces negative resistance in R15 has served as a model of neurotransmitter inactivation of an 'endogenous' bursting pacemaker mechanism (Boisson and Gola, Comp. Biochem. Physiol., 1976; Wilson and Wachtel, Science, 1978). We report here that DA at much lower concentrations (1-5 uM) greatly enhances the net outward current during a depolarizing voltage clamp pulse that follows a train of spikes, but causes little or no decrease in the inward current during a depolarizing clamp pulse not preceded by a train of spikes.

R15 was studied in vitro in the isolated abdominal ganglion of Aplysia californica. The cell was voltage clamped to a holding potential of about -60 mV. Every 30 sec the cell was clamped to a depolarizing voltage (ca. -40 mV) for 3 sec, which produced a persistent inward current (Ii). Every 5 min (i.e. every 10th pulse) a 2 sec train of 12-40 spikes (in current clamp) preceeded the depolarizing clamp pulse, which now produced a net outward current (Io).

Bath application of 1-5 uM DA caused a slight (0-5 nA) decrease in Ii, whereas Io was increased by up to 33 nA. The increase in Io by DA was 2.5 to over 25 times larger than the decrease in Ii by DA, and therefore showed a wide range of Io enhancement versus Ii decrease. In one preparation, 1 uM DA increased the Io from 0.0 to +15.5 nA, but decreased the Ii by only 0.7 nA (from -12.0 to -11.3 nA). Increasing the number of spikes/train increased Io in normal saline and in DA, and therefore increased the relative Io enhancement versus Ii decrease in DA. Measurement of spike duration during the spike train showed no observable change in DA. DA induced activity was reversed in normal saline wash.

These results demonstrate a different action of DA on R15 that occurs at low concentrations: the enhancement of a spike-evoked current rather than the reduction of the persistent inward current. Whether the DA enhanced, spike-evoked net outward current represents the activation of an outward current or the inactivation of an inward current remains to be determined.

61.13 A FUNCTION OF ARACHIDONIC ACID IN THE PHOSPHATIDYLINOSITOL CYCLE. D.T. Dudley* and A.A. Spector* (SPON: C.V. Gisolfi), Dept. of Biochemistry, Univ. of Iowa, Iowa City, IA 52242.

The inositol phospholipids are believed to play a major role in mediating the signal transduction for a variety of membrane receptors. Additionally, inositol phospholipids contain a large amount of esterified arachidonic acid (20:4). We are investigating the role of 20:4 in these phospholipids and its relationship to the phosphatidylinositol (PI) cycle. Results in Y79 retinoblastoma and GH3 pituitary cells show that PI has a unique ability to incorporate and retain 20:4 as compared to the other major cell phospholipids. Y79 cell phospholipids can be depleted of 20:4 by maintenance in serum-free medium. When 20:4 depleted cells are then enriched in 20:4 by addition of 20:4 to the medium, they exhibit an increased labeling of PI and phosphatidic acid with ^{32}Pi when stimulated with either carbachol (CCh) (for PI, 3.7±0.41 for enriched and 1.86±0.41 for depleted, fold labeling over control, n=5) or the calcium ionophore A23187 (15.17±0.46 vs 3.7±0.41, n=5). The CCh stimulated labeling can be blocked by atropine but not by d-tubocurarine, suggesting involvement of muscarinic receptors. Therefore, the formation of PI and PI cycle activity appear to be increased when cells are enriched with 20:4. When GH3 cells are labeled to constant specific activity with ^{32}Pi or ^{14}C 20:4, 75% of the phosphatidylinositol 4,5-bisphosphate (PIP_2) is labeled with ^{32}Pi, but only 30% with ^{14}C 20:4. When these cells are then stimulated with thyrotropin-releasing hormone, which activates the PI cycle, there is an equivalent percentage decrease in the amount of ^{32}P (54.9±1.3, n=3) and ^{14}C (58.9±1.5, n=3) in the PIP_2 fraction. This shows that while PIP_2 containing 20:4 is sensitive to hormonal stimulation, there is no preferential degradation of PIP_2 containing 20:4. Since PI labeling is increased when cells are enriched with 20:4, but the 20:4 content does not facilitate hydrolysis, it appears that 20:4 may have a role in redirecting the diacylglycerol intermediate back to PI. (Supported by NIH grant AM28516).

61.14 COMPARISON OF MEMBRANE PROPERTIES AND MECHANISMS OF SYNAPTIC TRANSMISSION IN EMBRYONIC CHICK LUMBAR SYMPATHETIC AND CILIARY GANGLIA. S.E. Dryer and V.A. Chiappinelli. Dept. of Pharm., St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

We have examined membrane properties and patterns of slow and fast synaptic transmission in embryonic (18-21 d.i.) chick autonomic ganglia as a prelude to more detailed developmental electrophysiological investigations. We find that the properties of ciliary ganglia neurons are markedly different from those of the sympathetic cells.

Membrane Properties. Chick ciliary ganglia neurons have short duration action potentials and can fire at very high frequencies (100 Hz). Input resistance is 35-90 MΩ. Application of large hyperpolarizing current pulses produces a marked Cs^+-sensitive anomalous rectification that becomes more pronounced with larger pulses. Exposure to 3 mM Ba^{+2} increases input resistance and induces multiple spiking but does not affect spike duration. This suggests that chick ciliary neurons contain Im and Ih channels. In contrast, chick sympathetic neurons have longer duration action potentials and can only follow at 20 Hz. Input resistance is high (70-260 MΩ). Application of small hyperpolarizing pulses reveals the presence of Im channels. No Ih channels are detected. Addition of 3 mM Ba^{+2} to the superfusate evokes multiple spiking but also broadens the action potential duration as much as 100 fold. The mechanism of delayed rectification in chick sympathetic neurons may therefore differ from that in ciliary neurons.

Synaptic Transmission. Chick ciliary ganglia cells are singly innervated. A combined electrical-chemical synapse is often present. A single preganglionic stimulus will evoke one or two action potentials in the postganglionic cells. No slow e.p.s.p.s are observed. In contrast, chick sympathetic neurons are multiply innervated. Simultaneous activation of more than one preganglionic fiber is required to evoke action potentials. Two types of non-cholinergic slow e.p.s.p. can be evoked by repetitive stimulation of the preganglionic nerves. One of these persists at the potassium equilibrium potential and is therefore not due to inhibition of Im. The second slow e.p.s.p. is associated with an increase in input resistance and may be due in part to inhibition of Im. This slow e.p.s.p. is mimicked by pressure injection of substance P, which is therefore a candidate neurotransmitter in chick sympathetic neurons. Supported in part by NS17574 to V.A.C.

61.15 THE VOLTAGE DEPENDENT ACTION OF L-GLUTAMATE ON MOUSE SPINAL CORD NEURONS. G.L. Westbrook and M.L. Mayer. Laboratory of Developmental Neurobiology, NICHD, NIH, Bethesda, Md. 20205

Despite the widely accepted view that acidic amino acids are excitatory neurotransmitters in the mammalian CNS, the conductance mechanism underlying their action has been poorly understood. For example, L-glutamate has been reported to cause increases, decreases, or no change in neuronal input resistance; while responses to aspartate show a region of negative slope conductance when examined under voltage clamp(MacDonald,et al.,1982). Recent reports indicate that the negative slope conductance of L-glutamate and NMDA is due to voltage-dependent channel block by Mg^{++} (Nowak,et al.,1984; Mayer & Westbrook,1984). However we have found that L-glutamate currents in mouse spinal neurons, although voltage-dependent, may activate two distinct conductances: one highly voltage-sensitive,the other voltage-insensitive.

Spinal cord neurons dissociated from 13d embryonic mice were grown in culture for 2-4 weeks. Neurons were impaled with two CsCl(1 M) microelectrodes (60-80 MΩ) and voltage clamped in a bath containing 5 mM Ca^{++}, and either "0" or 1 mM Mg^{++}. Amino acids(.01-1 mM) in recording medium were delivered by brief pressure pulses (10-250 msec) from pipets (1-2 μm tip) near the soma. Current-voltage(I-V) plots were constructed between -70 and +20 mV from peak amino acid-induced currents, or from voltage jumps performed in the presence and absence of a steady amino acid-induced current.

I-V plots of L-glutamate responses in 1 mM Mg^{++} solutions were voltage-dependent such that the slope conductance was close to zero between -70 and -30 mV. At membrane potentials more positive than -30 mV, the slope conductance became steeply positive. This voltage-dependence was intermediate between that observed with highly voltage-sensitive agonists, aspartate and NMDA; and voltage-insensitive agonists, kainate and quisqualate. In the presence of the NMDA receptor antagonist 2-APV(250 μM), or in Mg^{++}-free solutions the I-V relationship for L-glutamate responses became linear suggesting that the voltage-sensitivity of L-glutamate results from activation of NMDA receptors. Paradoxically, in 10 Mg^{++}, L-glutamate I-V plots were also linear since all inward current flow through the voltage-sensitive(i.e. Mg^{++}-sensitive) channels was blocked, and only voltage-insensitive channels contributed to the response.

Such mixed agonist behavior could lead to markedly different properties at glutaminergic synapses depending on the type and distribution of the postsynaptic receptors.

61.16 EVIDENCE FOR TWO GLUTAMATE SENSITIVE RECEPTOR SPECIES ON THE POSTSYNAPTIC GIANT AXON OF THE SQUID GIANT SYNAPSE. E.F. Stanley, Dept. Neurol., Johns Hopkins U. Balto., MD. 21205

Treatment of the squid giant synapse with glutamate (GLU) is known to block synaptic transmission, possibly by desensitization of the receptors activated by the (as yet unidentified) endogenous transmitter (ET) (Kelly and Gage, 1969, J Neurobiol 2:209). Iontophoresed GLU depolarizes the postsynaptic giant axon which also desensitizes with repeat application (Miledi 1967 J Physiol 192:379). In order to test whether these two actions of GLU are via the same receptor type, the rates of desensitization of the EPSP and the directly evoked depolarization have been compared using a technique of arterial infusion to introduce GLU to the syapse. With this technique GLU both depolarizes the postsynaptic giant axon and also blocks the EPSP (Stanley 1983 Biol Bull 165:533).

GLU was infused into the giant synapse via the arterial system while recording intracellularly from the postsynaptic giant axon at the giant synapse. GLU evoked a depolarization of the postsynaptic giant axon which slowly desensitized back to the resting level. Infusion of GLU during the recording of EPSPs, resulted in the gradual decline in the amplitude of the EPSPs to undetectable levels. However, the decline in the EPSPs occurred much more rapidly than the desensitization of the direct GLU response. Similar findings were observed with the GLU agonist quisqualate (QUIS), but the difference in the timing of desensitization was even more evident: the EPSP was completely abolished before the QUIS-induced depolarization had begun to desensitize.

These results suggest that the action of GLU is via (at least) 2 different receptor types; a slowly desensitizing receptor that is independent of the giant synapse, and a rapidly desensitizing receptor that is activated by the ET. This finding may explain the anomaly that the depolarization induced by iontophoresed GLU has a different reversal potential from that of the EPSP (Miledi 1969 Nature 223:1234), a result that has questioned the identification of GLU as the giant synapse transmitter. It is likely that GLU receptors on the postsynaptic axon that are accessible to iontophoresis are those that are distant from the giant synapse itself, i.e. extrasynaptic GLU receptors. Such receptors may represent the slowly desensitizing type. Whether the rapidly desensitizing receptors have the same reversal potential as the EPSP requires further study. Thus, it remains possible that the ET is GLU.

61.17 CEREBELLAR PURKINJE CELLS IN VITRO; MEMBRANE PROPERTIES AND THE EFFECTS OF ETHANOL.

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Ethanol affects neuronal function by producing hyperpolarization, reduction in input resistance and perhaps an increase in membrane capacitance. Some of these actions may be due to an increase in intracellular Ca^{2+}. These actions have been seen in intracellular experiments in the hippocampus and in extracellular experiments in the cerebellum.

Intracellular recordings were obtained from 25 Purkinje cells to date in slices of the rat cerebellar vermis. The Purkinje cells had a mean resting membrane potential (V_m) of -62.5 mV ±SD 7.7, an input resistance (R_N) of 14.9 MOhm ± 5.3 and an action potential duration of less than 0.8 ms at the baseline and less than 0.3 ms at half height. Typically, Purkinje cells fired action potentials (AP) at between 50 and 140 Hz. Many cells showed bursting behaviour, where the fast APs were suddenly replaced either by a stable V_m about 5 mV more negative accompanied by an apparent increase in R_N or by smaller slower spikes. In the former case, the new V_m remained for up to 1 min before becoming suddenly more positive and initiating fast APs. In the latter case, the slow spikes either gave way to to fast spikes or to a hyperpolarization that was accompanied by an apparent decrease in membrane resistance.

Hyperpolarizing pulses could change the state of the cell and showed that R_N was strongly dependent on the membrane potential, with an apparent increase in R_N with moderate hyperpolarization and then a decrease with large hyperpolarizations. In some cells, injected currents that were hyperpolarizing when the cell was not active were depolarizing when the cell was firing, which may indicate a region of negative slope in the current/voltage relationship of the cell.

Ethanol was applied to the cells either by pressure ejection or by perfusion. There was little difference in the effects at 20mM and 100mM. Ethanol reduced or stopped the firing of the cells. This effect was sometimes accompanied by a hyperpolarization and/or a decrease in R_N. Ethanol also increased the threshold for the climbing fibre response.

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61.18 EFFECTS OF KAINIC ACID LESIONS ON THE CALMODULIN-DEPENDENT PHOSPHATASE ACTIVITY ASSOCIATED WITH CALCINEURIN IN RAT STRIATUM.

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Calcineurin, a Ca^{2+} and calmodulin-dependent phosphatase (CM-PTase), dephosphorylates several proteins as well as non-protein phosphoesters. Calcineurin is a major calmodulin-binding protein in brain and has been shown to be present in high concentrations in the striatum. In order to determine the localization of calcineurin in the striatum, the effect of destruction of intrinsic neuronal cell bodies on the PTase activity was investigated. Male Wistar rats weighing 200-250 g were anesthetized with sodium brevital and 0.5 and 1.0 ug of kainic acid were injected into the left striatum (AP = +2.0, L = +3 V = -4.5 mm). 14 days after kainic acid lesioning, rats were sacrificed and striata removed and frozen over dry ice.

In order to estimate the extent of intrinsic neuronal cell body destruction, glutamic acid decarboxylase (GAD) was assayed by measuring CO_2 formed from L-(1-^{14}C)-glutamate. CM-PTase was assayed by measuring the release of ^{32}Pi from phosphothreonyl inhibitor-1, a heat stable protein inhibitor (21K), specific for protein phosphatase-1, a Mg^{2+} ATP-dependent phosphatase.

	RAT STRIATUM	
	CM-PTase (cpm/ug prot/2 min)	GAD (nmole CO_2/mg prot/h)
Control	688.4 ± 49.0	93.2 ± 10.0
0.5 ug kainic acid	227.1 ± 27.2*	20.5 ± 3.9*
1.0 ug kainic acid	56.4 ± 23.6*	9.3 ± 2.2*

Each value is the Mean ± SE of 5-7 rats. * = $p < 0.001$

0.5 and 1.0 ug of kainic acid reduced striatal CM-PTase activity by 67% and 92% and striatal GAD activity by 78% and 90%, respectively. Thus CM-PTase activity in the striatum appears to be localized exclusively in intrinsic neuronal somata and dendrites rather than in nerve terminals or fibers of passage. We hypothesize that the phosphatase activity of calcineurin may be involved in the postsynaptic mechanisms of neurotransmitter action in the striatum.

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61.19 CHANGING POST-SYNAPTIC RESPONSIVENESS IN INTERNEURONS?

M. Hauser*, C.L. Keenan and H. Koopowitz. Developmental and Cell Biology, University of California, Irvine, CA 92717.

Several identified interneurons (b-BRA, w-BRA, SC and SIC cells, Keenan and Koopowitz, J. Comp. Phys., in press) in the brain of the marine polyclad flatworm Notoplana acticola are responaive to vibratory stimuli. Upon repeated presentation of vibration, the response habituates. This process is blocked by the removal of calcium or the addition of magnesium or cadmium to the bathing medium. This suggests that habituation in these cells is post-synaptic. Changes in the post-synaptic properties of the membrane concomitant with habituation indicate decreasing input resistance. This may signal opening of specific ion channels in the post-synaptic cell membrane. Habituation is rapid; the response decreases to less than half of the final value following the second stimulus. The rate and degree of response decrement has been demonstrated to be inversely related to both interstimulus interval and stimulus intensity. The decline in responsiveness, measured as area of the EPSP (mV sec) is an exponential function. The extent of habituation appears to be dependent upon the simulus intensity, and further decline after reaching the minimum response in negligible. In addition to vibration, these cells also decrease in their response to repeated injection of depolarizing current. The kinetics of declining responsiveness is different in the two situations. Habituation to vibratory stimuli occurs more rapidly (2 trials to reach 50% decrease of response) in comparison to that for current injection (7 trials to reach the 50% criterion). In addition, the decline in responsiveness to repeated current injection (measured as the number of action potentials per second) is a linear, rather than an exponential function. We postulate that these phenomena are mediated by changes in calcium-activated potassium channels, and present data describing the calcium and potassium dependence of the observed response decrements.

69.20 **POST-SYNAPTIC HYPERPOLARIZATIONS IN "IN VITRO" NEOCORTICAL NEURONS.**

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Intracellular recordings were performed in neurons located in the deep layers of neocortical slices (500-700 m thick) obtained from the rat sensorimotor region and maintained "in vitro" at 35 ± 1°C. Stimuli delivered to the pia, the underlying white matter or within the layers of the neocortical slice evoked post-synaptic potentials which were mainly depolarizing at the resting membrane level of healthy, non-spontaneously discharging neurons impaled with K-acetate filled microelectrodes. By depolarizing these cells up to 30 mV with applied steady current, hyperpolarizing post-synaptic potentials were disclosed. These stimulus-induced hyperpolarizations: (a) were usually preceded or concomitant with an EPSP; (b) lasted up to 1s depending upon the stimulation intensity as well as the level of polarization. Also the early part of the hyperpolarizing potential was inverted in polarity at a more positive level than the late one. In a few cases, by setting the neuron to a certain level of polarization, two distinct components could be separated: the first, short-lasting (50-120ms) peaked in amplitude 10-25 ms after the stimulus while the second, long-lasting component displayed an amplitude maximum at a latency varying between 80 and 200 ms. The membrane conductance at the soma, as calculated with short (20-50ms) hyperpolarizing and depolarizing intracellular pulses of current increased during the overall duration of the hyperpolarization , though the largest increase could be consistently observed in the first phase of the stimulus-induced hyperpolarizing potential. Similar conductance changes were also seen when the resting membrane level was set close to the equilibrium potential for the hyperpolarization to minimize any rectifying property of the membrane. In intracellular recordings performed with KCl-filled microelectrodes the early part (up to 100 ms) of the hyperpolarization was readily inverted to a depolarizing potential, but the late part appeared only slightly affected. By increasing the extracellular K^+ concentration from the control value of 3.25 mM up to 11.25 mM and counteracting the depolarizing shift consequent to this procedure by polarizing the neuron at the same level as in the control situation, the late hyperpolarization could be reduced.

These findings demonstrate that, similarly to what reported in "in vitro" neocortical neurons of guinea pig (Connors et al, J. Neurophysiol., 48, 1982, 1302), hyperpolarizing post-synaptic potentials in the neocortex display two components. The present evidence supports that these components might be associated with two different underlying ionic mechanisms.

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61.21 CALMODULIN BLOCKERS AND ANTIBODY TO CALMODULIN DEPENDENT PROTEIN KINASE ENHANCE AFTERHYPERPOLARIZATION IN CAT MOTONEURONS. C.Y. Yim, Department of Anaesthesia Research, McGill University, Montreal, Quebec, Canada H3G 1Y6.

Membrane hyperpolarization (AHP) following an action potential (AP) has been shown to be due to increase in K^+ conductance resulting from an influx of Ca^{++} during the AP. Since calmodulin (CAL) is known to mediate a number of cellular effects of Ca^{++}, a possible mechanism by which Ca^{++} activates Ca^{++} dependent K^+ channels is its activation of certain protein kinases through CAL and the subsequent phosphorylation of channel proteins. This hypothesis is tested in the present study by investigating the effects of CAL blockers (trifluoperazine (TFP) & pimozide), CAL dependent protein kinase (CAL-PK) and antibody to CAL-PK (CAL-PK-Ab) on AHP when injected into spinal motoneurons.

Intracellular recordings were obtained from L6 & L7 spinal motoneurons of pentobarbital anaesthetized cats. Neurons were antidromically activated by stimulation of the respective ventral roots. Double- or triple-barrel glass micropipettes were used for recording and pressure injection of drugs. TFP & pimozide injected at 1.25 atm. for 15 s produced an average of 20% increase in the AHP in 10 of 12 cells tested. The increase was gradual and leveled of 4-5 min after injection. The resting membrane potential (RMP) of 5 out of the 10 cells that showed an increase in AHP was hyperpolarized by 3-7 mV whereas the other cells showed no change in RMP or a slight decrease of 2-4 mV. Effects of TFP or pimozide were not reversed over 30 min after injection. CAL-PK-Ab injected at 1.67 atm. for 15-30 s increased the AHP in 5 out of 7 cells by an average of 11% over 4-5 min, but the remaining 2 cells showed 4-6% decrease. CAL-PK injected at 1.67 atm. for 30 s produced an average of 8% decrease in AHP 5 min after injection in 6 out of 9 cells tested. No significant change in AHP was observed in the remaining 3 cells. Neither CAL-PK-Ab nor CAL-PK produced consistent hyperpolarization or depolarization of RMP.

Results of these experiments show that CAL may not have a direct role in the activation of Ca^{++} dependent K^+ channels. Blockade of CAL or CAL-PK did not reduce but augments AHP. Also, the time course of the effects of the blockade suggests that CAL may be involved more so in the homeostasis of intracellular Ca^{++} rather than mediating the rapid electrophysiological effects of the cation. The augmentation of AHP and slight hyperpolarization of RMP could be explained by the possible interference of the Ca^{++} pump that normally transfers Ca^{++} out of the cell. (supported by MRC of Canada.)

61.22 EFFECTS OF ACUTE AND SUBACUTE ADMINISTRATION OF PYRIDOSTIGMINE (PYR) ON MUSCLE CONTRACTILITY AND NEUROMUSCULAR TRANSMISSION. S.S. Deshpande[1], M. Adler[2], R.E. Foster[2], E. Toyoshima[3] and E.X. Albuquerque[1]. Dept. Pharmacol. & Exp. Ther.[1] and Neurology[3], Univ. Maryland Sch. Med., Baltimore, MD 21201 & Neurotoxicology Branch[2], USAMRICD, APG, MD 21010.

The effect of PYR was investigated on endplate potential (EPP), junctional acetylcholine (ACh) sensitivity and muscle contractility in rat diaphragm and extensor digitorum longus (EDL) nerve-muscle preparations. PYR (5-10 μM) potentiated the indirect elicited (0.1 Hz) twitch. A progressive depression of twitch occurred with higher stimulation frequencies ($>$ 1 Hz). Similar results were obtained when PYR (10 mg/ml) was released (5.9 μl/hr) from subcutaneously implanted Alzet osmotic minipumps for 14 days. For these experiments, in vivo twitch and tetanic tensions were determined in the presence of PYR and after removal of the minipumps. The PYR-induced alterations were maximal by one day of implantation as demonstrated by potentiation of single twitch tension and depression of contractions during repetitive nerve stimulation. These alterations persisted without changes in severity during the 14 day treatment. Recovery occurred within 24 hr of drug withdrawal and was more rapid ($<$ 1 hr) if the muscles were removed from the animals and bathed in physiological solution. To investigate the mechanisms underlying the alterations in muscle contractility, experiments were performed on intact (for junctional ACh sensitivity) and cut (for EPP) diaphragm muscles. In the absence of PYR, trains of EPPs (1-50 Hz, 50 pulses) showed little frequency-dependent decrements. After addition of PYR (10 μM), the last EPP decreased by 25, 44, 60 and 87% relative to the first at 1, 10, 20 and 50 Hz respectively. This depression was accompanied by a depolarization of ~1 mV at 1 Hz and ~5 mV at 50 Hz. The EPP decays were prolonged but did not undergo further changes during repetitive stimulation. These effects were reversible. PYR (2 μM) produced 30% depression in amplitude of junctional ACh potential by the end of a 40 pulse train at 4 Hz. This was accompanied by ~1 mV sustained membrane depolarization. These effects were enhanced at 10-25 μM PYR. In conclusion, the inability of muscle to sustain tetanic contraction under PYR appears to be partly due to postsynaptic desensitization and also due to interaction with the ACh receptor-ionic channel complex (Mol. Pharmacol. 25, 102, 1984). (Supported by U.S. Army Medical Research and Development Command Contract No. DAMD 17-81-C1279)

61.23 EPSPs EVOKED IN CAT MOTONEURONES BY Ia FIBRE ACTIVATION ARE ENHANCED BY INTRACELLULAR TETRA-ETHYL AMMONIUM (TEA) IONS. P.G. Nelson, S.J. Redman* and J.D. Clements.* Experimental Neurology Unit, John Curtin School of Medical Research, Australian National University, Canberra, 2601, Australia.

Composite and single fibre Ia EPSPs were recorded in cat spinal motoneurones before and following intracellular injection of TEA. Ia afferents were activated by muscle stretch, and recordings of afferent spike activity in dorsal root filaments were used with spike-triggered techniques to obtain single fibre EPSPs. The amount of TEA injected into the neurone was monitored by measuring increases in the duration of the antidromic action potential.

Marked increases ($\leq$ 72%) in the amplitudes of single fibre EPSPs were produced by TEA injections for EPSPs having shape indices characteristic of synapses located on or near the soma. No change in EPSP time course occurred with this amplitude increase, and no consistent change in passive membrane properties was seen. For EPSPs with shape indices indicative of more distal synaptic locations, a prolongation of the EPSP time course was sometimes seen. In some instances, this prolongation could be reversed by steady hyperpolarizing current. The prolongation by TEA of composite EPSPs elicited in one motoneurone by stimulation of two different muscle nerves was accompanied by a greater-than-linear summation of the two EPSPs when both muscle nerves were stimulated simultaneously. (Linear summation was observed before TEA injection.) This nonlinear summation could be blocked by small, steady hyperpolarizing currents. The prolongation of more distally generated EPSPs and the effects of hyperpolarizing currents on this prolongation indicates that a voltage dependent membrane current contributes to the generation of these EPSPs in the presence of TEA.

The large augmentation of single fibre EPSPs (of somatic origin) by TEA injection seems most plausibly related to a positive shift in the driving potential for these EPSPs. The maximum observed increase in amplitude requires a shift in E_{rev} to E_{Na}. We hypothesize that TEA causes a selective decrease in the K^+ permeability of synaptically activated channels.

61.24 MUSCARINIC AND PEPTIDERGIC ACTIONS ON C CELLS OF BULLFROG SYMPATHETIC GANGLIA. Stephen W. Jones. Dept. Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

The large B cells of bullfrog sympathetic ganglia are known to be depolarized by muscarinic agonists, corresponding to the slow EPSP; and by peptides related to mammalian luteinizing hormone-releasing hormone (LHRH), corresponding to the late, slow EPSP. These actions are largely due to inhibition of a voltage dependent K^+ conductance, the M-current. The smaller C cells have been reported to have a late, slow EPSP, but a muscarinic IPSP. Pharmacological differences between B and C cells were studied by single electrode voltage clamp.

C cells (identified by a slow, $<$ 0.5 m/sec orthodromic conduction velocity) had a Ba^{2+} sensitive M-current with kinetics comparable to that of B cells. However, 20 μM muscarine produced little or no M-current inhibition in most C cells. In contrast, B cells were uniformly sensitive to muscarine, with an $I_{50} \sim 1$ μM. This demonstrates that blockade by muscarinic agonists cannot be used as a defining criterion for M-current. Clear correlates of muscarinic IPSPs have not been observed to date.

LHRH inhibited the M-current in B cells with an $I_{50} \sim 5$ μM. 10 μM LHRH produced variable inhibition of the M-current in C cells. Some C cells that showed little or no M-current inhibition to muscarine were more sensitive to LHRH than are B cells. Inhibition of M-current has also been observed during the late, slow EPSP in C cells.

In some B cells, muscarine and LHRH induce an inward current, associated with an increase in conductance, in addition to M-current inhibition. This effect contributes to the slow and late, slow EPSPs. This additional inward current could also be observed in C cells. In some C cells LHRH also caused an outward current, associated with a decrease in conductance, which was especially clear hyperpolarized to -60 mV (where M-current is inactive). This effect was not seen in B cells but was observed in some C cells at concentrations as low as 0.1 μM LHRH. Both of these non-M-current effects had extrapolated reversal potentials near 0 mV or more positive, and were not associated with slow voltage dependent relaxations--i. e., they appeared as changes in the apparent instantaneous conductance.

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61.25 COMPARATIVE EFFECTS OF EDROPHONIUM, PHYSOSTIGMINE AND METHANE SULFONYL FLUORIDE ON THE KINETICS OF END-PLATE CHANNELS. J.F. FIEKERS, Dept. Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, Vt.

The primary action of acetylcholinesterase agents is to inhibit the activity of acetylcholinesterase (AChE) at cholinergic synapses. Most effects of these agents are consideredindirect as a result of AChE inhibition, however direct actions of many of these agents have been reported but have not been well described. Previous results demonstrated (Fiekers, Soc. Neurosci. Abst., 1983) that neostigmine directly modified the ACh receptor channel complex. In the present study the effects of several anti-esterase agents, edrophonium (edr), physostigmine (phy) and methane sulfonyl fluoride (MSF) were studied on mepc amplitude, mepc decay and ACh-induced end-plate current fluctuations recorded in voltage clamped snake costocutaneous neuromuscular junctions. In low concentrations (1-25uM) edr produced a concentration dependent increase in both mepc amplitude and γmepc. Higher concentrations (25-100uM) decreased mepc amplitude. At all concentrations, mepc decay was described by a single exponential. Fluctuation analysis demonstrated that concentrations of edr above 30uM produced power density spectra (pds) which required the sum of two Lorentzian components- one faster and one slower than the control. The second, slower time constant increased and the fast time constant decreased with membrane hyperpolarization. Low concentrations of phy (1-10uM) increased mepc amplitude and γ-mepc. Higher concentrations (above 50uM) decreased mepc amplitude and decreased γmepc. Mepc decay increased with membrane hyperpolarization so thatthe voltage dependence was reversed above 50uM when analyzed between -60mV and -140mV. Pds relations were well fitted by a single Lorentzian at all membrane voltages and concentrations of phy. Phy produced a progressive decrease in γ_n, mean channel lifetime, with increasing conc. above 10uM. MSF (10mM, one hour; one hour wash) increased peak mepc amplitude and γmepc without change in the voltage dependence (γ_{mepc},γ_n). Pds were well described by a single Lorentzian. After complete inhibition of AChE by MSF, exposure to edr or phy (50uM) decreased mepc amplitude. Current fluctuation spectra obtained with 50uM edr exhibited 2 time constants similar to those obtained prior to MSF. Phy also decreased γ_n in MSF. These results show that phy and edr have direct actions on the end-plate receptor channel complex which are unrelated to AChE inhibition. (supported by MDA).

STRUCTURE AND FUNCTION OF NEUROENDOCRINE CELLS

62.1 CHROMOGRANIN: STRUCTURE AND LOCALIZATION. R. Hogue Angeletti*, J. Nolan*, J. Settleman* and J.Q. Trojanowski. (SPON: M.L. Schmidt). Division of Neuropath., Univ. of Pennsylvania Medical School, Phila., PA 19104

Chromogranin is the principal protein found in the adrenal medulla chromaffin granule, and is secreted upon nervous stimulation. It has recently been found to be present in several other endocrine and neuroendocrine tissues. We have approached the study of chromogranin using protein chemical, molecular biological, immunohistochemical and immunochemical tools. The current data on the primary structure of chromogranin will be presented. These sequence data will be related to the results of the Ca++-dependent breakdown of chromogranin. Several additional tissues have been found to contain chromogranin based on both immunohistochemical and immunoblotting studies of these tissues. Thus, the actual molecular properties of the new chromogranins in these other tissues can be related to the adrenal chromaffin cell chromogranin. (NS-13201)

62.2 THE ORNOSOME: A NEW ORGANELLE IN SECRETORY GRANULES OF ADRENAL CHROMAFFIN CELLS. R.L. Ornberg*, Le Duong*, and H.B. Pollard (Spons: Peng Loh). Lab of Cell Biology and Genetics, NIADDK, NIH, Bethesda, Md. 20205

Bovine adrenomedullary cells have been examined using quick freezing and freeze-substitution techniques to reveal the existence of small, 80-100 nm diameter, electron translucent vesicles within the matrix core of most (57 $\pm$ 10%) chromaffin granules. These vesicles are very rarely observed in granules of cells prepared by aldehyde-osmium tetroxide fixation which explains why they have not been described until now. Typically one, but as many as five, vesicles have been seen between the dense core material and the limiting granule membrane. The vesicles are "true" vesicles in that they have a trilaminar unit membrane and are not simple invaginations of the granule since they contain no cytoplasm and do not appear as invaginations in serial sections. In addition, small vesicles identical in appearance to the granule vesicles are exocytosed along with catecholamines and granule contents from cells during release stimulated by nicotine. Rapid frozen isolated granules prepared on sucrose-metrizamide density gradient or 1.6 M sucrose shelf gradient also contain vesicles supporting the contention that vesicle-containing granules are true chromaffin granules. A curious feature of isolated granules is that the intragranular vesicles can be preserved by conventional aldehyde fixation. We call the intragranular vesicles "ornosomes" after their discoverer, and we are employing analytical electronmicroscopic methods to determine ornosome content and to assess their physiological function.

62.3 INTERACTION OF EXTRACELLULAR MATRIX (ECM), CYTOSKELETAL AGENTS AND POLYSACCHARIDES ON PC 12 CELL SHAPE AND DOPAMINE (DA) PROCESSING. C.L. Bethea and S.L. Kozak*. Reprod. Biol. & Behav., Oregon Reg. Primate Ctr., Beaverton, OR 97006.

PC 12 cells assume a flattened configuration on ECM, release significantly more DA and contain less intracellular DA than cells which are rounded on plastic. This difference is not due to an increase in cell attachment or growth on ECM (Soc. Neurosci. Abst. 5.3, 1983). To further explore the role of cell shape in DA processing we examined the effects of ECM and various agents on cell shape and DA release and content. PC 12 cell suspensions in medium containing vehicle or concentrations of cycloheximide, colchicine, cytochalasin B, heparin, dextran, or dextran sulfate were plated on ECM and plastic. Following overnight attachment, the medium was replenished. After 12 h (cycloheximide & cytoskeletal agents) or 24 h (polysaccharides), medium and cells were harvested for DA and DNA assays (DA values were normalized by µg DNA). Morphology was examined with phase LM and scanning EM. Cycloheximide (10^{-7}, 10^{-6}M) inhibited cell spreading, decreased DA release, but also decreased DA content in cells on ECM. Colchicine (10^{-5}M) and cytochalasin B (1.0 & 10.0 µg/ml) did not prevent cell spreading, but cell morphology was aberrant. Colchicine decreased DA release in ECM cultures and decreased DA content in ECM and plastic cultures. Cytochalasin B did not alter DA release, but decreased DA content in cells on both substrates. These compounds thus appeared toxic to DA processing regardless of cell shape. Heparin (1000 U/ml) caused an increase in rounded (phase refractile) cells on ECM, a decrease in DA release and an increase in DA content which is similar to rounded cells on plastic. Heparin did not affect DA release from cells on plastic, but did increase cell content. Dextran (10.0 mg/ml) also caused an increase in refractile cells on ECM and decreased DA release but did not affect content. Dextran sulfate (1.0 & 10.0 mg/ml) caused a dose-related increase in refractile cells on ECM, a decrease in DA release and an increase in cell content. The cells on plastic did not attach with dextran sulfate. These experiments suggest that these polysaccharides block cell spreading on ECM, and thus prevent the increase in DA release and the decrease in DA content which occurs when PC 12 cells flatten on ECM. In summary, ECM alters cell shape which influences dopamine secretion and storage. Supported by HD17269 and RR00163.

62.4 INTRACELLULAR ELECTROPHYSIOLOGICAL COMPARISION OF PHASIC AND NON-PHASIC NEURONS IN RAT HYPOTHALAMIC SLICES. R. D. Andrew and F. E. Dudek. Dept. of Anat., Queen's Univ., Kingston, Ont. K7L 3N6 and Dept. of Physiol., Tulane Univ., Sch. of Med., New Orleans, LA. 70112.

Oxytocinergic (OX) and vasopressinergic (VP) neuroendocrine cells have similar embryology, morphology and hormone biochemistry. However, the firing patterns that evoke hormone release differ markedly between the two populations of magnocellular neuroendocrine cells (MNC's). In vivo extracellular recordings in rat have shown that during dehydration, VP neurons are recruited to a phasic burst mode. In contrast, suckling evokes a synchronous, high-frequency burst by the OX population.

Despite these differences between bursting patterns in vivo, intracellular recordings showed several electrophysiological properties common to each of 31 magnocellular neuroendocrine cells (10 phasic, 21 nonphasic) recorded in coronal slices of rat hypothalamus. Whether phasic, silent, slow or fast-firing, all cells that were tested displayed 1) high input resistance (157 ± 47 MΩ for 9 phasic; 146 ± 48 MΩ for 21 non-phasic), 2) spike broadening during transition from slow (<3 Hz) to fast (>10 Hz) firing, 3) a brief but prominent post-train afterhyperpolarization, 4) a depolarizing afterpotential (DAP). The DAP tended to promote a burst in both phasic and some non-phasic MNC's. Thus as a group, MNC's have several important electrophysiological properties in common.

Although not identified immunohistochemically, it is likely that our phasic cells are VP and many (but not all) non-phasic cells are OX. Phasic cells were distinguished from non-phasic neurons by a slow oscillation in membrane potential. A burst usually accompanied the depolarizing phase, although in two cells the slow oscillation was still present after spiking ceased spontaneously.

These data suggest the hypothesis that OX cells lack the slow oscillation and therefore do not usually burst repetitively. However, like phasic cells, many OX cells have summating DAP's and can thus fire a triggered burst to brief depolarizing stimuli. Together with excitatory synaptic input, summating DAP's would be expected to promote the high-frequency discharge that evokes milk ejection in vivo.

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62.5 COMPLETE SERIAL RECONSTRUCTION OF LUCIFER YELLOW-FILLED RAT SUPRAOPTIC NUCLEUS (SON) NEUROSECRETORY NEURONS. J.C.R. Randle , C.W. Bourque and L.P. Renaud, (SPON: A.T. Tan). Neurosciences Unit, Montreal General Hospital and McGill University, Montreal, Canada H3G 1A4.

The magnocellular neurosecretory cells (MNCs) of the rat SON project to the neurohypophysis where they secrete vasopressin and oxytocin. Until recently, little was known of the cytoarchitecture of MNCs because of their resistance to Cox-Golgi impregnation. Immunohistochemical studies allow better definition but a full appreciation of the distribution of dendrites is impossible because many MNCs are stained simultaneously. Intracellular injection of the fluorescent dye lucifer yellow provides a means to visualize the complete cytoarchitecture of single SON neurons.

Lucifer yellow (LY)-CH was iontophoresed intracellularly into MNCs maintained in vitro in an arterially perfused explant of hypothalamus. Forty micron horizontal or coronal sections were cut and processed for visualization of LY fluorescence. Sections of the LY-filled cell were photographed with Kodak TRI-X Pan film and the cells were reconstructed by tracing the projected negative image on a Leitz-Ortholux microfilm projector.

Soma size and shape (round or elongated, mean short axis 12 µm, long axis 23 µm) were similar to previously reported values. The cells had 2-4 dendrites ranging in length from 40-625 µm that branched sparingly. Dendrites projected in all directions from the soma, but 95% eventually turned ventrally and ended in the glial lamina. Dendritic morphology was characterized by fairly even tapering (dia. 2 µm or less) and the presence of numerous spines and fine hair-like processes (length 1-15 µm; density about 40/100 µm of dendrite). The axon arose from the soma or more often from a proximal dendrite, 6 to 140 µm from the soma. Axons were followed 300-1700 µm as they projected dorsally and medially over the optic tract and then turned ventrally to the surface of the hypothalamus. Axons were distinguishable by their beaded appearance and the near absence of secondary processes.

These observations indicate that MNCs possess simple but elongated dendrites with numerous spinous processes. Moreover, a single axon often arising from a dendrite appears to have few collaterals.

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62.6 ULTRASTRUCTURE OF CATECHOLAMINERGIC SYNAPSES IN THE RAT SUPRAOPTIC NUCLEUS. C. D. Tweedle and G. I. Hatton. Dept. of Anatomy and Neurosci. Program, Mich. State Univ., East Lansing, MI 48824.

It was previously established at the light microscopic level that the supraoptic nucleus (SON) has a rich catecholaminergic (CA) innervation (predominantly noradrenergic). Histofluorescence combined with immunocytochemistry revealed that CA varicosities were in a position to represent axosomatic synapses (preferentially with vasopressin immunoreactive cell bodies) as well as possible synapses onto the dendrites of the magnocellular neurosecretory cells (J. Comp. Neurol. 193:1023-1033, 1980). To determine whether CA fibers indeed form conventional synapses on SON neurons, CA terminals were labeled by 5-hydroxydopamine treatment and examined ultrastructurally.

Young adult female rats (N = 4/group) were anesthetized and stereotaxically injected via the lateral ventricle (1. virgins; 2. 14 day lactating; and 3. animals whose drinking water was replaced by 2% saline for 10 days). 5-Hydroxydopamine hydrochloride (2.0 mg) dissolved in 5 µl of Ringer solution containing 0.2 mg/ml L-ascorbate was infused into each rat over a period of 20 minutes. Subsequently, animals were perfused with fixative and prepared for electron microscopy. Two virgins, two lactating and three saline-treated rats ultimately provided usable SON material. In all groups, labeled synapses could be seen on dendritic shafts, spines and, mainly in the ventral posterior part of the nucleus, onto somata and somatic spines. The synapses contained a mixture of labeled 50-75 nm clear, round vesicles and 100-150 nm dense core vesicles. All degrees of symmetry of the synaptic thickenings were seen. Close association of CA axonal profiles with glial cell processes, including presynaptic membrane thickenings, also occurred, suggesting neuron-glial communication.

In previous work (Brain Res. Bull. 8:197-204, 1982; Neurosci. Abstr. 9:860-861, 1983) we found that novel double synapses between the somata of SON cell bodies appeared after chronic stimulation (lactation and dehydration). Likewise, the number of double synapses between SON dendrites was increased at parturition and lactation. The present study revealed no labeling of experimentally-induced somatic double synapses, but did show labeling of many dendritic double synapses. This difference and other morphological criteria, suggest that even though double synapses are "plastic" at both the somatic and dendritic levels, they differ in content and perhaps in origin. Supp. by NIH Grant NS 09140.

62.7 SYNAPTIC EFFECTS OF MEDIAL STIMULATION ON LATERAL PARAVENTRICULAR NEURONS IN SLICES OF RAT HYPOTHALAMUS. D. M. Smith and F.E. Dudek. Dept. of Physiol., Tulane Univ. Sch. of Med., New Orleans, LA 70112.

Anatomical data indicate afferent projections and local connections to the lateral paraventricular nucleus (PVN) that pass through or originate in medial PVN (A.N. van den Pol, 1982, J. Comp. Neurol., 206:317). We have used extracellular electrical stimulation of the medial PVN area and single-cell recording in lateral PVN to study these synaptic inputs.

Coronal hypothalamic slices (500 μm) were prepared from mature rats. Extracellular recordings (N=43) were from PVN neurons that visually appeared to be in the lateral PVN, but some may have been medial or perifornical. Three paired stimulating electrodes were placed in the ipsilateral medial PVN parallel with the third ventricle, and the effects reported below were from at least one of the electrodes.

Direct activation, determined by the criteria of invariant latencies (1-4 msec) and all-or-none responses, was seen in 14% of the cells. Synaptic activation, identified on the basis of long and variable latencies (3-65 msec) with graded responses, was seen in 49% of the neurons. These data suggest polysynaptic pathways and multiple inputs. Graded synaptic potentials were also seen in an intracellular recording. Synaptic inhibition occurred in 2% of the cells. One cell was synaptically excited at low intensities, but was inhibited at high intensities. Electrical stimulation did not directly activate three neurons (7%), but spontaneous firing increased after a 1-Hz stimulus train, suggesting excitatory intranuclear connectivity. No clear response was seen in 26% of the recordings.

Although more intracellular recording is needed to elucidate synaptic mechanisms, and marking of recording and stimulating sites is necessary to identify the cells involved, these preliminary data indicate synaptic connections to lateral PVN from neurons located in, or passing through, medial PVN. The results suggest that the synaptic inputs are strongly excitatory, but inhibitory synapses are also present and could be significant. Future studies will be aimed at specific identification of the stimulated and recorded neurons, and a more rigorous analysis of the synaptic mechanisms.

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62.8 DISSOCIATION OF OXYTOCIN AND VASOPRESSIN IMMUNOREACTIVITY FROM THAT OF NEUROPHYSINS IN TRANSPLANTED HYPOTHALAMUS-PREOPTIC AREA. C.M. Paden and M.J. Durick*. Dept. of Biology Montana State University, Bozeman, MT 59717.

To our knowledge, oxytocin and vasopressin immunoreactivity in the brain and neurohypophysis has always been associated with immunoreactivity for their respective neurophysins. We report here that this is not always the case in transplants of fetal hypothalamus-preoptic area (HPOA).

Adult female rats received transplants of E17 HPOA onto the choroidal pia overlying the superior colliculus as previously described (Stenevi et al., Cell Tiss. Res. 205; 217, 1980). Eight weeks later animals were sacrificed by cardiac perfusion with PBS and Bouin's fixative. Ten micron serial paraffin sections were cut throughout the length of the transplant. Every tenth section was immunostained for vasopressin using rabbit-anti-vasopressin N1-F at 1:1000 dilution. Peroxidase labeling was performed using the Vectastain avidin-biotinylated HRP method with glucose oxidase generation of H_2O_2. Wherever vasopressin-positive neurons, beaded fibers, or terminal clusters were observed in the transplant, adjacent sections were stained with rabbit-anti-oxytocin N1-9 (1:500) or rabbit-anti-rat-neurophysins 4 (1:1000). While in many cases staining patterns were similar with the three antisera, neurophysin-positive cell bodies and fibers were sometimes seen in the absence of oxytocin or vasopressin immunoreactivity. This result could be due to the greater sensitivity of the anti-neurophysins sera. However, in several instances clusters of oxytocin or vasopressin-positive cell bodies or terminals were present through several serial sections in the complete abscence of neurophysin immunoreactivity. This occurred in spite of the presence of positive neurophysin staining elsewhere in the transplant on the same section.

Dissociation of oxytocin and vasopressin immunoreactivity from that of neurophysins was observed in transplants grown in both intact and adrenalectomized hosts, ruling out the possibility of glucocorticoid effects on peptide metabolism as the cause of this phenomenon. Our current hypothesis is that the ability or inability of transplanted magnocellular neurons to reach appropriate targets in the transplant or host brain may affect processing of their neurosecretory peptides.

This work was supported by NIH NS17974. We wish to thank Drs. Gaj Nilaver and Allan Robinson for generous gifts of antisera to nonapeptides and neurophysins, respectively.

62.9 EARLY ESTRADIOL-INDUCED CHANGES IN NUCLEI OF VENTROMEDIAL NEURONS OF RAT HYPOTHALAMUS. K.J. Jones, D.W. Pfaff, and B.S. McEwen. The Rockefeller University,New York,NY 10021.

A small, discontinuous period of estradiol (E_2) treatment (2 1h segments separated by 4h-13h) can activate female sexual behavior in ovariectomized(ovx) rats by a mechanism partly dependent on increased protein synthesis (Parsons et al., Endocrinol. 110:613&620,1982). In this study, we ultrastructurally and morphometrically examined nuclei of neurons in rat hypothalamic ventromedial nucleus (VMN), an essential anatomical component in the lordosis response, after continuous 2h or discontinuous (2h on/7h off/2h on)E_2 treatment in female rats ovx for 5-7 days. Among 12 ovx rats, 6 received 5mm silastic capsules containing E_2, with 6 sham-operated ovx rats serving as controls. The rats were perfused, VMN groups dissected with the aid of a vibratome, and the tissue blocks processed for routine TEM. For ultrastructural work, thin 70nm-sections were cut and stained, and EM's of 25 neurons per rat systematically collected for a total of 300 neurons. For quantitative measurements, 1μm thick, toluidine blue stained sections were used. Drawings of 50 nucleoli and nuclei per animal were made with a Zeiss camera lucida microscope. Using a BioQuant Image Analyzer, nucleolar and nuclear area, and nuclear perimeter measurements were taken and averaged per animal. Nuclear shape was calculated by a form factor equation:$FF=4\pi x area/perimeter^2$. The data were analyzed with 2-way ANOVA and Student-Newman-Keuls test at $p<0.05$. After 2h of E_2, ultrastructurally, differences from the controls included an increased tendency toward nuclear roundness, and pronounced decreases in the heterochromatin in the nucleoplasm and lining the nuclear envelope. Significant increases in nucleolar and nuclear area, and nuclear perimeter, and changes in nuclear shape, from ellipsoid to spherical, were observed. After 2h on/7h off/2h on of E_2, ultrastructurally, altered nuclear shape was less dramatic, and there was a decreased incidence of the single large heterochromatin clump attached to the nuclear envelope together with an increase in nucleolus-associated chromatin (cf., Cohen et al., Cell Tiss. Res. 235:485,1984). With BioQuant measurements, nuclear area and perimeter were still significantly elevated but nucleolar area had returned to control levels and there were no longer nuclear shape changes. Based on these observations, it would appear that ultrastructural and morphometric signs consistent with alterations in RNA synthesis are present within the 1st 2h of E_2 and during short, behaviorally-sufficient E_2 treatment paradigms. Supported by MH15125.

62.10 HYPOTHALAMIC AND LIMBIC SYSTEM ESTRADIOL (E_2) CONCENTRATING NEURONS THAT PROJECT TO THE AMYGDALA. J.I. Morrell and D.W. Pfaff. The Rockefeller University, New York,NY 10021.

To determine exactly where steroid hormone-concentrating neurons fit into neuroendocrinologically or behaviorally relevant neural circuits, we have been examining their efferent connections, using the combined steroid autoradiographic fluorescent dye retrograde tracing method. Previous experiments uncovered E_2 concentrating neurons in the preoptic area (POA), bed nucleus of the stria terminalis (bnst) and hypothalamus that project to the midbrain (Morrell and Pfaff, Sci., 217, '82; Morrell et al. Peptides, '84). This study examines whether E_2 concentrating neurons also project within the limbic system to parts of the amygdala which themselves have large numbers of E_2 concentrating neurons.

The amygdala of nine young ovariectomized adrenalectomized female rats were injected under stereotaxic guidance with 35 or 50 nl of True Blue or DAPI (10%). After two days, to allow for retrograde labeling of neurons, the rats were injected I.P. with .8 ug/250 gm body weight ^3H-E_2 (SA-134 Ci/mM); two hours later, after nuclear binding of the E_2 had occurred, the animals were sacrificed by perfusion with saline, followed by 4% paraformaldehyde, and then 15% sucrose PBS. Steroid autoradiograms were prepared, exposed for 8 mo, photodeveloped, fixed, and then systematically examined with a microscope using standard white light for the presence of silver grains over cell nuclei, and U.V. light (360 nM) for the presence of fluorescent granules in neuronal soma and proximal processes.

After dye injections into the medial amygdala, the majority of retrogradely labeled E_2-concentrating neurons were found in the ipsilateral ventromedial nucleus (ventrolateral subdivision, VL-VM), anterior hypothalamic area (AHA), bnst, and mPOA. Additionally, doubly labeled neurons were found in the lateral hypothalamus, periventricular and ventral premammillary nuclei, diagonal bands of Brocca, and subfornical organ. Doubly labeled neurons were rare in the septum and arcuate nucleus. Each of these areas also contained many neurons that were retrogradely labeled but not E_2 concentrating and E_2 concentrating neurons that were not retrogradely labeled. Contralaterally a few doubly labeled neurons were found in the VL-VM, AHA, MPOA and BNST. Doubly labeled neurons were rare after injections centered in the lateral or rostral amygdala, although retrogradely labeled neurons were found in the thalamus. Thus E_2-neurons can address limbic E_2-sensitive cell groups as well as other brain regions. (Supported by HD 16327).

62.11 PROJECTIONS OF VENTROMEDIAL HYPOTHALAMIC NEURONS TO THE MIDBRAIN CENTRAL GRAY: AN ULTRASTRUCTURAL STUDY. S.K. Chung*, D.W. Pfaff, R.S. Cohen. Dept. of Anatomy, University of Illinois at Chicago, Chicago, IL 60612 and Dept. of Neurobiology and Behavior, The Rockefeller University, New York, NY 10021.

The mechanism for lordosis behavior is thought to consist of two components: a spinal-brainstem-spinal reflex action dependent on cutaneous stimulation of lordosis relevant areas and hormone-dependent influences initiating in the medial hypothalamus. Through their connections to the midbrain, neurons in the ventromedial nucleus (VMN) may influence the circuitry for female reproductive behavior. Recently, it was shown that some estrogen-concentrating neurons in the VMN send axonal processes to the midbrain (Morrell, J.I. and Pfaff, D.W., Science, 217:1273, 1982). In order to further demonstrate the projections of the VMN neurons to the midbrain central gray (MCG), we performed electrolytic (2ma/10sec) and chemical, i.e., kainic acid (1µg/0.5µl), lesions in the VMN and examined the ultrastructure of these projections in the MCG. Survival times ranged from 27 1/2 hours to 8 days in the former case and 1 week in the latter. Control MCG tissue shows a variety of synaptic types with axodendritic synapses appearing to be the most predominant. Most of the synaptic endings contained many clear vesicles and some contained dense-cored vesicles as well. Most synapses were asymmetric possesing thick postsynaptic densities, some of which showed underlying subjunctional bodies and the dendrites showed well-preserved microtubules. As a result of electrolytic lesions, degenerating endings were seen in the MCG. Characteristics of degeneration include shrunken, dense axons and endings, clumped synaptic vesicles, abnormally large, dark mitochondria and membranous sacs of various sizes. In addition, degenerating cell bodies and postsynaptic processes were seen. Chemical lesions produced similar aspects of axonal and presynaptic degeneration in the MCG. Postsynaptic dendritic processes often appeared swollen, devoid of microtubules and contained enlarged mitochondria. The significance of the abnormal appearance of postsynaptic cell bodies and processes seen with electrolytic and chemical lesions is presently under investigation.

Supported by Campus Research Board Award from Univ. of Illinois at Chicago and NIH Grant HD 05751.

62.12 VASCULAR VOLUME AND AMINO ACID UPTAKE IN RAT CIRCUMVENTRICULAR ORGANS. P.M. Gross [1], R.G. Blasberg* [2], J.D. Fenstermacher [1] and C.S. Patlak* [3]. Dept. of Neurological Surgery[1], SUNY at Stony Brook, NY 11794, Dept. of Nuclear Medicine[2], NIH and Theoretical Statistics & Mathematics Branch[3], NIMH, Bethesda, MD 20205.

Circumventricular organs (CVOs) are specialized periventricular tissues having high capillary densities and fenestrated endothelial cells that make these structures likely target sites for circulating compounds such as hormones, immune complexes, and water-soluble drugs. Quantitative values for vascular volume and rates of blood-to-tissue transfer of plasma solutes in CVOs have not been obtained before probably because of the very small size of these structures. We used quantitative autoradiographic methods to determine plasma and erythrocyte volumes, and blood-to-tissue rates of transfer for a small neutral amino acid, alpha-aminoisobutyric acid (AIB, MW=103 daltons), in the pineal gland, subfornical organ, median eminence, organum vasculosum of the lamina terminalis (OVLT) and lateral ventricular choroid plexi. Conscious Sprague-Dawley rats were given the following labeled tracers through a femoral venous catheter: ^{51}Cr-erythrocytes, ^{125}I-albumin, and ^{14}C-AIB to determine erythrocyte volume (V_e), plasma volume (V_p) and tissue amino acid uptake (K) in separate 5 min, 2 min, and 12 sec studies, respectively. Arterial blood was sampled and assayed for whole blood and/or plasma radioactivity. Coronal sections of frozen brain were cut, autoradiographic images on X-ray film were obtained, and the images were analyzed with a computerized microdensitometer and image-processing system. Computations of V_e, V_p and K were made from measured tissue and plasma or blood radioactivities; K was corrected for plasma radioactivity. A rank-order for total vascular volume ($V_e + V_p$) was discerned: pineal gland < subfornical organ = median eminence = OVLT < choroid plexi, with values in a range of 28 to 107 ul/g, or 3 to 10 times the vascular volume of cerebral gray matter regions (about 11 ul/g). Uptake of AIB in the CVOs (368 to 710 ul/g/min) was 400 to 500 times larger than in cerebral gray matter. The results illustrate 1) examples of the marked heterogeneity of vascular volume (capillary density) and endothelial permeability in the nervous system and 2) that the unique characteristics of the vasculature in CVOs are probably important for penetration to the CNS by circulating humoral factors that elicit neuroendocrine responses.

PEPTIDES: RECEPTORS I

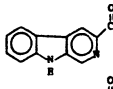
63.1 MULTIPLE TACKYKININ RECEPTORS: SUBSTANCE P AND NEUROMEDIN K. D. E. Wright*[1], L. J. Post*[2], H. I. Jacoby*[2], and J. L. Vaught*[2], (SPON: A. J. Bean). Departments of [1]Chemical and [2]Biological Research, McNeil Pharmaceutical, Spring House, Pa 19477.

Neuromedin K, a peptide isolated from the porcine spinal cord, is a recently discovered member of the tachykinin family. Since structural differences exist between neuromedin K (NK) and substance P (SP), a study was undertaken to evaluate possible pharmacological differences. In the guinea pig ileum (GPI) NK and SP are equipotent [ED_{50} (that dose which produces 50% contraction)= 0.3 (0.17-0.46)x 10^{-9} M for NK and 0.22 (0.15-0.30)x 10^{-9}M for SP]. However, the SP-antagonist spantide, [D-Arg[1], D-Trp[7,9], Leu[11]]-SP, shows a 4-fold preference for inhibition of SP-induced GPI contractions compared with those induced by NK [pA_2=6.1 against NK and 6.7 against SP]. Neuromedin K produces reciprocal hind-limb scratching when injected intrathecally in mice (onset <2 min, duration <5 min) but is much less potent than SP [SD_{50} (that dose which produces scratching in 50% of the animals)= 5.8(3.9-9.6)ng/mouse for SP and 304(158-1667)ng/mouse for NK]. The SP-antagonist [D-Pro[2], D-Trp[7,9]-SP inhibits both SP (20ng) and NK (500ng) induced scratching [ID_{50} (that dose of antagonist which inhibits agonist-induced scratching by 50%)= 4.6(2.9-6.9)ng/mouse for SP and 2.6(0.8-4.8)ng/mouse for NK]. An NK-analog (NKA-I) shows significant antagonism of the immediate scratching response (scratching seen <2 min) induced by NK(500ng) and SP(20ng), but SP-induced scratching requires a 10-fold higher concentration of NKA-I for inhibition (ID_{50}=0.5ng/mouse for NK and 5ng/mouse for SP]. Interestingly, NKA-I produces a delayed (onset ≥10 min) scratching response [SD_{50}=10ng/mouse] not characteristic of tachykinins and in the GPI shows agonist activity [ED_{50}= 12x10^{-9}M] while being devoid of any antagonist effects from 8x10^{-7}M to 2x10^{-5}M. The present data indicate the possible existence of separate NK and SP receptors in both central nervous and peripheral systems.

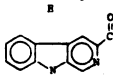
63.2 ACTIVITY OF SELECTED β-CARBOLINE PEPTIDES AT THE BENZODIAZEPINE RECEPTOR. J. C. Kauer* and J. G. Davis, Central Research Dept., Du Pont Experimental Station. Wilmington, DE 19898.

The discovery of the benzodiazepine (Bz) receptor by Braestrup and Squires in 1977 led to a search for an endogenous ligand. Several promising candidates have been isolated including ethyl β-carboline-3-carboxylate which shows high Bz receptor affinity and antagonizes Bz actions (Braestrup, 1980). It was suggested subsequently that this β-carboline residue might have been cleaved (during purification) from a β-carboline peptide which was the true endogenous ligand.

We have synthesized seven β-carboline peptides by the mixed anhydride procedure. These compounds (e.g., 1 and 2) were derived from the two known N-terminal tryptophan-containing neuropeptides DSIP (delta sleep inducing peptide) and CCK-4 (cholecystokinin tetrapeptide).



1



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These peptides, their derivatives, and corresponding tryptophan-terminal peptides were tested for competing activity in a Bz-receptor assay using ^{3}H-flunitrazepam (Fz) and rat brain membranes under standard conditions. Compound 1 above inhibited Fz-binding at 1µM while DSIP itself exhibited no binding. No activity was observed for the CCK-4 analog 2. Thus β-carboline attachment to the DSIP sequence and not to the CCK-4 sequence resulted in an active peptide ligand for the Bz receptor.

63.3 ^{3}H-ELEDOISIN BINDS EXCLUSIVELY TO A SP-P TYPE SITE IN RAT SALIVARY GLAND. S.H. Buck, E. Burcher*, and W. Lovenberg*. Section on Biochemical Pharmacology, NHLBI, Experimental Therapeutics Branch, NINCDS, Bethesda, MD 20205 and Deakin University, Vic. 3217, Australia.

Substance P (SP) and related tachykinins are potent sialagogues in mammals. Physalaemin (P) is more potent than SP while eledoisin (E) is equipotent to SP in stimulating salivation in vivo and in vitro (Rudich and Butcher, Biochim. Biophys. Acta 444, 704, 1976; Brown and Hanley, Brit. J. Pharmacol. 73, 517, 1981). Lee et al. (Mol. Pharmacol. 23, 563, 1983) have determined that ^{3}H-SP binds exclusively to a high affinity (K_D = 1 nM) SP-P type site (P > E) in rat submaxillary gland. P was equipotent to and E was less potent than SP in inhibiting ^{3}H-SP binding. We have examined the binding of ^{3}H-E in rat submaxillary gland.

Radiolabeled E was prepared by tritiation of 2-dehydro-PRO-E to a specific activity of 30 Ci/mmole. Binding assays were conducted with crude membranes in the presence of Mn^{++} as previously described (Lee et al., op cit.; Buck et al., Life Sci. 34, 497, 1984).

With a 2% tissue concentration, specific binding (displaceable by 1 uM unlabeled E) of ^{3}H-E reached equilibrium by 60 min and was stable for up to 90 min at 25° C. At 2 nM ^{3}H-E, specific binding comprised 85% of total binding. Scatchard analysis of saturation data indicated a B_{max} of 9 fmole/mg tissue and a K_D of 4 nM. The Hill coefficient was approximately 1.0 indicating a single class of binding sites. In competition studies of ^{3}H-E binding, SP (IC_{50} = 0.2 nM) was the most potent inhibitor and E exhibited an IC_{50} of 2 nM. For the various tachykinins, the order of potency in inhibiting ^{3}H-E binding was SP > P > substance K ≥ E > kassinin. The displacement curves for all the tachykinins in inhibiting ^{3}H-E binding were shifted leftward by approximately one order of magnitude compared to the inhibition of ^{3}H-SP binding.

Our results show that ^{3}H-E binds to a site in rat submaxillary gland which is present in the same number as the site to which ^{3}H-SP binds and which has the pharmacology of a SP-P receptor. ^{3}H-E has a lower affinity than ^{3}H-SP for this site which is consistent with it being the SP-P type. The results also indicate that ^{3}H-E at nM concentrations can label SP-P receptors. The relative potencies of SP, P, and E in inducing salivation are not in complete agreement with the relative potencies obtained in ligand binding assays in salivary gland membranes suggesting that additional factors are important in intact tissues.

63.4 HIGH AFFINITY SUBSTANCE K BINDING IN GUINEA-PIG INTESTINE. E. Burcher*, S.H. Buck, C.W. Shults, T.N. Chase, M.L. Swenberg*, W. Lovenberg* and T.L. O'Donohue. (SPON: F. Porreca). B.H.S., Deakin Univ., Vic. 3217, Australia; Experimental Therapeutics Branch, NINCDS and Section on Biochemical Pharmacology, NHLBI, NIH, Bethesda, MD 20205.

It has been suggested that the peripheral actions of tachykinins are mediated by two classes of receptors, SP-P and SP-E. Tachykinins are approximately equipotent on SP-P receptors, whereas the order of potency on SP-E receptors is kassinin (K) ≥ eledoisin (E) >> physalaemin (P) > substance P (SP). A recently identified mammalian peptide, substance K (SK), structurally very similar to K, might be the endogenous mammalian ligand for SP-E receptors (Nawa et al., Nature 306, 32, 1983). In these experiments we investigated SK binding in the guinea-pig intestine, using ^{125}I-Bolton Hunter SK (BH-SK), purified by reverse phase HPLC.

Autoradiography was carried out using 24 µm slide-mounted sections of guinea-pig stomach and intestine. After pre-incubation in 50 mM Tris HCl pH 7.4 with bovine serum albumin (BSA), tissues were incubated with BSA, protease inhibitors, 3 mM Mn^{++} and 0.1 nM BH-SK for 2 h. Non-specific binding was defined by the addition of 1 µM SK. High-density specific binding was seen in the outer muscle layers of the colon, ileum, jejenum and duodenum. No specific binding was seen in the stomach, or in the mucosa or submucosa of any region.

Longitudinal muscle from guinea-pig small intestine was homogenized and resuspended in tricine buffer pH 7.4 containing BSA, protease inhibitors, 3 mM Mn^{++} (Buck et al., Life Sci. 34, 497, 1984) and 0.1 nM BH-SK for 90 min. High affinity saturable specific binding (i.e. displaced by 1 µM SK) was seen. This binding was inhibited by SK (IC_{50} 1 nM) >> E > SP > K > P. This pharmacological characterization indicates that BH-SK is not binding to a SP-P receptor. The data also suggest that BH-SK is not binding to a definitive SP-E site, which is consistent with our preliminary finding that ^{3}H-E binding is very low in guinea-pig longitudinal muscle. This probablity of low numbers of SP-E receptors is supported by the results of Watson et al. (Eur. J. Pharmacol. 87, 77, 1983) in the guinea pig ileum. We conclude that BH-SK is binding to a third type of tachykinin receptor, the SP-K receptor, although the possibility that it is also binding to a small population of SP-E receptors cannot be excluded.

63.5 ^{3}H-SUBSTANCE P BINDING SITES IN THE DEVELOPING RAT BRAIN. M.L. Swenberg*, S.H. Buck, and W. Lovenberg* (SPON: E. Ross). Section on Biochemical Pharmacology, NHLBI, NIH, Bethesda, MD 20205.

In the course of investigating the levels of the neuropeptide, substance P (SP), in the developing rat brain of Sprague-Dawley rats, we observed that SP-like immunoreactivity (SPLI) could be detected in low levels as early as day 11 of gestation (G11) in whole brain homogenates. Beginning on G14-15, the amount of SPLI increased dramatically until it reached the maximum whole-brain level by G22-23. SPLI appeared to fall at birth and then increased transiently again during P7-21 (Gilbert and Emson, Brain Res. 171, 166, 1979; Swenberg and Lovenberg, Soc. Neurosci. Abst. 7, 509, 1981). The prenatal changes in levels of SPLI were observed in most brain regions while the postnatal changes were most prominent in the midbrain and brainstem. In considering possible differences in roles for SP in the immature versus mature brain, we have examined ^{3}H-SP binding and compared changes in this binding to changes in SPLI levels.

Crude brain membranes from prenatal (G) and postnatal (P) rats were prepared as described by Lee et al. (Mol. Pharmacol.) 23, 563, 1983) and Buck et al. (Life Sci. 34, 497, 1984). A 4% tissue suspension (based on initial wet weight) was then incubated for 20 min at 25° C in 20 mM tricine (pH 7.4) containing BSA, bacitracin, chymostatin, leupeptin, 5 mM $MnCl_2$, and ^{3}H-SP, followed by filtration. Specific binding was defined as the difference between the presence and absence of 1 uM unlabeled SP.

Substantial amounts of low affinity (K_D > 10 nM) ^{3}H-SP binding were observed on G15 through G18 with the additional appearance of high affinity (K_D = 0.5 nM) binding at G20 through G23. High affinity binding persisted at steady high levels (approximately 200 fmole/mg protein) through birth and into the first two postnatal weeks. Low affinity binding subsided markedly during the same postnatal period. By P21, high affinity ^{3}H-SP binding had fallen to near the adult level of approximately 50 fmole/mg protein. SPLI was transiently elevated at G21-22 and during P7 through P21.

The low affinity binding we have observed may be an immature binding site, a low affinity receptor, or binding to a transport molecule. The prenatal development of high affinity ^{3}H-SP binding parallels an increase in SPLI while the postnatal transient rise in SPLI may be associated with the reduction to adult levels of transiently high ^{3}H-SP binding. The regulation of SP receptors by the peptide may thus be different in the immature and mature brain.

63.6 EFFECT OF IONS AND GUANINE NUCLEOTIDES ON THE RAT BRAIN SUBSTANCE P RECEPTOR. P.M. Narang*, S.W. Bahouth*, D.E. Brundish* and J.M. Musacchio. Dept. of Pharmacology, New York Univ. Med. Ctr., New York, NY 10016.

[^{3}H]Physalaemin ([^{3}H]Phy) was used to characterize the substance P (SP) receptor in rat brain membranes. The binding of [^{3}H]Phy is specific, saturable and reversible. It requires the addition of peptidase inhibitors, the presence of monovalent cations and it is increased by 100 percent by the addition of 2.5 mM $MnCl_2$. Under these conditions, Scatchard analysis demonstrated a single, noninteracting high affinity site with a K_d = 3.6 nM and a B_{max} = 75 fmole/mg protein. The nonspecific binding was only 15 percent of the total binding at 4.0 nM [^{3}H]Phy and subcellular distribution studies demonstrated that the specific binding was maximal in the synaptosomal fraction. Regional distribution studies demonstrated that binding was maximal in the olfactory bulbs, followed by the hypothalamus, striatum, hyppocampus, cortex and cerebellum, which was usually excluded from the assay. Competition experiments indicated that SP and Phy were equipotent, while SP(4-11), SP(3-11) and eledoisin were 13, 10 and 3.6 percent as active as Phy. These finding demonstrated that the receptor labeled by [^{3}H]Phy is of the SP-P type.

Gpp(NH)p and GTP completely inhibited the binding increase produced by 2.5 mM MnC_{12} and in the absence of divalent cations, decreased the binding of [^{3}H]Phy by only 15 percent. Preincubation of the membranes with NEM or PCMB for 30 min inhibited binding in a dose dependent manner. If the SP receptors were protected with 10 µM Phy, the sulphydryl reagents did not inactivate binding, but inhibited the effect of both, divalent cations and G nucleotides. These effects were irreversible, but could be prevented by reduced glutathione or DTT. Since the extent of the NEM inhibition was identical to that of the G nucleotides and G nucleotides became ineffective after the treatment, we concluded that NEM inactivates a G nucleotide binding regulatory protein. (Supported in part by PHS grants DA-02013, MH-29591 and MH-17785).

63.7 [^{3}H]SUBSTANCE P AND [^{3}H]PHYSALAEMIN BINDING TO THE RAT SUBMAXILLARY GLAND. S.W. Bahouth*, D. Lazaro*, D.E. Brundish* and J.M. Musacchio (SPON: R. Margolis). Dept. of Pharm., New York Univ. Med. Ctr., New York, NY 10016.

[^{3}H]substance P ([^{3}H]SP) and [^{3}H]physalaemin ([^{3}H]PHY) bind to a single class of noninteracting binding sites on rat submaxillary gland membranes suspended in high ionic strength media (0.2 M sodium sulfate, 20 mM HEPES, pH 7.4). The K_D of both labels was 2.7-2.8 nM and the B_{max} was 220-240 fmole/mg protein. The potency of the various SP analogs in inhibiting the binding paralleled their relative salivation potency, indicating that a physiologically relevant SP receptor was labeled.

When the membranes are suspended in a low ionic strength binding medium (0.3 M sucrose), [^{3}H]SP binds with a K_D of 0.14 nM and B_{max} 350 fmole/mg protein. The IC_{50} of SP and PHY in displacing 1 nM [^{3}H]SP was 1.4 and 150 nM respectively. The K_D of [^{3}H]PHY in 0.3 M sucrose was 3.3 nM and the B_{max} 300 fmole/mg protein. The IC_{50} of SP and PHY in displacing 2.5 nM [^{3}H]PHY was 0.18 and 7 nM respectively. The data of the displacement of [^{3}H]PHY and [^{3}H]SP was analyzed simultaneously by the LIGAND computer program. The K_D of SP and PHY was 0.06 and 4.4 nM respectively and the B_{max} = 300 fmole/mg protein. Thus in low ionic strength, PHY had one seventieth the affinity of SP, eventhough it is twice more potent as a sialogogue.

The addition of monovalent cations or the pretreatment of the membranes by the method of Lee et al. (Mol. Pharmacol. 23:563-569, 1983), reduces the affinity of SP but not PHY to the rat submaxillary gland. The charged groups in the amino terminal of the SP molecule (Arg^1 and Lys^3) are somehow responsible for the high binding affinity of SP and some of its fragments in low ionic strength. PHY, lacks these basic groups, and its K_D did not change as a function of ionic strength.

The B_{max} of [^{3}H]PHY and [^{3}H]SP increased by 20-30 percent in the presence of optimal concentrations of Mg^{2+} and Mn^{2+}, while 50 μM GTP and Gpp(NH)p reduced the B_{max} of both ligands to 140 fmole/mg, in the presence or absence of divalent cations. We conclude that divalent cations increase the population of receptors that are sensitive to guanine nucleotides. This work was supported in part by PHS grants DA-02013, MH-29591 and MH-17785 to J.M.M.

63.8 SUBSTANCE P RECEPTOR MEDIATED RESPONSES IN A RAT PANCREATIC ACINAR CELL LINE. M.D. Womack*, M.R. Hanley, and T.M. Jessell. Dept. of Neurobiology, Harvard Medical School, Boston, MA and Dept. of Biochemistry, Imperial College, London.

Many of the membrane and intracellular events that mediate the physiological actions of substance P (SP) on mammalian cells remain to be determined. A rat pancreatic acinar cell line, AR4-2J, exhibits a high density of SP binding sites (Hanley and Jessell, J.Physiol. In Press). To determine whether these receptors are functional, we have examined the membrane, intracellular, and secretory events evoked by exposure of AR4-2J cells to SP.

SP binding sites on intact AR4-2J cells were identified by labeling with ^{125}I Bolton Hunter SP (^{125}I-BHSP) (2200 Ci/mmol; 80pM). ^{125}I-BHSP binding to intact cells has an apparent Kd of 40pM with slow rates of association and dissociation. The binding site density was approximately 10,000 per cell. Binding of ^{125}I-BHSP was inhibited by SP (IC_{50}= 500pM) and by structurally related peptides. Physalaemin was more potent than SP whereas kassinin, eledoisin, and substance K were much less potent. SP free acid and SP-(7-11) were 3-4 orders of magnitude less potent than SP in displacing ^{125}I-BHSP.

AR4-2J cells contained 1.24 +/- 0.02 U amylase/ 10^5 cells (mean +/- s.e.m., n=6). Incubation of AR4-2J cells with SP (10pM-10nM) elicited a dose-related increase in amylase secretion. A half maximal increase in amylase secretion was obtained with 50pM SP. Incubation of AR4-2J cells with most other pancreatic secretagogues did not evoke amylase release. Bombesin (1μM) caused a significant increase in amylase secretion.

Intracellular recording revealed AR4-2J cells had resting potentials of about -60 mV. Pressure application of SP (100nM-100pM; 1 sec) evoked depolarizations of 20-40 mV which were maintained for 1 to 2 min.

The intracellular free calcium concentration $[Ca^{2+}]i$ in AR4-2J cells, measured with Quin-2 AM, was between 100 and 500 nM. Addition of SP (100pM-10nM) or physalaemin (1nM) induced a transient rise in $[Ca^{2+}]i$ whereas SP- free acid was ineffective. AR4-2J cells may be useful for determining SP-linked membrane transduction events that lead to calcium mobilization.

Supported by NIH grant NS 17369, a NATO travel grant and by Pfizer Inc.

63.9 MODULATION OF 3-H SUBSTANCE P BINDING TO LRM-55 CELLS BY CATIONS AND NUCLEOTIDES. R.E. Lepore*, W. G. Shain, and M.H. Perrone* (SPON: D.R. Haubrich). CNS Section Dept. of Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, N.Y. 12144 and Center for Laboratories and Research, N.Y. Dept. of Health, Albany, N.Y. 12201.

We have demonstrated the presence of 3-H substance P (SP) binding sites on LRM-55 glial cells by radioligand studies. These sites appear to be biologically active receptors based on the fact that SP inhibits isoproterenol-induced taurine release from these cells. In this report we describe the modulation of 3-H SP binding by cations and nucleotides. Cells were grown in monolayers, harvested, homogenized, and a 48,000xg pellet resuspended in 50 mM Tris. Incubations were for 20 min at 20° C. and were terminated by rapid filtration over GF/F filters presoaked in 0.1% polyethylenimine. Incubation with $MgCl_2$, $CaCl_2$, or $MnCl_2$ produced biphasic effects on 3-H SP binding--concentrations less than 1 mM enhanced binding but higher concentrations decreased binding. Additions of up to 30 uM GTP, GDP, GMP, Gpp(NH)p, ITP, ATP, ADP, or cAMP inhibited binding less than 15%. However, in the presence of 2 mM $MgCl_2$ and $CaCl_2$, 10 uM GTP decreased binding by 32%. NaCl produced dose-dependent decreases in 3-H SP binding with a 45% inhibition of binding at 150 mM. Divalent cations shifted competition displacement curves for SP analogues to the left. In the presence vs. the absence of 2 mM $MgCl_2$ and $CaCl_2$, the IC_{50} values for physalaemin (11nM vs 44nM), eledoisin (224nM vs 10,000nM), SP_{3-11} (12nM vs 123nM) and SP methyl ester (320nM vs 25,000nM) were significantly lower.

The results presented here demonstrate that divalent cations and guanine nucleotides modulate the binding of 3-H SP to its receptor in LRM-55 cells. Although the regulation of neurotransmitter binding to receptors coupled to adenylate cyclase is well documented, the SP receptor in LRM-55 cells does not appear to be linked to adenylate cyclase. Thus Rodbell's (Nature, 284 1980) suggestion that there is a nucleotide regulatory protein for all hormone-regulated systems is supported by these data.

63.10 GUANINE NUCLEOTIDE REGULATION OF SUBSTANCE P RECEPTORS IN RAT SMALL INTESTINE. K.E. Smith* and W.P. Hoss (SPON: R.D. Frisina). Center for Brain Research, Univ. of Rochester Medical Center, Rochester, NY 14642.

Substance P (SP) is a putative neurotransmitter of enteric neurons having potent stimulatory effects on gastrointestinal motility. We have previously reported the specific binding of ^{3}H-SP to a washed membrane preparation of rat small intestine (Trans. Am. Soc. Neurochem. Abstract 93, 1984). Briefly, ∿100 μg tissue protein is incubated for 30 minutes at 20°C with varying concentations of ^{3}H-SP (23.8 Ci/mmole, New England Nuclear) in 1 ml 50 mM Tris-HCl, pH 7.4 containing 4 μg leupeptin, 2 μg chymostatin, 50 μg bacitracin and 0.02% BSA. Nonspecific binding is determined in the presence of 2 μM unlabeled SP. Incubation is terminated by dilution and rapid filtration through glass fiber filters pretreated with 0.1% polyethylenimine.

Specific binding represents > 80% of total at concentrations up to 2 nM ^{3}H-SP and is saturable and reversible. Unlabeled SP displaces 2 nM ^{3}H-SP with an IC_{50} of 4 nM. Scatchard plots of specific binding are curved and can be resolved into a high affinity site with K_D ≈ 0.35 nM and B_{max} ≈ 0.3 pmoles/mg protein, and a low affinity site with a K_D in the nanomolar range. The divalent cations manganese, magnesium and calcium (10 mM) cause up to 60% inhibition of specific binding and zinc (1 mM) reduces binding by 95%.

Sodium (50 mM-200 mM) inhibits specific binding of 1 nM ^{3}H-SP in a dose-dependent manner ranging from ∿20% inhibition at 50 mM to ∿74% at 200 mM. Guanosine 5'-Triphosphate (GTP, 100 μM) alone has a small inhibitory effect, but in the presence of 50 mM and 100 mM sodium inhibition is increased to ∿43% and∿ 69%, respectively. This represents a ∿1.5 fold increase in inhibition over that of sodium alone, and is greater than can be accounted for by additive effects. Scatchard analysis of specific binding in the presence and absence of 50 mM sodium + 100 μM GTP reveals a significant reduction in binding affinity with a smaller reduction in B_{max}. These studies show that GTP and sodium modulate SP binding and suggest that a guanine nucleotide binding protein is involved in the mediation of SP effects in this tissue.

63.11 SUBSTANCE P BINDING SITES IN THE RAT SPINAL CORD: SUBCELLULAR DISTRIBUTION AND PHARMACOLOGICAL CHARACTERIZATION. E.E. Mena, M.J. Pagnozzi*, M.F. Gullak* and C.J. Pazoles. Central Research, Pfizer Inc., Groton, CT 06340.

Evidence from electrophysiological, immunohistochemical and behavioral studies indicate that Substance P (SP) may be a transmitter or modulator of sensory information in the spinal cord (SC). We have analyzed the subcellular distribution, pharmacological characteristics and kinetic properties of the SP binding sites in rat SC. (125-I) Bolton Hunter (BH)-SP binding was determined by a microfuge assay in 50 mM Tris-HCl, pH 7.4, containing divalent cations, protease inhibitors and 25 pM (125-I) BH-SP. Nonspecific levels were determined in the presence of 1 µM nonradioactive SP. SP binding sites were enriched nearly 5 fold in the synaptic plasma membrane (SPM) fraction when compared to the total SC particulate material (8.22 vs. 1.68 fmol/mg protein). The rank order of SP binding to subcellular fractions was: SPM>P3>P2>SC particulate material>P1>>myelin> mitochondria. No specific binding was detected in the synaptic junction (SJ) fraction. However, this binding site may be unstable to the detergent required to prepare SJs. Scatchard analysis of SP binding in rat SPMs revealed a single class of binding sites with K_d and B_{max} values of 288 pM and 48 fmol/mg protein, respectively. GTP was a potent inhibitor of this binding. The ability of several analogs of SP to displace (125-I) BH-SP from the SPM fraction from SC was examined. These compounds and their IC_{50} values were: SP1-11, 320 pM; (Sar^9) SP1-11, 380 pM; SP3-11, 1.3 nM; SP4-11, 3.2 nM; Physalaemin, 3.8 nM; SP-methyl ester, 4.8 nM; SP5-11, 12 nM; Eledoisin, 28 nM; SP6-11, 34 nM; Kassinin, 110 nM; SP-COOH, 120 nM; (N^{8}-methyl Phe^{8},Sar^9) SP, 510 nM; ($DArg^1$,$DPro^2$, $DTrp^{7,9}$,Leu^{11}) SP, 535 nM; ($DPro^2$, $DTrp^{7,9}$) SP, 710 nM; SP7-11, 2.3 µM; ($DPro^2$, $DTrp^{7,9}$) SP4-11, 3.1 µM; SP1-4, >10 µM; SP1-9, >10 µM. The results show that the concentration of SP binding sites in membrane fractions from the rat SC increases as the purity of synaptic components increases. (125-I) BH-SP binding to these sites can be displaced by SP analogs with an order of potency qualitatively similar to their reported ability to stimulate salivation and to depolarize spinal neurons. The results suggest that a physiologically relevant SP receptor has a synaptic localization in the rat SC and this receptor can be studied by in vitro methods.

63.12 AUTORADIOGRAPHIC LOCALIZATION OF RECEPTOR BINDING SITES FOR SUBSTANCE P IN THE GASTROINTESTINAL TRACT AND PERIPHERY OF THE GUINEA PIG. P.W. Mantyh, M. Goedert* and S.P. Hunt*. MRC Neurochemical Pharmacology Unit, MRC Centre, Medical School, Hills Road, Cambridge, CB2-2QH UK and C.U.R.E., V.A. Center/Wadsworth, Building 115, Room 217, Los Angeles, CAL, 90073, USA.

We have used the autoradiographic receptor binding technique to map the distribution of substance P (SP) receptors in several peripheral tissues including; stomach, uterus, duodenum, ileum, heart, blood vessels, esophagus, bronchus, lung, tongue, skin, penis, vagina, ovaries, colon, prostate gland, thyroid, kidney and a variety of peripheral ganglia. Frozen slide mounted tissue sections 20 um thick were preincubated for 10 min at 19°C in 50 mM Tris-HCl, pH 7.4 containing 0.005% (v/v) polyethylenimine followed by 10 min incubation at 19°C in 50 mM Tris-HCl, pH 7.4 containing 200 mg/l BSA, 2 mg/l chymostatin, 4 mg/l leupeptin 40 mg/l bacitracin, and 2 nM [^{3}H]-SP in the absence (total binding) and presence (non-specific binding) of 1 uM SP. At the end of the incubation, the slides were transferred sequentially through 4 rinses (10 sec ea) of 50 mM Tris-HCl pH 7.4, 0°C followed by 4 rinses (5 sec ea) of dH_2O and then dried quickly. Autoradiograms were generated using tritium sensitive-film or emulsion coated coverslips. Tritium sensitive film was then analysed by computer aided densitometry. Although specific [^{3}H]-SP binding was observed in the great majority of tissues, given the limitations of space we will only describe the specific binding observed in the gastrointestinal tract. SP receptors were widely distributed in the gastrointestinal tract. In the duodenum high densities of receptors were observed in the muscularis mucosa while lower densities were present in the longitudinal and circular muscle layers. In the ileum and colon SP receptors were also widely distributed although in these tissues the circular muscle appeared to have the heaviest concentration of receptors when compared to a more moderate density in the longitudinal muscle and muscularis mucosa.

In summary we have localized SP receptor binding sites in the guinea pig periphery using an autoradiographic technique with light microscopic resolution. SP receptor sites would appear to correlate well with previous studies which have shown SP-like immunoreactivity, SP actions and a functional SP response in these peripheral tissues. These results suggest that the SP receptor binding sites observed in the present study are those which mediate the effects of SP in the periphery.

PEPTIDES: RECEPTORS II

64.1 AUTORADIOGRAPHIC LOCALIZATION OF RECEPTORS FOR THYROTROPIN-RELEASING HORMONE (TRH). R. Dean,* H.I. Yamamura, E. Snowhill,* J.K. Wamsley (SPON: R.J. Mullen), U of UT Dept. Psych & Pharm, SLC, UT 84132, U of AZ Dept. of Pharm, Tucson, AZ.

Thyrotropin-releasing hormone (TRH) is a tripeptide that was originally described as a thyrotropin-releasing factor which influenced pituitary function. Subsequently, TRH has been localized in distinct neuronal populations within the CNS of a variety of vertebrate species where it is thought to subserve functions totally unrelated to its role in the pituitary (Jackson et al, 1974; Oliver et al, 1974; Winters et al, 1974). The results of these and other studies suggest a role for TRH as a neurotransmitter or a neuromodulator. Specific, saturable, high affinity receptor binding of TRH has been demonstrated using radioligand binding and immunohistochemical techniques.

It is important in receptor binding studies that the experiment be performed at equilibrium. To establish at what time equilibrium is reached in slide-mounted tissue sections, we performed an association experiment encompassing the following incubation times: 15 min, 30 min, 1, 2, 3, 4, 5, 6, 7 & 8 hrs. Our results demonstrate that equilibrium is reached between 4-6 hrs. All subsequent autoradiographic experiments employed a 6-hr incubation time. Coronal tissue sections, 10µ thick, were incubated in the presence of 2nM [^{3}H]-TRH (New Eng. Nuc.) in 20nM sodium phosphate buffer, pH 7.4, for 6 hrs at 4°C on ice in a cold room. Anatomically adjacent tissue sections were incubated under similar conditions with the added presence of unlabeled TRH to determine regions of non-specific binding. The incubation phase was followed by two 5-minute rinses in buffer. Slides were then dried under a stream of cold, dry, filtered air. Autoradiograms were obtained by apposing slide-mounted tissue sections to tritium sensitive film (LKB Ultrofilm) for 6 months. A high density of specific TRH binding sites were localized in: the molecular layer of the dentate gyrus, the cortex lining the superior border of the rhinal sulcus, the nucleus tractus diagonalis, the bed nucleus of the stria terminalis, the interpeduncular nucleus, entorhinal cortex and superficial layer of the superior colliculus. A lower, but still highly significant, density of receptors was found in laminae IV and VI of the parietal cortex, basal forebrain areas, subiculum, periaqueductal gray matter, principal nucleus of the trigeminal nerve, structures on the floor of the fourth ventricle and in the dorsal and ventral horns of the spinal cord. The present study thus greatly extends the number of discrete regions reported to exhibit specific TRH binding.

64.2 **AUTORADIOGRAPHIC LOCALIZATION OF THYROTROPIN-RELEASING HORMONE (TRH) RECEPTORS IN THE HUMAN SPINAL CORD.** A. Winokur, S. Manaker, C.H. Rhodes* and T.C. Rainbow, Depts. of Biology, Psychiatry, Pharmacology and Pathology, University of Pa. School of Medicine, Philadelphia, PA 19104.

Thyroptropin-releasing hormone (TRH) has been demonstrated to be widely distributed throughout the CNS, including spinal cord. TRH has been reported to affect neuronal activity within the spinal cord, depolarizing dorsal sensory and motor neurons and altering the firing rates of interneurons. In addition, specific receptors for TRH have been demonstrated within spinal cord tissue from many species. However, such receptors have not been demonstrated within the spinal cord of humans. In preliminary studies, TRH has been reported to be efficacious in the treatment of humans with amyotrophic lateral sclerosis and spinocerebellar degeneration. The present study localizes TRH receptors within the human spinal cord with quantitative autoradiography.

Regions of spinal cord were rapidly dissected from four individuals after their demise. Coronal sections 32 µ thick were cut at -18°C, thaw-mounted onto subbed slides, and stored at -70°C until use. Slides were warmed to 25°C, preincubated for 10 min in Tris/$MgCl_2$/BSA buffer (pH 7.4), immediately chilled to 4°C and allowed to air dry. Sections were then incubated with 300 µl of ice-cold buffer containing 320 µM bacitracin and 10 nM of (^{3}H)-MeTRH (3-methyl-histidine-TRH). Non-specific binding was defined as the binding of (^{3}H)-MeTRH in the presence of 10 µM TRH. After a 2-hr incubation, slides were washed with ice-cold buffer 4 times for 30 sec each, dipped in ice-cold distilled water to remove buffer salts, rapidly dried on a 60-70°C slide drier, and apposed to LKB tritium-sensitive Ultrofilm for 2 mos. Density values were converted into molar amounts using tritium brain mash standards.

Highest concentrations of TRH receptors (125-200 fmol/mg protein) were present in Lamina II, the substantia gelatinosa, of the human spinal cord. The motor neurons of the anterior horn, Lamina IX, contained a moderate density (50-100 fmol/mg protein). The remainder of the grey matter of the spinal cord, including the dorsal and ventral grey horns and the intermediolateral column, possessed low levels of TRH receptors (< 50 fmol/mg protein). No differences were noted between cervical, thoracic, lumbar and sacral concentrations of TRH receptors. No TRH receptors were localized to white matter. In general, this distribution of TRH receptors within the human spinal cord closely corresponds to the distribution seen in the rat spinal cord. However, humans possess a greater concentration of TRH receptors in Lamina IX than rats. The possibility that TRH receptors mediate the clinical effects of TRH on human spinal cord function requires further investigation.

64.3 SOMATOSTATIN ANALOGS WITH δ- AND μ-OPIATE RECEPTOR ACTIVITY. K. Gulya*, J.T. Pelton+,*, V.J. Hruby+,*, S.P. Duckles and H.I. Yamamura (SPON: Kelvin Gee). Depts. of Pharmacology and Chemistry+, Univ. of Arizona, AZ 85724.

Somatostatin, a cyclic tetradecapeptide, is known to interact with its own receptor and with others, including opiate receptors in the rat brain, giving rise to many different biological responses. A series of somatostatin ana - logs (most of them are cyclic compounds) and their penicillamine-substitued derivatives have been prepared by standard solid phase synthetic techniques and tested for their ability to displace ^{3}H -naloxone, ^{3}H - D-Ala^{2},D-Leu^{5} enkephalin and ^{125}I-CGP 23,996 (des-Ala^{1},Gly^{2}-desamino-Cys^{3}- Tyr^{11} - dicarba-somatostatin) binding from rat brain receptors.

While CGP 23,996, somatostatin and its cystine containing octapeptide displayed little or no preference for either receptor system, the substitution of penicillamine at posi - tions two or seven resulted in analogs that displayed opposite opiate receptor selectivity. While all the octapeptide analogs were less potent than somatostatin in inhibiting ^{125}I-CGP 23,996 binding to rat brain membranes, the least active analogs contained penicillamine at the second position. Substitution of penicillamine for cysteine at the seven position resulted in a small increase in binding affinity. When penicillamine was substituted for cysteine at the second position, however, a decrease of 200-fold potency in μ-opiate receptor selectivity was observed. The substitution of tyrosine for phenylalanine at position three resulted in an increase in opiate receptor activity which may be related to the known requirement for a phenolic hydroxyl moiety in the rigid opiate and enkephalin systems (IC_{50}=300 nM and 3700 nM in the case of μ- and δ-opiate receptors, respec - tively), but decreased somatostatin activity. The acyclic tetrapeptide fragment Ac-Phe-Trp-Lys-Thr which is the critical pharmacophore for growth hormone showed a 9-fold preference for δ-opiate receptors; its IC_{50} values were 5800 nM and 51,000 nM towards δ- and μ-opiate receptors. The introduction of an amide derivative at the eight position resulted in a very potent octapeptide which had an IC_{50} of 3.5 nM for the μ-opiate receptor and 950 nM for the δ-opiate receptor. In this latter case the displacement curve was shallow, and the n_H value was close to 0.3 indicating at least two states or sites of this receptor.

Work is in progress to further elucidate the nature of these changes and their connection with the adenylate cyclase system.

64.4 CENTRALLY MEDIATED HYPOGLYCEMIC EFFECT OF INSULIN: INVOLVEMENT OF SPECIFIC INSULIN RECEPTORS. Y. Shechter*and S. Amir (SPON: V.I. Teichberg). Departments of Hormone Res. and Isotope Res., The Weizmann Inst. of Sci., Rehovot, Israel.

Injection of insulin into the central nervous system (CNS) produces systemic hypoglycemia within minutes. This effect is mediated by activation of the parasympathetic system and involves the release of endogenous insulin from the endocrine pancreas. The receptors involved in the central effect of insulin have not been determined. Insulin receptors are widely distributed in the mammalian brain and studies have shown that these receptors are identical to the insulin receptors on classical target tissues by all criteria tested including specificity for insulins and insulin analogues. To determine the involvement of these receptors in the central hypoglycemic action of insulin, male ICR mice were injected intracerebroventricularly (ICV) with native insulin or several insulin analogues possessing differential activity at peripheral target sites - i.e. A chain (0% activity), succinyl$_3$ insulin (1% activity) and acetyl$_3$ insulin (10% activity) - and the plasma glucose was determined 15 min later. Plasma glucose of control mice receiving ICV saline injection was 189.6±3.7 mg% (n=10). ICV injection of 0.25 or 2.5 ug of the A chain fragment (0% activity) had no effect on the plasma glucose; a higher dose 25 ug, slightly though not significantly decreased the plasma glucose (178.6±7.1mg% n=6). Succinyl$_3$ insulin (1% activity) was not effective at 0.25 and 2.5 ug but it markedly decreased the plasma glucose at 25 ug (104.5±6.2 mg% n=6). Acetyl$_3$ insulin (10% activity) had no effect at 0.25 ug but it produced significant hypoglycemia at 2.5 ug (125.0±4.1 mg% n=10) and 25 ug (103.5±5.1 mg% n=6). Finally native insulin decreased the plasma glucose to 173.0±10.5 mg% (n=6) at 0.25 ug and caused profound hypoglycemia at 2.5 ug (100.4±3.6 mg% n=10) and 25 ug (88.6±2.2 mg% n=6). These results indicate that insulin analogues with graded potency at peripheral target tissues can differentially affect the plasma glucose following ICV injection. This may support the notion that the hypoglycemic response to centrally administered insulin is mediated through specific insulin receptors in the CNS.

64.5 QUANTITATIVE RECEPTOR AUTORADIOGRAPHY AND COMPUTER DIGITAL IMAGE MEASUREMENT OF [3H]-VASOPRESSIN BINDING IN RAT KIDNEY AND BRAIN. D. Baskin, F. Petracca, D. Dorsa (SPON: W. Stahl). Depts. Medicine Pharmacology, Psychology, Biological Structure, Univ. Washington, and VA Medical Center, Seattle, WA 98108

We have recently shown that [3H]-arginine vasopressin (AVP) binds specifically to renal medulla and cortex, and the lateral septum and amygdala of the rat brain. In order to characterize the pharmacology of AVP binding at these anatomical sites in situ, we labeled rat kidney and brain slices (frozen, unfixed) with 1-2 nm [3H]-AVP (43 Ci/mmol, New England Nuclear) and localized the binding sites with LKB Ultrofilm (30 days exposure). Specific binding (displaced by 1-2 uM unlabeled AVP) was observed in kidney medulla and cortex, in lateral septum, amygdala, olfactory tubercle, nucleus accumbens, and ventral tegmentum. Binding to renal medulla was characterized by labeling rat kidney slices with 1-20 nM [3H]-AVP alone and with 1 uM unlabeled AVP to determine nonspecific binding. The optical density (gray level) of the corresponding regions on the LKB film was measured with a CCD camera and macro lens, A/D converter, and microcomputer. The gray levels were converted to fmol AVP bound/mm sq, using a standard curve (gray level/pixel vs [3H]-AVP CPM/mm sq) that was based on [3H]-AVP binding to renal cortex slices. Analysis of computer-measured binding to renal medulla slices with EBDA and LIGAND programs, showed a Hill coefficient of 1.00, suggesting a single site with a Kd=5.7x10(E-9) mol and a site number of 1.56x10(E-12) mol/mm sq for the renal medulla AVP binding site. These values are similar to those obtained in previous results from renal medullary membranes. Specific binding of [3H]-AVP for various renal and brain regions were:

Region	Specific binding (fmol/sq mm)
Olfactory Tubercle	1.5
Amygdala	1.4
Ventral Tegmentum	1.5
Kidney Medulla	0.9
Nucleus accumbens	0.9
Lateral Septum	0.6
Kidney Cortex	0.5

These results suggest that computerized digital image analysis of LKB films can characterize the pharmacology of AVP binding in small, focal brain regions in situ, and thus identify potential targets of AVP action. The levels of specific binding for AVP in several brain regions are comparable to those in the renal medulla, indicating that AVP receptors are located in these regions.

64.6 CHARATERIZATION OF ANGIOTENSIN II BINDING SITES ON NEUROBLASTOMA X GLIOMA HYBRID CELLS. J.A. Weyhenmeyer and C.-J. Hwang*. College of Medicine, University of Illinois, Urbana, IL, 61801.

Angiotensin II (AII) has been implicated to have a number of physiological and pharmacological roles in CNS tissues, including an increase in blood pressure, fluid intake and hormone release. Recent immunochemical evidence has demonstrated a complete renin-angiotensin system in the neuroblastoma x glioma hybrid cell line, NG108-15 (Fishman et al., Sci. 214, 1981). We have examined the homogenous NG108-15 cell line to determine whether these neoplastic cells contain an AII binding site that shares similar biochemical characteristics with the AII receptor that is widely distributed in the mammalian CNS.

Mouse neuroblastoma x rat glioma hybrid cells (NG108-15) were grown and passaged in Dulbecco's modified Eagle's medium, supplemented with 10% fetal bovine serum. Cells were grown until confluent (3 to 4 days), harvested, and assayed in phosphate buffered saline containing 0.2% bovine serum albumin. AII binding in the NG108-15 hybrid cells was specific, saturable and reversible. Scatchard analysis, using concentrations of .003 to 1.0 nM iodinated AII and 150 nM unlabeled AII, revealed a linear plot with an affinity constant (K_d) of .313 nM and binding capacity (B_{max}) of 7.13 fm/mg protein. Kinetic studies demonstrated an association rate (K_{+1}) of 7.76 x $10^{6}M^{-1}sec^{-1}$ and dissociation rate (K_{-1}) of 4.18 x 10^{-4} sec^{-1}. Inhibition curves, using concentrations of $10^{-4}M$ to $10^{-11}M$ unlabeled AII, revealed high and low affinity components with IC_{50} values of .46 nM and 1.75 μM respectively. Saralasin had approximately equal displacement potency with AII at the high affinity component, while structurally unrelated peptides (eg., vasopressin, somatostatin, insulin, neurotensin and substance P) had no effect on AII binding.

Our results indicate that the NG108-15 cell line contains a highly specific AII binding site that closely resembles the kinetic characteristics of the AII receptor in the mammalian CNS. Further, our data suggests that this hybrid cell line contains a low affinity component similar to that described in the rat neostriatum by Simonnet et al. (Neurochem. Int. 4, 1982). We conclude that the NG108-15 should provide a model system for investigating the biochemical and molecular properties of the AII binding site.

This work was supported by NIH Grant HL 27757 to JAW.

64.7 REDUCED HIGH AFFINITY CHOLECYSTOKININ BINDING IN HIPPOCAMPUS AND FRONTAL CORTEX OF SCHIZOPHRENIC PATIENTS. S.M. Farmery*, F. Owen*, M. Poulter* and T.J. Crow* (SPON: T.W. Stone). Division of Psychiatry, MRC Clinical Research Centre, Watford Road, Harrow, HA1 3UJ, UK.

High affinity specific binding sites for cholecystokinin (CCK) have been demonstrated in the brains of several species, including man (S.E. Hays, F.K. Goodwin, S.M. Paul, Brain Res., 225: 452, 1981). In the present study CCK binding sites were assessed in membrane preparations from five regions of post mortem brain of controls and schizophrenic patients. CCK_{33} was iodinated by conjugation to ^{125}I-Bolton Hunter reagent (H. Sankaran, C.W. Deveney, I.D. Goldfine, J.A. Williams, J. Biol. Chem., 254: 9349, 1979). Binding of ^{125}I-BH CCK_{33} to crude membrane preparations was assessed by the method of Hays et al. All assays were carried out blind and in triplicate. The highest binding values were in the caudate nucleus, with less binding in the frontal cortex, temporal cortex, and amygdala, and the least binding in the hippocampus. Compared with controls ^{125}I-BH CCK_{33} binding was reduced by 40% ($p < 0.02$) in the hippocampus and 20% ($p < 0.01$) in the frontal cortex of the schizophrenic group. There were no significant differences between the groups in caudate nucleus, amygdala, or temporal cortex. It was not possible to carry out a saturation analysis to determine whether the increase in ^{125}I-BH CCK_{33} binding was due to a reduction in receptor number, or a change in affinity, because of the limited amount of brain tissue available. ^{125}I-BH CCK_{33} binding was unrelated to patients age, neuroleptic medication, delay between death and autopsy or sample storage time. The decrease in CCK binding observed in this study provides further evidence of receptor abnormalities in schizophrenia, and although the number of brains studied was small (16 control and 14 schizophrenic frontal cortex samples: 9 control and 9 schizophrenic hippocampus samples) the results suggest that further investigation of CCK binding in schizophrenics might be worthwhile.

64.8

WITHDRAWN

64.9 CRF BINDING SITES IN THE RAT BRAIN. F.M. Chen, M.H. Perrin*, J. Rivier and W.W. Vale. Peptide Biology Laboratory, The Salk Institute, La Jolla, CA 92037.

Physiological, anatomical and immunological evidence have suggested that CRF is not only essential for pituitary production of ACTH and β-endorphin-like peptides but that the neuropeptide may have broad functions within the central nervous system where it may play a key role in integrating physiologic responses to stress. In the present study, we have iodinated the CRF analogue [Nle^{21},Tyr^{32}]-oCRF, which releases ACTH from the pituitary with equal potency to rat CRF, in order to examine the CRF binding sites in the brain. Binding of [Nle^{21},^{125}I-Tyr^{32}]-oCRF to midbrain membrane pellets reached equilibrium by 60 min at 20°C or 2 hr at 0°C. Binding experiments were routinely carried out in 50 mM Tris-Cl (pH 7.4), 5 mM $MgCl_2$, 2 mM EGTA at room temperature for 60 min. Separation of bound from free was by filtration on GF/C filters. Furthermore, the binding was readily reversible by addition of excess unlabeled peptide. The affinity for the midbrain determined by competitive inhibition of radioligand binding was 2.7 (1.3-5.6) x 10^{-9} M and the number of sites, R^o, was 50 (26-95) fmol/mg protein. In addition, competition experiments also revealed a lower affinity site (K_D ~100 nM). The high affinity binding sites displayed pharmacological specificity: rat CRF, [$DTyr^3$, $DPro^4$,$Nle^{18,21}$]-α helical CRF^{3-41} and sauvagine were equipotent with [Nle^{21},Tyr^{32}]- oCRF while CRF^{7-41}, CRF^{9-41} and CRF^{1-39} were of much lower affinity. Unrelated peptides such as oxytocin, vasopressin and angiotensin II were inactive. A significant regional variation in the density of CRF binding was observed. High densities of binding sites were found in striatum > midbrain = brainstem > cortex = septum >> cerebellum. In conclusion, we have demonstrated high affinity, specific and saturable binding sites for CRF in the brain.

64.10 AN IMPROVED, RAPID FILTRATION TECHNIQUE FOR RECEPTOR BINDING STUDIES: CHARACTERIZATION WITH MU AND DELTA OPIATE RECEPTORS AND Tyr-MIF-1 BINDING SITES. J.E. Zadina and A.J. Kastin. V.A. Med. Center, and Tulane Univ. Sch. of Med., New Orleans, LA 70146.

The most commonly used methods for separation of bound and free radiolabeled ligand in receptor binding studies involve pouring the incubation material over glass fiber filters under vacuum. Except when centrifugation or dialysis are required for rapidly dissociating receptor-ligand complexes (for example, the GABA receptor), filtration is the method of choice. However, the conventional filtration manifolds developed for this purpose require laborious and time-consuming rinsing and handling of individual filters, and tend to be the rate-limiting step in the performance of binding studies. A commercially available (Skatron) cell harvestor was adapted for use in binding studies and compared against a widely used conventional manifold on a number of binding characteristics with ligands for the mu (^{3}H-naloxone) and delta (^{3}H-DADLE) opiate receptors and with ^{125}I-Tyr-MIF-1, which labels a binding site with a dissociation constant (Kd) in the range of 10^{-8} M.

A variety of suction heads can be used on the harvester, permitting use of a range of incubation tube sizes including 12 x 75 mm tubes, 1 ml tubes with microplate spacing, or 0.25 ml microplates. Twelve tubes can be filtered simultaneously and 8 sets of 12 tubes can be filtered before the filter mat is replaced in a simple and rapid operation. Both Whatman GF/B filter sheets and the less expensive filters available with the harvester (used double-ply) produced similar binding characteristics and coefficients of variation on the harvestor and the manifold. The Kd values were nearly identical for the two filter types and the two filtration systems. A somewhat reduced maximum number of sites (Bmax) observed after filtration on the harvestor reflects the smaller filter surface area relative to that of the manifold filter size. The filter surface area on the harvestor, in turn, is considerably larger than that of other manifolds with microplate spacing, allowing larger tissue concentrations to be used.

Although the results of conventional and harvestor filtration methods were similar, the time requirements differed considerably; with the conventional method and 2 experimenters, the time required to begin the incubations and filter 216 tubes was comparable to that required for one person to process 800 tubes with the harvester.

64.11 CORTICOTROPIN-RELEASING FACTOR (CRF) RECEPTORS: IDENTIFICATION IN BRAIN BY AUTORADIOGRAPHY. T.R. Insel*, E.B. De Souza, M. Perrin*, J. Rivier*, W. Vale and M.J. Kuhar (SPON: D.C. Jimerson). Dept. of Neurosci., Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205 and Peptide Biol. Lab, Salk Institute, LaJolla, CA 92138 USA.

A 41-amino acid peptide that fulfills the criteria of a physiological CRF has been previously isolated from ovine hypothalami, characterized, and then synthesized. This peptide releases proopiomelanocortin-derived peptides from anterior and intermediate lobes of the pituitary. We have recently identified and localized high affinity CRF receptors within these two lobes of the rat pituitary (De Souza et al., Brain Res. 296:202-207, 1984). In addition, there is immunohistochemical evidence that CRF is present in extrahypothalamic sites within the brain, and increasing pharmacologic evidence suggests that CRF may have selective behavioral and electrophysiologic effects. In the present study, we have used the radioiodinated analog of ovine CRF (oCRF), Nle^{21}, ^{125}I-Tyr^{32}-CRF (^{125}I-CRF) to identify, characterize, and map for the first time receptors for CRF in rat brain sections by in vitro labeling light microscopic autoradiography.

Quantitative analysis of the autoradiograms revealed that the binding of ^{125}I-CRF was saturable and on Scatchard analysis revealed a high-affinity component with an apparent dissociation constant (K_D) of 6.2 ± 0.8 nM. Blank values were obtained by adding unlabeled Nle^{21},Tyr^{32}-CRF. Rat CRF (1 µM) and oCRF (1 µM) inhibited 105.5 ± 6.3 and 80.8 ± 0.2%, respectively, of the specific ^{125}I-CRF binding in rat striatum (n = 3). The biologically weaker analogs, oCRF (1-39)-NH_2 and oCRF (1-22)-OH were less potent displacers (31.4 ± 0.9 and 29.4 ± 3.6%, respectively), and the unrelated peptide arginine vasopressin minimally displaced ^{125}I-CRF from rat striatum (n = 3).

CRF receptors were localized to the neocortex (Lamina I and IV) and hypothalamus (esp. median eminence) with slightly lower concentrations in amygdala (lat. nucl.), striatum, nucl. of diagonal band, ant. vent. nucl. thalamus, and cerebellum. This distribution generally correlates with the localization of CRF terminals obtained by immunocytochemistry. The presence of specific CRF receptors with a distribution similar to endogenous peptide supports a physiological role for endogenous CRF in the regulation of CNS function.

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64.12 DETERMINANTS OF CCK-8 BINDING TO CENTRAL RECEPTORS. M. Knight, C.A. Tamminga, P. Barone*, L. Steardo*, M. Beck* and T.N. Chase. Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20205.

Conformational characteristics of CCK octapeptide (CCK-8) which influence binding to central receptors are important to the design of potent and stable analogs. Gly a sterically unhindered amino acid residue allows more rotation of the peptide backbone at position 4. An analog with the substitution of $D\text{-}Ala^4$ was synthesized and found to be relatively equivalent in potency to CCK-8.

Peptide	$I.C._{50}$
CCK-8, CCK (1-8) NH_2	2.6×10^{-9}M
$D\text{-}Ala^4$ CCK (1-8) NH_2	9.0×10^{-9}M

These results indicate that the conformation due to the D-amino acid position of Gly interacts with receptors. Since $D\text{-}Ala^4$ CCK-8 is highly potent and stable to brain proteolysis this may be useful as a long lasting CCK analog for in vivo applications.

The relative importance of the amino and carboxyl end of CCK-8 as structural determinants of the interaction with central receptors was also evaluated by measuring the inhibition of specific binding of [^{125}I] CCK-33 to guinea pig cortical membranes by CCK-8 fragments.

CCK-8 Fragment Peptide Binding to CNS Receptors

C-term.	$I.C._{50}$	N-Term.	$I.C._{50}$
CCK-2	$> 10^{-3}$M	Ac CCK (1-4)	$> 10^{-3}$M
CCK-3	1.8×10^{-4}M	Ac CCK (1-5) NH_2	$> 10^{-4}$M
CCK-4	1.7×10^{-8}M	Ac CCK (1-6) NH_2	$> 10^{-4}$M
CCK-5	4.7×10^{-8}M	Ac CCK (1-7) NH_2	2.1×10^{-5}M

Of all the fragments tested the carboxyl peptides CCK-4 and CCK-5 bound most potently. The N-terminal fragments interacted with less affinity, the most potent being the heptapeptide CCK (1-7) NH_2 which contains most of the carboxyl sequence of CCK-8. The carboxyl half of the octapeptide thus appears to be the major determinant of the interaction with the central receptor.

In the pancreas CCK-3 and the heptapeptide have been found to be antagonists of CCK-8 activity. If their central activity corresponds to their receptor interactions, these peptides may prove to be inhibitory.

64.13 AUTORADIOGRAPHIC LOCALIZATION OF [^3H]-ARGININE VASOPRESSIN AND [^3H]-OXYTOCIN BINDING SITES IN THE RAT BRAIN, KIDNEY AND MAMMARY GLAND. F.W.van Leeuwen, J.J.van Heerikhuize* and P.J.van der Sluis*. Netherlands Institute for Brain Research, Zuider IJdijk 28, 1095 KN Amsterdam, The Netherlands.

Recently it has become clear that vasopressin (VP) containing cell bodies are distributed over at least 7 cell groups in the rat brain, while oxytocin (OXT) cell bodies remain restricted to the hypothalamic area (1,3). Both VP and OXT fibres are widely distributed throughout the brain and terminate in synaptic structures. One of the neurotransmitter criteria which have not yet been met for VP and OXT is that specific receptors interact with the substance in question and do so in close proximity to synaptic structures.

In order to reveal VP and OXT binding sites, the methodology developed by Kuhar c.s. (4) was followed using [^3H] Arg VP (NEN, S.A.: 45.3 Ci/mmol), [^3H] OXT, kindly supplied by Dr. Y.P.Wan, NEN, Boston, S.A.: 38 Ci/mmol) and tritium sensitive Ultrofilm. The purity of both [^3H] VP and [^3H] OXT was checked by isoelectric focussing (2). The rat kidney and mammary gland (of a lactating female) served as control.

After incubation with 5 nM [^3H] VP, autoradiography revealed grains both in the cortex (distal convolute) and even more so in the medulla (collecting ducts) of the kidney. In brain sections especially the lateral septum (dorsal part) showed high grain densities. In the pituitary the anterior and neural lobe showed labelling which is in agreement with the idea that VP influences hypophyseal functioning. Virtually no grains were seen in consecutive sections treated in identical fashion except for the addition of 5 µM unlabelled VP, although other areas (e.g. the dorsal hippocampus) showed a high aspecific labelling. [^3H] OXT revealed a very bright labelling in the mammary gland. In the brain the ventral hippocampus was labelled, while labelling was absent in the lateral septum. In control sections no labelling was seen. At present other brain areas are scanned for the presence of VP and OXT binding sites.

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64.14 ARGININE VASOPRESSIN INCREASES PROTEIN PHOSPHORYLATION BUT DECREASES PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE IN RAT HIPPOCAMPAL SYNAPTIC MEMBRANES. A. Hinko*, M.G. Costantini* and A.F. Pearlmutter*. Department of Biochemistry, Medical College of Ohio, Toledo, OH 43699.

We have shown previously that the hippocampus (HP) of the rat has specific binding sites for arginine vasopressin (AVP), a peptide known to facilitate learning and memory. We have extended our studies to examine the effect of AVP on protein and phospholipid phosphorylation in rat HP synaptic membranes (SPM). Purified SPM and SPM + cytosol were used in the protein and lipid phosphorylation experiments, respectively. Membranes were prepared according to the procedure of Dokas et al., Eur. J. Pharmacol. 88: 185-193, 1983. SPM samples were pre-incubated for 5 min at 30°C in tris-Mg^{2+} buffer, pH 7.4; AVP (100 µM) + Ni^{2+} (5 mM) or Ni^{2+} alone were then added. The samples were incubated for 0.5, 2 or 5 min. ATP [2 or 3 µCi (^{32}P)] was then added for protein or lipid phosphorylation, respectively. After 20 seconds the reactions were stopped and the phosphorylated proteins were separated and studied by polyacrylamide slab gel electrophoresis and autoradiography. Phospholipids were separated by thin layer chromatography. Following autoradiography, the lipid regions were visualized with I_2 vapor and counted. After 0.5 min, AVP increased phosphorylation of 5 protein bands of 57000, 50000, 19000, 17000, and 15000 m.w. In parallel experiments, AVP decreased the proportion of (^{32}P)-phosphatidylinositol 4,5-bisphosphate (PIP_2) to total (^{32}P)-phospholipids by 25% after 2 min of AVP treatment ($p<0.05$). An 18% decrease was seen after 5 min ($p<0.01$). The proportion of (^{32}P)-phosphatidylinositol 4-phosphate (PIP) and the level of total (^{32}P)-phospholipids increased slightly with AVP. The proportion of (^{32}P)-PIP_2 decreased in both control and AVP-treated samples as the length of incubation increased, while the proportion of (^{32}P)-PIP increased. These results suggest that the mechanism of AVP action in the rat HP involves an increase in phosphorylation of certain SPM proteins and activation of PIP_2 hydrolysis. This research was supported by NIH grant NS17848.

65.1 INFLUENCES OF THE RAPHÉ NUCLEI ON BRAIN GLUCOSE UTILIZATION. J. McCulloch*, E.T. MacKenzie*, A. Cuddenec*, D. Duverger*, A. Degueurce and B. Scatton. (Spon: S.E. Blackshaw). Wellcome Surgical Institute, University of Glasgow, Scotland, and Dept. of Biology, LERS, Bagneux, France, F92220.

The focal cerebral metabolic and vascular effects of stimulation or removal of the raphe projections have yet to be examined. In initial studies, we have examined the effects of lesions of raphe nuclei (median and/or dorsal) on local cerebral glucose utilization (CMR_{glu}) in the unanesthetized rat brain. Local glucose use provides an index of integrated function in each discrete region.

CMR_{glu} was determined in 57 brain regions of the central nervous system of conscious, lightly restrained rats by the use of the quantitative autoradiographic ^{14}C-deoxyglucose technique. Four groups were studied: sham operated, lesion of dorsal raphe nucleus, lesion of median raphe nucleus, and lesions of both nuclei. The efficacy of the electrolytic lesions (performed under anesthesia 14 days prior to the measurement of CMR_{glu}) was verified in the same animals by neurochemical microassay of 5-HT and 5-HIAA concentrations in the striatum and hippocampus dissected from sections adjacent to those used for autoradiography.

The most remarkable observation in the 3 groups of lesioned animals was the lack of change in CMR_{glu} in the vast majority of regions. Where significant observations were noted, these were decreases in CMR_{glu} with the single exception of increases in the anterior thalamus (and possibly cingulate cortex) following median raphe lesions. Median lesions significantly reduced CMR_{glu} (by 40 to 20% compared to sham-operated) in the following structures: dorsal tegmental nuclei, dorsal raphe nuclei, inferior olive, pontine grey, and substantia nigra. The most pronounced reductions in CMR_{glu} following dorsal raphe lesions were in the red nucleus and dentate gyrus. The effects of double lesions were approximately additive: CMR_{glu} was reduced in all the structures affected by lesions of one or other of the raphe nuclei.

No changes were noted in any neocortical structure in any of the lesion groups. This may indicate that the chronic reduction of 5-HT levels is accompanied by some compensatory changes in this or other neurotransmitter systems. Under the present experimental conditions, the serotonergic system is not the principal determinant of function (reflected by CMR_{glu}) in those structures known to receive raphe projections.

65.2 CHANGES IN HYPOTHALAMIC HISTAMINE (H_1) BINDING SITES IN PHYSIOLOGICALLY STRESSED RATS. B.J. Wilcox and V.S. Seybold. Department of Anatomy, University of Minnesota Medical School, Minneapolis, MN 55455.

Evidence exists implicating histamine in regulation of a variety of neuroendocrine functions in the mammalian brain,but little is known regarding its exact site of action. High densities of H_1 receptors have been reported in the paraventricular nucleus (PVN) and the supraoptic nucleus (SON) of rat hypothalamus, nuclei which are known to contain neurons which secrete vasopressin, oxytocin, and corticotropin releasing hormone (CRF). This suggests that histamine may have an action upon these neurons to influence the release of their hormonal products. To lend support to this hypothesis, the present study used receptor autoradiography to determine changes in H_1 binding sites in response to physiological stimulation known to cause release of vasopressin, oxytocin, and CRF.

Groups of four female Sprague Dawley rats were subjected to varying experimental conditions including lactation, dehydration, and adrenalectomy. Age matched normal and sham operated groups were also established. After 10 days, the animals were sacrificed by vascular perfusion with 0.1% paraformaldehyde. Ten um frozen sections were cut through the PVN and SON of each brain. The sections were then incubated in 5nM [^3H] pyrilamine and non-specific binding was determined with an excess of unlabeled pyrilamine. Emulsion coated coverslips were apposed and allowed to expose for 6 weeks. By examination under a microscope, the grains in the emulsion per 1444 um^2 area overlying the SON and PVN were counted and the data were analyzed by non-parametric statistical methods.

The results showed a significant reduction in grain density over the SON of lactating rats (52.97±11.95; mean±sem) and dehydrated rats (95.9±21.9) as compared to normal (140±10.9). Grain density over the PVN was also significantly reduced in lactating animals (25.7±3.11) as compared to normal (70.59±3.88). Grain densities over these nuclei in adrenalectomized animals were not different from sham operated controls.

These data are consistent with the hypothesis predicting down regulation of receptor populations on target cell membranes in response to increased stimulation by histamine. This supports the suggestion that histamine has an action at the level of the SON and PVN influencing release of vasopressin and oxytocin. Supported in part by NS19312.

65.3 FFFECT OF HYPOPHYSECTOMY AND SUBSEQUENT TREATMENT WITH OVARIAN STEROIDS ON HYPOTHALAMIC 5-HYDROXYTRYPTAMINE AND DOPAMINE SYNTHESIS IN OVARIECTOMIZED RATS. T.S. King, R.W. Steger* and W.W. Morgan. Depts. of Cellular and Structural Biology and OB/GYN, The Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX 78284.

Ovarian steroids have been shown to increase hypothalamic dopamine (DA) synthesis, an effect apparently mediated via the anterior pituitary gland (Eikenburg *et al., J. Neural Transm.* 40:235, 1977). The potential effects of ovarian steroids on hypothalamic 5-hydroxytryptamine (5HT) metabolism remains unsettled and controversial due to conflicting reports. Bilaterally ovariectomized adult Sprague-Dawley rats were divided into two experimental groups: non-hypophysectomized (NON-HYPOX) and hypophysectomized (HYPOX) rats. Half of the rats in each of these two groups was injected subcutaneously with 5 $\mu g \cdot 100g^{-1}$ of estradiol benzoate (E_2) in corn oil vehicle (VEH) at 0900h one and two days before the rats were to be killed and 5 $mg \cdot 100g^{-1}$ of progesterone (P) in VEH at 0900h approximately six hours prior to killing the rats. The other half received VEH injection alone. Thirty minutes before killing the rats, they were injected intravenously with 100 $mg \cdot kg^{-1}$ of NSD-1015 (3-hydroxybenzylhydrazine). Subsequent accumulations of 5-hydroxytryptophan (5HTP) and L-dihydroxyphenylalanine (L-DOPA), as indices for 5HT and DA synthesis respectively, were measured by separate assays using high performance liquid chromatography with electrochemical detection. Estimated 5HT synthesis in the median eminence (ME) and mediobasal hypothalamus (MBH) remained unchanged by E_2 + P treatment in NON-HYPOX rats. Similarly, synthesis of this indole in the ME and MBH was unaltered by these steroids in HYPOX rats. In contrast, an increase in DA synthesis due to E_2 + P treatment was apparent in the ME and MBH of NON-HYPOX rats. This effect was not observed in the ME or MBH of HYPOX rats. These observations confirm previous reports (Eikenburg *et al., J. Neural Transm.* 40:235, 1977) that ovarian steroids exert a negative, pituitary-mediated influence on hypothalamus DA synthesis. Under the experimental conditions of our study, hypothalamic 5HT synthesis appears to be unsensitive to the effects of ovarian steroids. We are currently examining the effects of alternative steroidal treatment regimens to alter hypothalamic 5HT turnover. (Supported by NIH grant RR07187 to TSK, NIH grant DA00755 and Research Scientist Development Award DA00083 to WWM and NIH grant HD10292 [Neuroendocrine Core].)

65.4 DISCRIMINATIVE STIMULUS PROPERTIES OF TRIFLUOROMETHYLPHENYLPIPERAZINE (TFMPP): EFFECTS OF SEROTONIN AGONISTS AND ANTAGONISTS. •K. A. CUNNINGHAM and J. B. APPEL. Behavioral Pharmacology Laboratory, Department of Psychology, University of South Carolina, Columbia, SC 29208.

Biochemical evidence suggests that TFMPP acts through central serotonin (5-HT) systems; that is, it decreases 5-HT turnover, inhibits K+ - evoked release of 3H-5-HT from rat hypothalamic synaptosomes and is one of the more potent non-indole inhibitors of 3H-5-HT binding (Fuller et al., Eur J Pharmacol, 1978, 52, 11; Martin and Sanders-Bush, Naun-Schmied Arch Pharmacol, 1982, 321, 165). However, TFMPP also appears to have effects that differ from those of typical 5-HT agonists; e.g., in drug discrimination situations, animals trained with DOM and saline respond on the saline-appropriate lever following TFMPP (Glennon et al., Eur J Pharmacol, 1984, 91, 189).

In the present experiment, male Sprague-Dawley rats (N=24) were trained to discriminate TFMPP (0.8 mg/kg). In substitution tests, m-chlorophenylpiperazine (MCPP)(0.8 mg/kg) and Ru 24969 (0.4-1.6 mg/kg) mimicked TFMPP; quipazine (0.2-1.6 mg/kg) elicited primarily saline-lever responding while LSD (0.1-0.8 mg/kg) produced intermediate results. Thus far, combination (antagonism) tests have shown that neither ketanserin (0.8-3.2mg/kg), pizotyline (1.6 and 3.2 mg/kg) nor spiperone (0.02-0.16 mg/kg) attenuate the TFMPP cue. Metergoline (0.4-6.4 mg/kg) decreased drug-lever responding by as much as 40 %.

While the possibility that the stimulus properties of TFMPP are not related to 5-HT receptor stimulation cannot be ruled out, these data suggest that TFMPP acts through a mechanism (or mechanisms) similar to those of the novel (5-HT1 ?) agonists MCPP and Ru 24969; this mechanism can be differentiated from those underlying the LSD, quipazine and DOM cues, which are attenuated by putative 5-HT2 antagonists. It is interesting that these "antagonists" do not block the stimulus properties of 1-5-hydroxytryptophan (5-HTP) as well as TFMPP. Alternatively, since TFMPP does not potently inhibit 5-HT reuptake or monoamine oxidase activity (Fuller et al., JPET, 1981, 218, 636), the drug may act as a more direct 5-HT receptor agonist than quipazine or LSD.

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65.5 SEROTONIN FORMATION IN RAT STRIATUM AND SUBSTANTIA NIGRA: STIMULATION BY FORSKOLIN AND 8-THIOMETHYL CYCLIC AMP.
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Forskolin, an activator of adenylate cyclase (AC) in intact cells as well as homogenate and soluble enzyme preparations, stimulates the catalytic moiety of AC without uncoupling the inhibitory subunits (N_i) required for receptor mediated inhibition of AC. We previously reported that forskolin stimulates conversion of tyrosine to DA in slice and synaptosomal preparations of striatal and other CNS tissue (Katz et al, Brain Res.,264: 173,1983). This effect of forskolin appeared to be mediated by cyclic AMP and we have now found it to involve a stable activation of tyrosine hydroxylase (TH). We report here that forskolin as well as certain cyclic AMP analogues stimulate serotonin (5HT) synthesis in rat striatum and substantia nigra (SN). Evidence is presented that this stimulation involves activation of tryptophan hydroxylase (Tryp H).

Synaptosomes were prepared from rat striatum and SN and incubated in a Krebs-bicarbonate medium. 5HT formation was measured by following the release of $^{14}CO_2$ from 1-^{14}C-tryptophan. Also, conversion of ^{3}H-tryptophan and ^{3}H-5-hydroxytryptophan to ^{3}H-5HT was assessed by chromatographic isolation of the 5HT formed.

Forskolin and 8-thiomethyl cyclic AMP both were found to stimulate conversion of tryptophan, but not 5-hydroxytryptophan, to 5HT by striatal and SN synaptosomes. Isoproterenol, vasoactive intestinal peptide and PGE_1 did not influence striatal 5HT formation. Half-maximal stimulation of striatal 5HT formation by forskolin occurred at less than 1 uM, as was the case also for forskolin stimulation of TH. Forskolin did not influence synaptosomal uptake of tryptophan. Forskolin-stimulated 5HT formation in striatum was still present following 6-OH-DA lesion of the SN. 5HT itself inhibited the forskolin-stimulated 5HT formation.

It is concluded that 5HT formation in striatum and SN is regulated by cyclic AMP and AC, most likely due to direct activation of Tryp H by cyclic AMP. It is proposed that stimulation by forskolin provides an approach for elucidation of presynaptic receptors involved in regulation of 5HT formation.

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65.6 SELECTIVE NEUROTOXIC LESIONS OF DESCENDING MONOAMINERGIC PATHWAYS IN THE RAT.
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The monoaminergic innervation of the spinal cord derives from descending projections from the brain stem. Neurotoxic lesions of these pathways have been extensively used, yet little information has been available concerning the selectivity of the lesions and the doses of neurotoxin required for optimal effects.

In the present study, saline, 6-hydroxydopamine (6-OHDA), 5,6-dihydroxytryptamine (5,6-DHT) or 5,7-dihydroxytryptamine (5,7-DHT) were administered into the lumbar subarachnoid space through a chronically indwelling catheter (dose range of neurotoxins: 0.6-80 µg). The lesions were evaluated 2-3 weeks later by measuring the in vitro uptake of (3H)-noradrenaline (3H-NA) and (14C)-5-hydroxytryptamine (14C-5-HT) into synaptosomal preparations from the frontal cortex, brainstem, cervical spinal cord and lumbar spinal cord of each animal.

There was no difference in uptake between saline injected and non-catheterized controls and no significant changes in cortical uptake after any of the treatments. 6-OHDA (5-80 µg) reduced the 3H-NA uptake in the lumbar spinal cord by approximately 90% with no effects on 14C-5-HT uptake. 5,6-DHT (20-80 µg) reduced the uptake of 14C-5-HT by 90 %, 3H-NA uptake was unaffected by lower doses but reduced by 45-55 % after 40-80 µg. 5,7-DHT (10-80 µg) reduced 3H-NA uptake by 90-95% and 14C-5-HT uptake by approximately 80 % (5-80 µg). In each instance, the submaximal effect of the neurotoxin was dependent on the dose given.

It is concluded that intrathecal administration of suitable doses of neurotoxins may produce extensive selective lesions of descending noradrenergic and serotonergic pathways.

65.7 PROLONGATION OF ANTIDROMIC LATENCY AND SPORADIC CONDUCTION BLOCKS IN PRESUMABLY SEROTONERGIC DORSAL RAPHE NEURONS DURING REPETITIVE ACTIVATION.
K.Watabe, T.Shinba* and T. Satoh*. Dept. Physiol., Aichi-Gakuin Univ. Dent. Sch., Nagoya, 464 Japan.

It has been demonstrated that in the noradrenergic neurons the antidromic latency is gradually prolonged to reach an asymptote during repetitive 10Hz activation. In the present study we examined if the projection neurons in the cat dorsal raphe (DR), which is the mixture of serotonergic and non-serotonergic cells, respond in a manner similar to the noradrenergic neurons during repetitive antidromic activation.

Single unit activities of 32 projection DR neurons were recorded. During the antidromic 10Hz stimulation 25 neurons showed a prominent latency prolongation. Five of them(type S2) showed a greater prolongation than the rest (type S1). The degree of maximal prolongation in latency was not significantly correlated with the latency upon single stimulation. Seven neurons showed almost no change (type NS). In some type S1 and S2 neurons a conduction block often occurred when the latency prolongation was maximal. In one type S2 neuron, during 10Hz stimulation, the antidromic spikes reappeared with a much shorter latency than that before the conduction block. When 10Hz stimulation was switched down to 1Hz stimulation, the prolonged latency was gradually restored to the normal level in parallel with the recovery of spontaneous discharges, which had been suppressed completely during 10Hz stimulation. In both the antidromic spike and slow mass potential their amplitude and duration were greater during 10Hz stimulation as compared to 1Hz stimulation, and the contour of the slow mass potential became less irregular. These results suggest that the type S1 and S2 neurons are presumably serotonergic, because they tended to behave like noradrenergic neurons as referred to above. If this is the case, the type NS neurons would be non-serotonergic. Latency prolongation observed in the type S1 and S2 neurons during 10Hz stimulation seems to be explained by postulating hyperporalization of both the axonal and somatic membranes after discharges.

65.8 ROLE OF AUTORECEPTORS IN REGULATING THE ACTIVITY OF SEROTONIN CONTAINING DORSAL RAPHE NEURONS IN MOUSE BRAIN SLICES IN VITRO.
M.E. Trulson and T. Crisp. Dept. of Pharmacol., Marshall Univ. Sch. of Med., Huntington, WV 25701.

It has been hypothesized that the activity of serotonin (5HT)-containing dorsal raphe (RD) neurons is regulated, in part, by autoreceptors located on the somatodendritic region. This hypothesis is based on the findings that manipulations that increase synaptic 5HT depress activity of RD units in vivo, while manipulations that decrease synaptic 5HT tend to elevate the firing rate of RD units. However, using the in vivo preparation, it is not possible to determine which effects are due to the action of 5HT on autoreceptors, and which are due to alterations in the afferent input to the RD. Therefore, we examined this issue using midbrain tissue slices in vitro, since this preparation removes virtually all afferents to the RD. Adult Swiss-Webster mice were decaptitated and the brains sectioned into 400 micron coronal slices. The slices were incubated in standard Yamamoto's solution under an oxygen-saturated atmosphere. Electrophysiological recordings were performed with either single or multibarrelled micropipettes, according to standard techniques. RD units maintained their characteristic slow rhythmic discharge rate (3.24 spikes/sec) and a cell incidence of 1.12 cells/track when recorded 2 hr after incubation, even though 5HT and its major metabolite (5HIAA) disappeared from the slice within this time. Depletion of 5HT by pretreatment of the mice with PCPA (800 mg/kg/day for 3 consecutive days, i.p.) did not change the firing rate of RD neurons recorded from tissue slices obtained from these animals (3.05 spikes/sec). Administration of a monoamine oxidase inhibitor (tranylcypromine, 1mM) to the incubation medium containing slices obtained from normal mice resulted in a cell/track ratio of 0, and an increase of 5HT in the tissue slice 137% above the levels found in fresh tissue. When 5HT (1 mM) was added to the incubation medium containing normal slices and recordings were intitiated 5 min later, the cell/track ratio was 0. However, after waiting one hr, both cell/track ratio (0.66) and discharge rate (3.14 spikes/sec) had significantly increased. These data suggest that excess 5HT depresses the activity of RD neurons, apparently by an action on autoreceptors, but that a deficiency, or normal amount, of 5HT does not influence the spontaneous activity of these cells.

65.9 SINGLE UNIT ACTIVITY OF SEROTONIN-CONTAINING NEURONS IN THE NUCLEUS RAPHE DORSALIS, RAPHE MEDIANUS, AND RAPHE PALLIDUS RECORDED FROM MOUSE BRAIN SLICES IN VITRO. T. Crisp, C.J. Frederickson and M.E. Trulson. Dept. of Pharmacol., Marshall Univ. Sch. of Med., Huntington, WV 25701 and Lab. Neurobiol., Univ. of Texas-Dallas, Richardson, TX 75080.

Previous studies have characterized the electrophysiological activity of single serotonin (5HT)-containing neurons in the nucleus raphe dorsalis (RD), raphe medianus (RM) and raphe pallidus (RPA) in awake, behaving animals. Neurons in all three nuclei displayed a slow, rhythmic discharge pattern characteristic of 5HT cells. However, RPA units fired at a significantly faster rate than those in either the RD or RM. To determine whether the differential firing rates among nuclei are intrinsic to the individual cells or due to differential afferent inputs, we recorded the activity of single units from the three nuclei in brain slices in vitro. Adult Swiss-Webster mice were decapitated and the brainstem was sectioned into 400 micron coronal slices. Slices containing the various raphe nuclei were incubated in standard Yamamoto's solution under a moist atmosphere saturated with oxygen. Single unit recordings were made using standard electrophysiological techniques. In agreement with our previous studies on the RD, these neurons discharged at a rate of 3.36 spikes/sec, and showed a cell/track ratio of 1.14. RM neurons exhibited a similar discharge rate of 3.40 spikes/sec but the cell/track ratio for these cells was significantly lower (0.53). RPA neurons, on the other hand, discharged at a significantly faster rate (5.07 spikes/sec) than either RD or RM neurons, and exhibited a cell/track ratio intermediate between the other two sets of neurons (0.76). When the incubation medium was altered to contain high magnesium (10 mM) and low calcium (0.5 mM), a procedure known to block all synaptic transmission, neither the discharge rates nor cell/track ratios were significantly changed (RD, 3.84 spikes/sec, cell/track =0.92; RM 3.61 spikes/sec, cell/track ratio=0.50; RPA, 5.15 spikes/sec, cell/track ratio=0.95). Cells in all three nuclei were inhibited by 5HT and LSD applied iontophoretically at low ejection currents. These data suggest that the differential discharge rates of 5HT-containing neurons in three major nuclei are determined by the intrinsic properties of the cells, rather than the differential inputs to the various nuclei.

65.10 DORSAL RAPHE UNIT ACTIVITY IN FREELY MOVING CATS: EFFECTS OF MONOAMINE OXIDASE INHIBITORS. V.M. Trulson and M.E. Trulson. Dept. of Pharmacol., Marshall Univ. Sch. of Med., Huntington, WV 25701.

Neurochemical studies have shown that one of the major effects of monoamine oxidase inhibitors (MAOIs) is to elevate brain serotonin (5HT). Furthermore, electrophysiological studies using single unit recordings have shown that MAOIs inhibit the activity of 5HT-containing dorsal raphe (RD) neurons (Aghajanian, et al., Science, 169,1100,1970). These latter studies, however, were conducted in rats that were anesthetized, and, therefore, long term recordings of these neurons were not possible. In the present study we examined the dose-response effects of two MAOIs (tranylcypromine, TCP, 2,4 and 8 mg/kg; and pargyline, PGL, 10,25 and 50 mg/kg, i.p.) on the activity of 5HT containing RD neurons in freely behaving cats, using methods described previously (Brain Res. 163,135, 1979). In addition, we examined the neurochemical effects of the intermediate dose of each drug on brain 5HT levels. TCP and PGL both produced dose-dependent decreases in the activity of RD neurons. The onset of the depression of unit activity occurred within 15 minutes post-injection, and persisted for approximately 4 hr at the lowest dose, and for more than 6 hr at the moderate and high doses. The maximal depression of unit activity occurred at approximately 2 hr post-injection, and was virtually complete at the high dose level. Unit activity returned to baseline levels within 12-16 hr post-injection. While unit activity had returned to baseline levels within 16 hr of injection, brain 5HT levels were still significantly elevated at this time (TCP, +86%; PGL, +67%). These data demonstrate that RD unit activity is decreased for more than 6 hr following moderate to high doses of MAOIs. Interestingly, however, RD unit activity returned to baseline levels in the presence of brain 5HT concentrations that normally produce a large suppression of unit activity. The decrease in unit activity following manipulations that elevate synaptic 5HT is apparently mediated by an action of 5HT on autoreceptors. Our data suggest that unit activity, after a prolonged period of depression, will return to baseline levels even though brain 5HT is still elevated. One interpretation of these data would be that the autoreceptors become tolerant to 5HT during prolonged exposure.

65.11 KETANSERIN'S EFFECTS ON NEURONAL RESPONSES TO SEROTONIN (5-HT) IN THE PREFRONTAL CORTEX, LATERAL GENICULATE AND DORSAL RAPHE NUCLEUS. J.M. Lakoski and G.K. Aghajanian. Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Evidence for multiple 5-HT receptors in brain tissue has been provided by physiological, behavioral and receptor binding studies; the presumed 5-HT antagonist ketanserin has emerged as the ligand of choice for studying $5HT_2$ binding sites, revealing a high concentration of $5\text{-}HT_2$ binding sites in the prefrontal cortex (PFC). Utilizing microiontophoretic techniques we investigated the physiological effects of ketanserin on 5-HT-induced responses recorded extracellularly in the PFC, the lateral geniculate nucleus (LGN) which also receives a serotonergic input, and on 5-HT-containing neurons in the dorsal raphe nucleus (DRN).

In spontaneously active cells recorded in the PFC of adult male Sprague-Dawley rats, ketanserin (2-20 nA) failed to antagonize the inhibitory effects of 5-HT recorded in cerveau isolé or chloral hydrate anesthetized preparations (22/22 cells tested; pure excitatory responses to 5-HT were rarely observed). Paradoxically, the inhibitory response produced by 5-HT (but not GABA, tryptamine or norepinephrine (NE)) was potentiated (> 20% baseline firing; 46% of cells tested) in cells where ketanserin alone did not alter spontaneous rates. In the DRN of chloral hydrate anesthetized animals ketanserin also failed to attenuate the inhibition of serotonergic neurons by 5-HT. The systemic administration of ketanserin (5 mg/kg, ip.) had effects similar to those observed in the microiontophoretic experiments in both the PFC and DRN. In the LGN (transected preparation) ketanserin potentiated 5-HT's inhibitory effect at iontphoretic currents where ketanserin alone did not alter spontaneous rates. However, ketanserin was also found to attentuate the excitatory responses produced by NE, an α_1-adrenoceptor mediated response in the LGN.

In summary, ketanserin appeared to enhance rather than antagonize the inhibitory response to 5-HT in the brain regions examined. The potentiation by ketanserin of inhibitory responses to 5-HT but not GABA, tryptamine or NE may be consistent with ketanserin's proposed interaction at a specific $5\text{-}HT_2$ binding site. The physiological interaction of ketanserin with an α_1-adrenergic site suggests that caution must be used ascribing a ketanserin-mediated response solely to an interaction at a $5\text{-}HT_2$ binding site. (Supported by USPHS Grants MH-17871, MH-14276, and the State of Connecticut).

65.12 EFFECTS OF L-DOPA ON FROG MOTORNEURONS: A PHARMACOLOGICAL CHARACTERIZATION. G.P. Ryan, J.C. Hackman, C.J. Wohlberg and R.A. Davidoff. Neurophysiology Laboratory, VAMC and Departments of Neurology and Pharmacology, Univ. of Miami School of Medicine, Miami, Florida 33101.

L-DOPA, the metabolic precursor of dopamine, is capable of eliciting locomotion in spinalized animals. The mechanism of this phenomenon is unknown. To help define the role of L-DOPA in locomotion we have characterized its actions on lumbar motorneurons by recording the membrane potential of motor axons contained in the 9th ventral root (VR) by sucrose gap in the isolated, hemisected frog spinal cord continuously superfused with HCO_3^--buffered 15°C Ringer's.

L-DOPA depolarized VRs--an effect dependent upon both dose (0.1-2mM) and duration (15-90sec) of application. Repeated administration of L-DOPA (2mM, 90sec) at 10 min intervals for 2 hrs. resulted in a progressive increase in potential change. Thus, the L-DOPA response exhibited potentiation. The stereoisomer D-DOPA (2mM, 90sec) had negligible effects on membrane potential.

The L-DOPA-depolarization does not involve adrenergic, dopaminergic (DA) or serotonergic (5HT) receptor activation, since application of the adrenergic antagonists yohimbine (1uM), prasozin (1uM), and propranolol (10uM), the DA antagonists fluphenazine (10uM) and metoclopramide (10uM), and the 5HT antagonist methysergide (10uM) were without effect on the VR depolarizations produced by L-DOPA (2mM, 90sec).

To determine whether the L-DOPA response involved activation of excitatory amino acid receptors we applied D,L-x-aminoadipate (AAD, 1mM) and glutamic acid diethyl ester (GDEE, 1mM). AAD significantly (about 40%) reduced the depolarization but GDEE was ineffective (<10%). (-)Baclofen (10um), which prevents the presynaptic release of excitatory amino acids, also reduced the depolarization (about 25%).

The L-DOPA-depolarization appeared to involve a metabolic process since bathing the cord with 2,4-dinitrophenol (50 uM), sodium cyanide (2mM), and lowering the temperature to 10°C all produced a substantial reduction in response.

Superfusion of the cord with Ringer's containing TTX (1.25uM) or Mn^{++}(1.5mM) significantly reduced the L-DOPA depolarization indicating that the effect is the result of both a direct action on motorneurons and indirect on interneurons.

In sum, L-DOPA-depolarizations appear to result in part from activation of both excitatory amino acid receptors and a metabolic process. Supported by VAMC Funds (MRIS 1769) and USPHS grant # NS17577 and HL07188 and a grant from the National Parkinson Foundation.

65.13 SEROTONIN AND METABOLITES IN THE FROG CNS: AN HPLC STUDY
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The filum terminale (FT) in the fog spinal cord is that region caudal to the last spinal root. The upper portion of the FT has been shown to contain large numbers of descending 5HT immunoreactive fibers which terminate in the neuropile, which consists largely of astrocyte like glia. The 5HT immunoreactivity disappears with cord transection below L-10 or treatment with the neurotoxin 5-6 dihydroxytryptamine: In this study, we examined the distribution of serotonin (5HT) and its metabolite, 5HIAA, in the frog CNS, by HPLC and electrometric detection. Frogs were dissected and tissues were frozen immediately and subsequently homogenized in 0.05N Perchloric acid bubbled with N_2 prior to use. Supernatants were analyzed on a reverse phase Ultrasphere C-18 column and an LC-4B amperometric detector (Bioanalytical Systems, Inc.). The sensitivity of the method was 140 femptomoles for 5HT and 5HIAA, and 250 femptomoles for tryptophan. High levels of serotonin (35pg/mg. prot.) are found in the FT, with intermediate levels of 26 pg/mg. prot. in the lumbar enlargement, combined thoracic and cervical cord, and in the medulla. The combined brain stem-cerebellum contains 40pg/mg prot. of 5HT, and 34 and 22 pg/mg prot. are seen in the mesencephelon and telencephalon respectively. The 5HIAA/5HT ratio is roughly 0.4 in all regions examined. The IP administration of the Monoamine oxidase inhibitor pargylline (75 mg/Kg) for two hours elevates the 5HT levels by 10-40% in all regions except the FT. The combined I.P. administration of pargylline and 5HTP (30mg, Kg) elevates 5HT levels by 300-400% in all areas examined, and the 5HIAA/5HT ratio drops to 0.12 with pargylline alone or combined with 5HTP. It is interesting to note that the 5HT levels in the FT, which in its most caudal portion is largely glial, are not elevated in response to pargylline, and are not as markedly elevated as other CNS areas in response to pargylline and 5HTP administration.
SUPPORTED BY Welch Grant H-504, NS 17696, NS 11255, and CA 18877.

65.14 IN VIVO EFFECTS OF VARIOUS SEROTONIN ANTAGONISTS ON 5-HYDROXYTRYPTOPHAN AND DIHYDROXYPHENYLALANINE ACCUMULATION IN RAT BRAIN.
W.C. Boyar*, L.L. Martin* and B.S. Glaeser (SPON: R.A. Lovell). Neuroscience Res., Res. Dept., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

Several serotonin antagonists (e.g. cinanserin, pirenperone, metitepine and metergoline) were evaluated by in vitro receptor binding assays and the in vivo measurement of 5-hydroxytryptophan (5-HTP) accumulation. In the ^{3}H-ketanserin binding assay for 5-HT_2 receptors (Life Sci. 33:2011, 1983), all four compounds displaced ^{3}H-ketanserin from rat fronto-parietal cortex membranes, with IC_{50} values of 5 nM-cinanserin; 1 nM-pirenperone and 0.3 nM-metitepine and metergoline. These IC_{50} values were consistent with the known serotonin antagonist properties of these compounds. However, in vivo evaluation of these compounds by the measurement of 5-HTP and dihydroxyphenyalanine (DOPA) accumulation did not produce consistent results as expected for serotonin antagonists. Levels of 5-HTP and DOPA in the fronto-parietal cortex and striatum were measured by HPLC/EC techniques (J. Chromatography 213:663, 1981 and Brain Res. 195:123, 1980) after animals had been treated with the aromatic amino acid decarboxylase inhibitor, NSD-1015. Pirenperone and metitepine (20 mg/kg i.p.) increased 5-HTP accumulation by 35% and 43%, respectively, 2 hr after administration in the fronto-parietal cortex. In contrast, in the same tissue, cinanserin (30 mg/kg i.p., 2 hr) produced a 15% decrease in 5-HTP accumulation whereas metergoline (20 mg/kg i.p., 1 hr) caused a 52% decrease in 5-HTP accumulation. All drugs except cinanserin produced significant elevations in striatal DOPA accumulation. These data are consistent with a hypothesis that there may be two different types of serotonin antagonists. One type of antagonist may interact with a serotonin receptor activating a feedback mechanism which produces the expected increases in 5-HTP accumulation, whereas another type of antagonist may interact with a serotonin receptor without activating the feedback mechanism such that an apparent agonist effect on the receptors may prdominate, i.e. the antagonist may be acting as a partial agonist.

BIOGENIC AMINES II

66.1 A SIMPLE, YET SENSITIVE AND REPRODUCIBLE METHOD FOR THE DETERMINATION OF HISTIDINE DECARBOXYLASE.
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Numerous studies are supportive of a neurotransmitter role for histamine (HA) in mammalian brain. The primary synthetic pathway for the synthesis of HA in brain is via histidine decarboxylase (HDC). Because the activity of HDC in brain is quite low, high sensitivity assays are necessary for its determination. Available assays all have shortcomings such as being very time-consuming, requiring several microcuries of substrate, not being reproducible, or being coupled to another enzyme. We have developed a direct assay which is simple to do and is also sensitive, reproducible, and does not require large amounts of radioactivity. The assay measures the formation of ^{3}H-HA from ^{3}H-His (Amersham L-[2,5-^{3}H]His, 40-60 Ci/mmole). Tissue is homogenized on ice in 5 to 20 volumes of 20 mM potassium phosphate at pH 7.2 containing 0.1 mg/ml phenylmethylsulfonylfluoride with either a probe sonicator or a polytron. For subcellular fractionation determinations the homogenizing buffer also contains 0.3 M sucrose and is done with a motor-driven Teflon pestle. The reaction is carried out in 400 µl polyethylene tubes in a 40 µl volume which has 20 µl of enzyme homogenate or homogenizing buffer for blanks, 10 µl of additions, and 10 µl of "mix". The mix contains 1 µCi of ^{3}H-His, 400 µM α-methyldopa, 400 µM amodiaquin, 40 µM PLP and 0.2 M potassium phosphate at pH 7.2. The reaction is initiated with the mix, is briefly centrifuged in a microfuge and incubated for 2 h at 45°C. Termination is with 10 µl containing 2 N NaOH, 1 mg HA/ml and 50% ethanol. Depending on desired sensitivity 100 or 200 µl of H_2O saturated butanol is added to extract ^{3}H-HA. Assay tubes are capped, vortexed at top speed for at least 10 sec and centrifuged for 5 min in a microfuge. Ten to 50% of the butanol extract is spotted onto Whatman LK5D thin layer plates. Plates are dried for 5 to 10 min at 120°C, allowed to cool at room temperature, and developed in $CHCl_3$:methanol:conc. NH_4OH at 12:7:1. HA is visualized with ninhydrin spray, scraped into polypropylene or polyethylene vials, shaken for 10 min with 0.5 ml of 1 mg HA/ml ethanol and counted in 5 ml of toluene fluor. This HDC assay is linear with time and tissue, is inhibited by α-fluoromethylhistidine and yields regional and subcellular distributions in rat brains similar to those reported. With our HDC method more than 100 tubes per day can be assayed.
Supported by Grants MH 36787, HD 03352 and NS 19312.

66.2 PHARMACOLOGICAL CHARACTERIZATION OF SEROTONIN-1 BINDING SITES IN THE RAT SPINAL CORD: COMPARISON WITH CORTEX.
V. Fardin*, D.L. NELSON and H.I. YAMAMURA (SPON: P. Consroe). Dept. Pharmacol., Univ. of Arizona, Tucson, AZ 85721.

Limited data are available on the pharmacology of serotonin-1 (5HT-1) binding sites in the spinal cord (SC) compared to the brain. Thus, the abilities of several agonists and antagonists to inhibit the binding of [^{3}H]5HT in crude washed homogenates of rat spinal cord and cortex (CTX) were examined. Incubations were carried out at 37°C for 10 min. No ascorbate was added to the incubation medium, which contained a final concentration of 2nm [^{3}H]5HT. For 5HT and spiperone, the IC_{50} values and the slopes of logit-log plots were not statistically different between CTX and SC (IC_{50}: 10-15 nM for 5HT, 250-300 nM for spiperone). In both tissues spiperone produced shallow inhibition curves with slope values of about 0.4. Although the IC_{50} values were not significantly different for metergoline in the two regions, the slopes of logit-log plots were significantly lower in the SC (0.64 ± 0.06) than in the CTX (0.90 ± 0.01). Furthermore, when the agonist 5-methoxytryptamine (5MEOT) was examined, it showed differences in potency: in the CTX, the IC_{50} was approximately 15 nM while in the SC, 100 nM 5MEOT produced a maximal inhibition of 50% in specific binding, with higher concentrations causing no further decrease. Clear regional variations between CTX and SC were also found by using 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (PTSD) and the tetrahydropyridine analogue of 5HT, RU 28253. Finally, under our conditions of assay it was found that ketanserin, an antagonist at 5HT-2 binding sites, was able to displace [^{3}H]5HT binding at relatively low concentrations. Nonlinear regression analysis of the data revealed in both SC and CTX the presence of at least two populations of sites, ketanserin being more potent in the SC than in the CTX (IC_{50} values for the high and low affinity sites are respectively 1.6 ± 0.7 nM and 1.4 ± 0.6 µM for the SC, 489.9 ± 182.4 nM and 47.6 ± 18.3 µM for the CTX). This study revealed striking differences in the pharmacological profiles of 5HT-1 binding sites between cortex and spinal cord. Moreover, the biphasic inhibition curves obtained for PTSD, RU28253 and ketanserin, as well as the shallow curve obtained with spiperone, are consistent with the concept of multiple 5HT-1 binding sites in the spinal cord. (Supported by NIH grant NS16605 and a French fellowship from the Ministere des Affaires Etrangeres)

66.3 AUTORADIOGRAPHIC LOCALIZATION OF 5-HT_1 BUT NOT 5-HT_2 RECEPTORS ON CANINE AND HUMAN BASILAR ARTERIES. S.J. Peroutka* (SPON: R. Grzanna). Dept. Neurosci., Johns Hopkins Univ. Sch/Med., Balto., MD 21205.

Serial mounted sections of human and canine basilar arteries were incubated with 8 nM ^{3}H-LSD and the slides were processed for autoradiography. In the absence of inhibitors, specific grain density was 10 $\pm$ 0.9 grains/625 microns^2 ($p < 0.001$) in human and 21 $\pm$ 4 grains/625 microns^2 ($p < 0.02$) in canine basilar arteries. This amount of specific binding represented 33% and 43% of total binding observed in human and canine vessels, respectively. When ^{3}H-LSD was incubated with 300 nM 5-HT in order to restrict radioligand binding to 5-HT_2 receptors, specific grain density was significantly reduced to 2 $\pm$ 0.5 grains/625 microns^2 in human and 4 $\pm$ 2 grains/625 microns^2 in canine arteries. These values were not significaltly greater than nonspecific binding (defined as ^{3}H-LSD grain density observed in the presence of 300 nM 5-HT + 30 nM spiperone). In the presence of 30 nM spiperone, ^{3}H-LSD selectively labels 5-HT_1 receptors. Under these conditions, significant amounts of specific binding could be detected in human (10 $\pm$ 0.8 grains/625 microns^2; $p < 0.001$) and canine (22 $\pm$ 2 grains/625 microns^2; $p < 0.02$) basilar artery segments. No significant difference ($p > 0.05$) was observed between the specific grain density in the presence of 300 nM 5-HT and the specific grain density in the presence of 300 nM 5-HT + 30 nM spiperone.

Because the specific binding of ^{3}H-LSD suggested the labeling of 5-HT_1 but not 5-HT_2 receptors, we attempted to confirm the presence of 5-HT_1 receptor sites with the binding of ^{3}H-5-HT. Specific grain density using 4 nM ^{3}H-5-HT was defined as the excess over blanks taken in the presence of 300 nM 5-HT, and represented 37% and 33% of binding observed in human and canine arteries, respectively. Significant amounts of specific grain counts were observed in both human (24 $\pm$ 3 grains/625 microns^2; $p < 0.001$) and canine (14 $\pm$ 4 grains/625 microns^2; $p < 0.02$) basilar artery segments. The labeled sites were homogeneously located throughout the medial layer (tunica media) of the blood vessel. In the canine preparation, these data confirm the conclusion (drawn from physiologic studies) that contraction of the canine basilar artery is mediated via 5-HT_1 receptors. (Supported by USPHS MH25951, DA00266 and a McKnight Foundation grant.

66.4 1-PHENYL-1,3,8-TRIAZASPIRO[4.5]DECAN-4-ONE AS A POTENTIAL MODEL FOR IDENTIFYING A SUBGROUP OF SEROTONIN-1 BINDING SITES. D. L. Nelson. Dept. Pharmacol. & Toxicol., Col. of Pharmacy, Univ. of Arizona, Tucson, AZ 85721.

During the examination of a variety of different molecular classes for activity at serotonin-1 (5HT-1) binding sites, it was found that the neuroleptic spiperone appeared to differentiate between subtypes of these sites, whereas other butyrophenones like pipamperone and haloperidol did not. This suggested that the 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (PTSD) portion of the molecule might be responsible for spiperone's unique actions at the 5HT-1 sites. When PTSD was examined at 5HT-1 sites defined by the high-affinity binding of [^{3}H]5HT, it was found to produce shallow curves for the inhibition of [^{3}H]5HT binding in both rat cortex (CTX) and corpus striatum (CS) (slopes of logit-log plots = 0.18 in CTX and 0.23 in CS), and it showed regional differences in potency. In CS the IC_{50} was approximately 2 μM, while in CTX 30 μM PTSD produced a maximum of 40% inhibition of specific binding. In both tissues there was significant inhibition of [^{3}H]5HT binding at relatively low concentrations (< 100 nM) of PTSD. In CS the curves for inhibition of [^{3}H]5HT binding by PTSD were almost identical to those produced by spiperone, while in the CTX the curves were shallower and never reached the same level of inhibition as those for spiperone. When examined at 5HT-2 sites defined by the binding of [^{3}H]ketanserin in rat frontal cortex, PTSD produced steep (logit-log slope = 0.94 $\pm$ 0.02) inhibition curves with an IC_{50} value of 2.7 $\pm$ 0.2 μM, indicating that PTSD is much less potent than spiperone ($K_d \simeq 0.5$ nM) at these sites. PTSD was also different from spiperone in potency at [^{3}H]8-hydroxy-2-(di-n-propylamino)tetralin ([^{3}H]PAT) binding sites, which represent a subgroup of 5HT-1 sites. Spiperone produced relatively steep (logit-log slope = 0.87 $\pm$ 0.06) curves for the inhibition of the binding of [^{3}H]PAT in rat CTX with an IC_{50} value of 117 $\pm$ 23 nM, while PTSD produced measurable inhibition of [^{3}H]-PAT binding only at concentrations greater than 1 μM and only achieved a 30-40% inhibition of specific binding at 100 μM. The data suggest that PTSD has relatively high affinity for a small proportion of the 5HT-1 sites and low affinity for 5HT-2 sites. This suggests that this compound and/or its analogues may be useful in the characterization of specific subtypes of the 5HT-1 binding sites. (Supported by NIH grant NS16605)

66.5 HIGH AFFINITY ^{3}H-SEROTONIN BINDING SITES ON INTACT BRAIN ASTROGLIAL CELLS. P.M. Whitaker-Azmitia and E.C. Azmitia. Dept. of Psychiatry and Behavioral Sciences, SUNY, Stony Brook, NY and Dept. of Biology, New York University, New York, NY.

The presence of serotonin receptors on astroglial cells was first demonstrated in 1979 by Hertz et al. (Can. J. Physiol. Pharmacol. 57:223) but sine that time little has been done to further characterize these sites, pharmacologically or functionally. We proposed to study these sites in the C_6 clonal cell line and in primary cultures of astroglial cells.

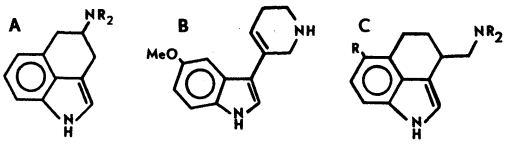
The C_6 cell line was maintained in F_{10} media supplemented with 2.5% fetal calf serum and 15% horse serum. The cells were grown for one week at 37° with 5% CO_2. The cells were harvested into Hank's Balanced Salt Solution for use in the binding assay. Intact cells were incubated with 18 different concentrations of radioligand (0.2 to 125 nM) with or without unlabelled serotonin to define non-specific binding (20 to 12,5000 nM) for 2 hours on ice. Saturation analysis revealed a single class of binding site with a K_D of 8.7 nM and a B_{max} of 316 fmoles/mg protein. Competition studies of tryptamine (bufotenine, 5-methoxytryptamine and psilocybin) and ergot (LSD and dihydroergotamine) derivatives indicated that these sites have the same pharmacological profile as that reported in brain homegenates.

Primary astroglial cultures were derived from newborn rats. Cerebral hemispheres were minced and disassociated with EDTA and plated with F_{10} media supplemented with 2.5% fetal calf serum and 15% horse serum. The cultures were maintained for two weeks in this media. At the end of this period the cells were either left to grow for a further week or they were matured with the addition of 0.25 mM dibutyryl cAMP. Using the procedure described for C_6 cells, saturation analysis of these cells indicated the presence of serotonin receptors of approximately the same affinity as those observed in the C_6 line (about 7 nM) but in greater number, depending on the maturation state of the cells.

We are currently investigating the role these receptors may play in brain metabolism or in neuronal development and plasticity (see abstract this meeting Azmitia and Whitaker-Azmitia).

66.6 RIGID ANALOGS AND THEIR USE IN DETERMINING PHARMACOPHORIC DIFFERENCES BETWEEN SEROTONIN BINDING SITES. E.W. Taylor*, B. Weck* and D.L. Nelson (SPON: H.E. Laird). Depts. of Pharmacol. & Toxicol. and Pharm. Sci., Univ. of Arizona, Tucson, AZ 85721.

Although a number of tryptamine (TRYPT) derivatives with flexible side chains are capable of discriminating between different types and subtypes of serotonin (5HT) receptors, most such compounds are not potent enough to be useful as selective ligands or drugs. Thus the development of selective 5HT-1 antagonists or 5HT-2 agonists may be contingent upon the determination of the precise side chain conformation of 5HT recognized at each receptor site (i.e., the pharmacophore for that receptor). We have undertaken the comparative study of various conformationally constrained or rigid 5HT analogs as a means of determining this information. Certain ergolines, e.g., metergoline, can be regarded as rigid analogs of 5HT and are also highly recognised at both 5HT-1 and 5HT-2 sites. However, binding studies with 2 partial ergolines, RU 27849 (**A**, R=H) and RU 28306 (**A**, R=Me) showed that both of these compounds were 3 X less potent than their non-rigid analogs (TRYP and DMT) at the 5HT-1 site . At the 5HT-2 site both compounds were several times more potent than their non-rigid analogs. These results are consistent with an ergoline-like pharmacophore for the 5HT-2 site, but suggest some other conformation may be optimal for 5HT-1 binding. Also, the partially constrained analog RU 28253 (**B**) was very potent at both 5HT sites: it can assume an ergoline-like conformation (not shown) or, by rotation, one such as that shown as **B**. An analog such as **C**, which can approximate only the non-ergoline conformations of **B**, would be useful to test the hypothesis that **B** might be acting in this manner at 5HT-1 sites. At least one such analog has now been synthesized. Its pharmacological activity will also be presented, and the relevance to the above results will be discussed. (Supported by NIH grant NS16605)



66.7 SOLUBILIZATION OF SEROTONIN 5-HT_2 RECEPTORS FROM RAT BRAIN
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Serotonin 5-HT_2 (S2) receptors have been solubilized by digitonin or by lysolecithin and labeled by several tritiated ligands (Ilien et al., FEBS Lett. 138, 311 (82); Chan and Madras, Eur. J. Pharmacol. 83, 1 (82)). In the current investigation we used the sensitive new 5-HT_2 ligand ^{125}I-LSD to label rat frontal cortex receptors which were solubilized by the zwitterionic detergent CHAPS. Carrier-free ^{125}I-LSD (2175 Ci/mmole) is 30-70 fold more sensitive than tritiated radioligands in assays for these solubilized receptor sites while CHAPS is a preferred detergent due to its high CMC, small micelle size and net electrical neutrality.

A prelabeling methodology which takes advantage of the slow dissociation rate of ^{125}I-LSD at low temperatures was used to label the solubilized receptors. Rat frontal cortex membranes were labeled with 1 nM carrier-free ^{125}I-LSD for 15 minutes at 37°C, cooled to 4°C and solubilized with 10 mM CHAPS. Approximately 25% of the serotonin 5-HT_2 receptor sites were obtained in soluble form in the supernatant following ultracentrifugation of this sample at 115,000 x g. Most of the non-solubilized sites were recovered in the membrane pellet when CHAPS concentrations of up to 50 mM were used. This observation contrasts with other receptor systems where high concentrations of CHAPS caused destruction of receptor sites. Using 1 µM ketanserin to define specific binding, 60% of the ^{125}I-LSD binding to solubilized sites occurred at serotonin 5-HT_2 receptors. The dissociation rate of ^{125}I-LSD from solubilized receptor sites was very slow, approximately 5% after 60 minutes at 4°C. When ketanserin was included in the prelabeling incubation, it potently inhibited binding to the solubilized sites while domperidone was a very weak inhibitor. This indicates that ^{125}I-LSD remains bound to the serotonin receptor site during the solubilization process. When rat frontal cortex membranes were solubilized in the absence of ligands and post-labeled with ^{125}I-LSD, approximately 3% of the membrane sites were labeled with a 35% specific to total binding ratio.

These studies demonstrate that CHAPS solubilizes serotonin 5-HT_2 receptors with an efficiency quite similar to the ionic detergent lysolecithin. ^{125}I-LSD is an effective and sensitive radiolabel for these solubilized receptor sites.

66.8 SOME EFFECTS OF DEUTERIUM SUBSTITUTION IN THE ALKYL SIDE CHAIN OF β-PHENYLETHYLAMINE.
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The most important pathway for catabolism of β-phenylethylamine (PE) is oxidative deamination by monoamine oxidase (MAO). The rate of PE deamination by MAO can be slowed by substitution of deuterium (d) for the α-hydrogen in the alkyl side chain of PE. It has also been observed that α,α,β,β-d_4 PE is more potent behaviourally than PE. This increased potency is probably due to the greater resistance of d_4 PE than PE to degradation by MAO. In order to determine whether this was the case, male Wistar rats were injected intraperitoneally with an equimolar mixture of d_4 PE and PE, and the d_4 PE/PE ratios in the rat brains or regions thereof measured mass spectrometrically. In the first set of experiments, a mixture of 25 mg/kg each of d_4 PE and PE was injected i.p. into rats and the animals killed after 10-300 min. There were no significant differences in the d_4 PE/PE ratios in the various brain regions; however, the ratios changed significantly with time. The data from the brain regions were pooled to give whole brain d_4 PE/PE ratios. These ratios were 3.0±0.3, 9.6±2.0, 41.9±5.9 and 14.6±5.0 (mean ± SEM, n=4) at 10, 20, 45 and 60 min, respectively. In the second set of experiments, a mixture of 5 mg/kg each of d_4 PE and PE was injected i.p. into rats pretreated 24 h earlier with 50 mg/kg pargyline. In these experiments, the actual amounts of d_4 PE and PE in the whole rat brain, as well as the d_4 PE/PE ratios, were determined by using d_9 PE as the internal standard. The d_4 PE/PE ratios were 3.0±0.9, 3.1±0.5, 11.4±1.8 and 13.8±1.8 at 10, 20, 45 and 60 min, respectively. It is clear from these data that much more of the systemically administered d_4 PE than of the PE reached the brain. In addition, the attenuation of the d_4 PE/PE ratios by pretreating the rats with pargyline demonstrates that the greater levels of brain d_4 PE than PE are due to the differential action of MAO on d_4 PE and PE. These data demonstrate that deuterium substitution may be a useful method of increasing the brain levels of centrally acting compounds. (Supported by Saskatchewan Health)

66.9 PHARMACOLOGICAL CHARACTERIZATION OF SEROTONIN STIMULATED PHOSPHATIDYLINOSITOL METABOLISM IN RAT BRAIN.
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Evidence suggests that the $5HT_1$ serotonergic binding site is functionally linked to adenylate cyclase in the adult rat brain, but a biochemical effector system which is linked to the $5HT_2$ site has not been found. We have used a modification of the method of Berridge, Downes and Hanley (Biochem. J. 206: 587, 1982) to investigate serotonin (5HT) stimulated phosphatidylinositol (PI) hydrolysis in rat cerebral cortex. This method exploits the ability of lithium to inhibit myo-inositol-1-phosphatase and allows direct measurement of accumulated ^{3}H-myo-inositol phosphates which have been hydrolyzed from membrane phosphoinositides prelabelled with ^{3}H-myo-inositol.

Increasing concentrations of 5HT resulted in an accumulation of increasing amounts of ^{3}H-inositol-phosphates. The concentration-response curve was hyperbolic and the response was near maximal at 500 µM (EC_{50}=80 µM). The selective $5HT_2$ antagonists ketanserin and pizotifen caused a concentration dependent inhibition of the stimulatory effect of 250 µM 5HT with IC_{50} values of 145 nM and 1 µM, respectively. Ketanserin did not inhibit KCl stimulated PI turnover, suggesting that its inhibitory action is not due to interaction with a non-receptor component of the phosphoinositide metabolic machinery. The addition of 10 µM concentrations of atropine, phentolamine, or triprolidine did not block the effect of 250 µM 5HT suggesting that 5HT's action is not due to stimulation of muscarinic, alpha-adrenergic, or H_1 histaminergic receptors.

The hydrolysis of PI has been proposed to be a multifunctional transducing mechanism for generating a number of intracellular signals including calcium fluxes, prostaglandin synthesis, stimulation of guanylate cyclase, and activation of protein kinase C. The present results suggest that 5HT stimulated PI hydrolysis in rat cerebral cortex may be linked to the $5HT_2$ recognition site. This is consistent with recent reports that 5HT stimulated calcium fluxes (Pletscher & Affolter, J. Neural Transmission 57: 233, 1983) and prostaglandin synthesis (Coughlin, Moskowitz and Levine, Biochem. Pharmacol. 33: 692, 1984) may be linked to the $5HT_2$ site in peripheral systems. (Supported by NIH Training Grant GM-07628, NIH BRSG Grant RR05424 and ADMHA Research Grant MH-34007).

66.10 A TRIFLUOROMETHYLPHENYL PIPERAZINE DERIVATIVE DISPLAYING HIGH AFFINITY AND SELECTIVITY FOR 5-HT_{1a} RECEPTOR SITES.
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A trifluoromethylphenyl piperazine derivative (BrTFMPP) was synthesized and displays a high affinity for 5-HT_{1a} sites relative to its affinity for 5-H_{1b} and 5-H_2 sites. In rat cortical membranes, the radioligand ^{3}H-5-HT produces a binding isotherm consistent with the labeling of a single population of sites displaying a Kd of 2.3 nM and a B_{max} of 217 fmol/mg protein. This population of sites, however, can be discriminated into two subtypes based upon their differing affinities for the neuroleptic spiperone (5-HT_{1a} - Kd = 24 nM, 5-HT_{1b} - Kd = 19,000 nM). BrTFMPP displaces ^{3}H-5-HT in a manner consistent with a two-site model of ligand-receptor interaction with mean affinities for the high and low affinity sites of 1 nM and 180 nM, respectively.

Evidence indicates that BrTFMPP distinguishes the same two subpopulations of ^{3}H-5-HT binding sites as does spiperone. The proportion of high affinity (35%) and low affinity (65%) sites for each compound are similar. In the presence of 1 µM spiperone, a concentration which saturates the 5-HT_{1a} sites, BrTFMPP displaces ^{3}H-5-HT in a manner consistent with displacement from a single class of sites which has a Kd for BrTFMPP (145 nM) which matches the Kd for BrTFMPP for its low affinity site in the absence of spiperone. Correspondingly, the inhibition of ^{3}H-5-HT binding by spiperone in the presence of 30 nM BrTFMPP, a concentration which saturates its high affinity sites, is consistent with displacement from a single class of sites having a Kd for spiperone of 21,000 nM. This Kd matches the Kd for spiperone for its low affinity site in the absence of BrTFMPP.

The radioligand ^{3}H-spiperone produces a binding isotherm in rat cortical membranes consistent with the labeling of a single population of sites (5-HT_2) displaying a Kd of 0.5 nM and a Bmax of 70 fmol/mg protein. Displacement of ^{3}H-spiperone by BrTFMPP is consistent with a single class of 5-HT_2 binding sites and displays a Kd for BrTFMPP of 40 nM.

Therefore, BrTFMPP displays a selectivity for 5-HT_{1a} sites versus both 5-HT_{1b} and 5-HT_2 sites, and thus a radiolabeled derivative should be a useful ligand for studying the 5-HT_{1a} receptor. (Supported by NIMH Grant MH 37020.)

66.11 SELECTIVITY OF SEROTONIN AGONISTS AND ANTAGONISTS FOR 5-HT_1 RECEPTOR SUBTYPES. M.A. Sills, B.B. Wolfe and A. Frazer, Departments of Pharmacology and Psychiatry, University of Pennsylvania and VA Medical Center, Philadelphia, PA 19104.

Recent studies of the 5-hydroxytryptamine$_1$ (5-HT_1) receptor indicate that multiple subtypes of this receptor exist. The results from these experiments show that certain serotonin compounds, such as spiperone, produce shallow inhibition curves of ^{3}H-5-HT binding to rat frontal cortical membranes when measured in the absence of GTP (J. Neurochem. 36:220, 1981). The component of binding inhibited by low concentrations of spiperone was designated the 5-HT_{1A} receptor whereas the 5-HT_{1B} receptor was that component insensitive to spiperone. However, if multiple states of the 5-HT_1 receptor exist, this would complicate interpretation of competition curves of ^{3}H-5-HT binding and lead to erroneous conclusions regarding 5-HT_1 subtype selectivity, particularly for serotonin agonists. Recently, we have provided evidence that there are multiple states of the 5-HT_1 receptor (Mol. Pharmacol., in press). In the presence of 1mM GTP, the high affinity state was eliminated. Therefore, we examined the ability of a series of serotonin agonists and antagonists to inhibit 15nM ^{3}H-5-HT binding in rat frontal cortex in the presence of 1mM GTP. At least 20 concentrations of each compound were examined. The results were analyzed by non-linear regression analysis (MLAB) and indicated that 8 agonists and 4 antagonists showed selectivity for the 5-HT_1 receptor subtypes. The subtype selectivity for each of the selective compounds was determined using 2uM spiperone to selectively inhibit most of the 5-HT_{1A} component and 2uM 1-(m-trifluoromethylphenyl)piperazine (TFMPP) to selectively inhibit the 5-HT_{1B} component. The results of these experiments indicate that spiperone, pizotifen and the indole agonists 5-methoxy-N,N-dimethyltryptamine and N,N-dimethyltryptamine are selective for the 5-HT_{1A} receptor whereas RU 24969 and the piperazine agonists TFMPP, 1-(m-chlorophenyl)piperazine and quipazine show a higher affinity for the 5-HT_{1B} receptor. The degree of selectivity ranged from 20-110 fold. No selective 5-HT_{1B} antagonist was found. These results reveal a difference in selectivity for the two structurally dissimilar groups of serotonin agonists, namely that selective indole agonists show a higher affinity for the 5-HT_{1A} receptor as opposed to the piperazine agonists, which are selective for the 5-HT_{1B} receptor. (Supported by Research Funds from the Vet. Admin. and USPHS Grants MH 29094, GM 07517 AND GM 31155).

66.12 IN VIVO BINDING OF ^{125}I-LSD TO SEROTONIN 5-HT_2 AND DOPAMINE D2 RECEPTORS IN MOUSE BRAIN. U. Scheffel,* H.N. Wagner, Jr.* and P.R. Hartig (SPON: M. Larrabee). Div. of Nuclear Medicine, Johns Hopkins Med. Inst. and Dept. of Biology, Johns Hopkins Univ., Baltimore, MD.

Previous in vitro studies have shown that ^{125}I-LSD binds primarily to serotonin 5-HT_2 (S2) receptors in rat frontal cortex and exhibits a lower affinity binding to dopamine D2 receptors in striatum. The present study was undertaken to characterize the in vivo binding of ^{125}I-LSD to receptor sites in mouse brain with a view towards development of I-LSD as a ligand for imaging serotonin receptors in man.

The temporal distribution of ^{125}I-LSD was determined in various mouse brain regions following intravenous (tail vein) injection of carrier-free ^{125}I-LSD (2 µCi; 14 ng/kg). ^{125}I-LSD levels fell rapidly over a 60 minute time interval after injection. At 2 minutes little regional selectivity was observed. A peak in regional selectivity (tissue to cerebellum ratio) was reached at 7 minutes in the striatum and at 15 minutes in frontal cortex and remaining cortical regions. At fifteen minutes the tissue to cerebellum ^{125}I-LSD ratios (normalized per mg wet tissue) in the four highest brain regions were: frontal cortex (2.6), olfactory tubercles (2.4), striatum (2.3), and cortex (2.0).

Intravenous injection of ketanserin (4-2500 µg/kg) 15 minutes before injection of ^{125}I-LSD inhibited ^{125}I-LSD binding in all brain regions tested, except in the cerebellum. In frontal cortex, ketanserin caused a steep dose-dependent inhibition while the striatum was less affected at all dose levels.

A variety of serotonergic and dopaminergic drugs were tested as blocking agents for ^{125}I-LSD binding. Serotonergic drugs potently inhibited ^{125}I-LSD binding in the frontal cortex, cortex and olfactory tubercles. Striatal binding was inhibited by serotonergic and dopaminergic drugs indicating that ^{125}I-LSD binds to both receptor types in this tissue.

These studies indicate that ^{125}I-LSD binds to serotonin 5-HT_2 receptors and, to a lesser extent, to dopamine D2 receptors in vivo. Based on these findings, labeled I-LSD derivatives may prove useful for tomographic imaging of serotonin 5-HT_2 receptors.

66.13 PURIFICATION AND CHARACTERIZATION OF HISTIDINE DECARBOXYLASE FROM FETAL RAT LIVER. S. Levine, D.H. Park and T.H. Joh. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Histamine is formed in a single-step biosynthetic pathway which consists of the decarboxylation of the common amino acid, L-histidine; a reaction catalyzed by histidine decarboxylase (HDC, EC 4.1.1.22). Neurochemical, neurophysiological and neuropharmacological evidence suggests that histamine is a transmitter in the central nervous system. However, it is only recently that workers have sought an immunocytochemical probe for the identification of the putative histaminergic pathway. In the present study, we have attempted to purify and characterize HDC using conventional biochemical techniques. We present a procedure which yields a highly purified enzyme with an excellent recovery of total enzyme activity.

In summary, HDC was purified from fetal rat liver (17-18 days of gestation). The purification procedure included homogenization in 3 volumes of 100 mM potassium phosphate buffer containing 1mM DTT, 1mM EDTA and 10uM pyridoxal phosphate (PLP), centrifugation at 100,000 g for 1 h, 25-45% ammonium sulfate fractionation followed by subsequent column chromatographies on DEAE-cellulose, chromatofocusing on polybuffer exchanger 94 (Pharmacia) and Bio-Gel A-0.5m. These purification steps provided a more than 2,000-fold enrichment in the specific activity of the enzyme and a 40% recovery of the total enzyme activity. This represents a significant improvement in purification procedures previously reported for HDC.

The final purification step involves preparative polyacrylamide gel electrophoresis. The enzymatically active protein eluted from the gels showed a protein band with a subunit molecular weight of 51,000 daltons on SDS-PAGE.

Further characterization of the purified enzyme showed that the apparent Km value for L-histidine was $2.5x10^{-4}$M under the following conditions: 50uM-10mM L-histidine, 10 uM PLP and pH 6.8 and that the isoelectric point for fetal liver HDC is estimated to be 5.7 as determined by chromatofocusing.

The purified enzyme will be used to develop an antibody probe to study the regulation of HDC at the molecular level as well as the anatomy of the histamine system. (Supported by NIH Grant HL18974 and MG24285. S. Levine is the recipient of a scholarship from the Inst. de Invest. Clin., Maracaibo, Venezuela.)

66.14 PURIFICATION AND CHARACTERIZATION OF TRYPTOPHAN HYDROXYLASE FROM RAT BRAINSTEM. D.H. Park and T.H. Joh. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Tryptophan hydroxylase (TPH, EC 1.14.16.4) catalyzes the conversion of tryptophan to 5-hydroxytryptophan, the first step of serotonin biosynthesis. While the enzyme has been partially purified by several laboratories, including ours, its instability during purification has not permitted the enzyme to be purified to homogeneity in sufficient quantity for the biochemical characterization. In the present study, we have purified the enzyme by different procedures, allowing characterization of the highly purified enzyme.

Rat brainstems were homogenized in 50 mM tris-acetate buffer, pH 7.5, containing 2 mM DTT, centrifuged at 100,000 g for 1h, and the supernatant freeze-dried. The enzyme was further purified by sequential chromatography over Sepharose 4B, metal chelating affinity, chromatofocusing and Sephadex G200 superfine columns. Polyacrylamide gel electrophoresis of the purified enzyme showed three completely separated protein bands, with one of the bands containing TPH activity (2.7 cm from the top in a 7.5% acrylamide gel at pH 8.2 for 3h electrophoresis). The enzymatically active protein in gels was eluted and used for production of antibodies. The enzyme at this stage of purification was highly unstable and it was difficult to assess the degree of purification. The stability of the purified enzyme was not improved even in the presence of glycerol, Tween 20 and EDTA.

The molecular weight of the enzyme estimated by a sucrose density gradient centrifugation was 200,000. The isoelectric point of the enzyme estimated by chromatofocusing using Pharmacia polybuffer exchanger and polybuffer 74-Cl, pH 4.0, was 4.9. K_ms for tryptophan and 6MPH$_4$ of the highly purified enzyme were both $1.6x10^{-4}$M. (Supported by NIH Grant HL18974, MH24285 and NS19002.)

67.1 INDUCTION OF NEW GLUTAMATE BINDING SITES IN RAT HIPPOCAMPAL MEMBRANES BY TRANSIENT EXPOSURE TO HIGH CONCENTRATIONS OF GLUTAMATE, HOMOCYSTEATE OR D-AMINOADIPATE. M.Kessler*, M.Baudry and G.Lynch(SPON: M.Nieto-Sampedro).Center for the Neurobiology of Learning and Memory, Univ. of California, Irvine, CA 92717.

An increase in the number of synaptic glutamate receptors has been suggested to underlie synaptic potentiation in the hippocampus induced by repetitive electrical stimulation. One candidate mechanism for such an increase was found in the irreversible activation of glutamate binding caused by micromolar calcium concentrations via the calcium activated protease calpain I(Baudry et al.,Science 212, 937).

We present results suggesting that there might be a second mechanism leading to a long-lasting increase in the number of glutamate receptors in hippocampal membranes. The number of Cl^--dependent glutamate binding sites is increased up to fourfold, if membranes are preincubated for several minutes in high concentrations (0.1-10 mM) of L-glutamate, homocysteate, quisqualate or D- or L-aminoadipate and then washed extensively before starting the binding assay. The induced binding gradually reverts to a normal level with a T1/2 of several hours, if the washed membranes are left at 35°C. Short exposure to 50 uM tyrosyl-glutamate also leads to an increase in Cl^--dependent glutamate binding with an equally slow reversal. Preincubation with the glutamate analogs NMDLA and kainate does not induce new binding sites.

The induced sites seem to be identical to the well characterized, predominant form of Na^+-independent glutamate binding sites:binding to these sites displays a K_d of 1 uM, is Cl^--dependent,is inhibited by low concentrations of Na^+, and is further increased after transient exposure to Ca^{2+}. Binding is inhibited by micromolar concentrations of homocysteate, quisqualate, APB and D-aminoadipate.

Since the induction of binding sites does not depend on calcium and is not inhibited by leupeptin, this mechanism of induction by glutamate must differ from the previously characterized activation mechanism by calcium.The necessity of using high concentrations of glutamate or glutamate analogs to induce new binding sites suggests the presence in hippocampal membranes of a glutamate binding site with low, millimolar affinity that is functionally related to the known high-affinity sites. Whether the induction of binding sites represents a true increase in functional synaptic receptors or a conversion of receptors to a desensitized high-affinity state remains to be investigated.

67.2 INHIBITION BY POTASSIUM OF Na^+-DEPENDENT ^{3}H-GLUTAMATE BINDING AND HIGH-AFFINITY UPTAKE IN RAT HIPPOCAMPUS. K. Kramer* and M. Baudry. Department of Psychobiology, UC Irvine, Irvine, CA 92717.

Glutamate is considered as a major excitatory transmitter in the mammalian central nervous system. Inactivation of its effect is mediated through a Na^+-dependent high affinity uptake mechanism. It is thus of importance to elucidate the factors involved in the regulation of glutamate uptake. In this report we present data indicating that low concentrations of potassium exert an inhibitory effect on both Na^+-dependent ^{3}H-L-glutamate binding and high affinity uptake.

High affinity ^{3}H-L-glutamate uptake was measured in the presence of Na^+ in synaptosomal fractions, while Na^+-dependent ^{3}H-L-glutamate binding was measured in synaptic membranes prepared from rat hippocampus.

Potassium inhibits Na^+-dependent ^{3}H-L-glutamate binding at concentrations between 0.2 and 100 mM with maximal inhibition representing more than 95%. The potassium concentration eliciting half-maximal inhibition is about 1.2 mM. This effect is due to a decrease in the maximal number of sites with no change in the apparent affinity of glutamate for the site. The inhibitory effect of potassium is irreversible and may be due to competition between sodium and potassium at the ionic locus of the glutamate recognition site.

Potassium exerts a biphasic effect on high-affinity ^{3}H-L-glutamate uptake: low concentrations (0.5 to 5 mM) stimulate while higher concentrations (10 to 100 mM) inhibit uptake with half-maximal inhibition at about 30 mM K^+. In the presence of ouabain (100 µM) half-maximal inhibition occurs at about 12 mM K^+. The inhibitory effects of potassium at various sodium concentrations also suggest competition between Na^+ and K^+ at the carrier level.

The inhibitory effect of K^+ on Na^+-dependent ^{3}H-L-glutamate binding and high affinity uptake is shared by rubidium but not by cesium or lithium. Studies of the effects of K^+ in different brain regions indicate that K^+ is more potent in inhibiting Na^+-dependent ^{3}H-glutamate binding in telencephalic than in non-telencephalic structures, whereas the effects on high-affinity uptake are not significantly different.

The present results indicate complex interactions between K^+ and Na^+ in the regulation of glutamate binding to the carrier and its translocation. They suggest that such interactions may play some important role in glutamate inactivation, especially under circumstances when external K^+ and Na^+ concentrations are altered, such as during epileptic discharges. (Supported by NSF grant BNS-81-12156.)

67.3 INFLUENCE OF VARIOUS CONDITIONS AND AGE ON THE SPECIFIC BINDING OF [^{3}H]2-AMINO-7-PHOSPHONO HEPTANOIC ACID TO RAT BRAIN IN THE PRESENCE AND ABSENCE OF PHENYLALANYL-L-GLUTAMATE. J.W. Ferkany and J.T. Coyle, NIA, Lab. Neurosci., Bethesda, MD 20205 and the Johns Hopkins Medical School, Dept. Psychiatry, Div. Child Psych., Baltimore, MD 21205.

Previously, we have demonstrated that a related series of L-glutamate containing dipeptides, of which phenylalanyl-L-glutamate (PG) is representative, markedly enhances (up to 1000%; EC_{50} = 5-10 uM) the specific binding of the NMDA-type antagonist [^{3}H]APH to rat brain membranes in vitro. This action of PG is due to a peptide-induced increase in the apparent number (Bma) of [^{3}H]APH binding sites. We now describe the effects of various assay conditions and treatments on the specific binding of [^{3}H]APH in the presence and absence of PG.

Rat forebrain membranes were prepared according to themethod of Enna and Snyder (1976) and stored frozen (-80°C) until use. Routinely, assays were performed in Tris citrate buffer (0.05 M, pH 7.3, 37°C) in the presence of 50 nM [^{3}H]APH with or without 0.1 mM APH to determine non-specific binding. Reactions were terminated by centrifugation.

Specific binding of [^{3}H]APH to extensively washed brain homogenates achieved equilibrium within 90 min at 37°C and, in the presence of PG had a sharply defined pH optimum of 7.3. Preincubation (30 min, 37°C) increased the Bma for [^{3}H]APH binding by 40 %; however, PG (100 uM) induced an additional (445%) increase in the Bma for the ligand. The rank order of potency of several excitatory amino acid analogues to inhibit [^{3}H]APH binding was identical in the presence and absence of PG although the potency of some compounds declined. Treatment of membranes with Triton X-100, deoxycholate or neuroaminadase abolished binding of the ligand in the presence and absence of PG while phospholipase A2 and D as well as chymotrypsin and trypsin had only modest effects on ligand binding. Inclusion of Na^+ (5 mM) in the assay abolished [^{3}H]APH binding, an effect which was not mimicked by Ca^{++}, Mg^{++}, Li^+, K^+, I^-, Cl^-, formate or nitrate. Specific binding of [^{3}H]APH achieved adult levels by 25 days postpartum; and the action of PG to enhance [^{3}H]APH binding paralleled the appearance of [^{3}H]APH binding sites.

These results confirm our previous report that PG markedly enhances the Bma for [^{3}H]APH binding and suggest that the mediator of PG in this regard is intimately associated with the [^{3}H]APH binding site. Supported by USPHS research grant NS-13584, RSDA MH-00125 and USPHS fellowship NS-06798.

67.5 A TRANSIENT, DENSE, POSTNATAL EXPRESSION OF GLUTAMATE BINDING SITES IN GLOBUS PALLIDUS. J.T. Greenamyre, J.B. Penney, F. Silverstein*, M.V. Johnston and A.B. Young. Univ. of Michigan, Neurosci. Lab. Bldg., 1103 E. Huron, Ann Arbor, MI 48104.

Glutamate (GLU) is believed to be a major excitatory neurotransmitter in the mammalian central nervous system. Using an autoradiographic technique to label putative GLU receptors with L-[^{3}H]glutamate (30 nM-2.5 µM), we have previously shown an excellent correlation between the location of GLU binding sites and terminal fields of glutamatergic pathways (Greenamyre et al., J.Neurosci.,In press). In rats and humans, adult globus pallidus (GP) shows little GLU binding relative to striatum, which is thought to receive massive glutamatergic cortical input. We report here that during postnatal development there is a transient, dense expression of GLU binding sites in the globus pallidus of both rats and humans. In rats, on the first postnatal day (PND1), the level of GLU binding in GP ([GLU]=40 nM) was approximately the same as in adult rats. On PND3, GLU binding in GP had increased by 50% and on PND7 binding was more than 3-fold higher than in adults or PND1 pups. At this time there was more binding in GP than striatum. Also, in a 6 week old human infant, binding in GP exceeded striatal binding and was >4-fold higher than adult human GP. In rats, by PND14 GLU binding was decreasing in GP, with a convex band of heavier binding remaining on the medial border of the GP adjacent to the internal capsule. GLU binding was further decreased on PND21 and PND28. Striatal GLU binding increased progressively to adult levels from PND1 to PND21.

To examine whether the apparent decrease in GP binding in from PND7 to adult was due to increased attenuation of β-particles caused by myelination, the binding of [^{14}C]glutamate was examined in adult rats. A pattern of binding similar to that seen with [^{3}H]glutamate in adult rats was obtained, indicating that the developmental pattern of binding observed with [^{3}H]glutamate is not due to myelination.

Thus, in rats and humans GLU binding in the early postnatal period is dramatically increased relative to adults. This may represent a developmental phenomenon common to mammalian brains and the possibility of transient glutamatergic innervation of GP is being investigated. These findings may have implications for the selective vulnerability of the GP during the perinatal period.

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67.6 LIGAND REQUIREMENTS FOR ANTAGONIST ACTIVITY AMONG PHOSPHORUS-CONTAINING GLUTAMATE ANALOGUES IN HIPPOCAMPAL EXCITATORY PATHWAYS. R.K. Freund, S.L. Crooks*, J.F. Koerner and R. L. Johnson*. Depts. of Biochemistry and Medicinal Chemistry, Univ. of Minnesota, Minneapolis, MN 55455.

L-2-amino-4-phosphonobutanoic acid (L-APB) is a potent antagonist of hippocampal excitatory pathways with a preference for the lateral perforant path [Koerner and Cotman, Brain Res., 216 (1981) 192]. Omega-terminal substitutions on L-APB altered the potency and pathway specificity while retaining antagonistic activity [Freund et al., Brain Res., 291 (1984) 150]. This prompted us to synthesize and examine the pharmacology of methylated and cyclic analogues of L-APB. These compounds were bath-applied to the submerged rat hippocampal slice. Evoked synaptic field potential amplitudes were recorded with an extracellular electrode placed in the terminal field of lateral or medial perforant path synapses with dentate granule cells. The following compounds were tested: α-methyl-, β-methyl-, γ-methyl-, and N-methyl-APB (α-MeAPB, β-MeAPB, γ-MeAPB, N-MeAPB); cis- and trans-4-phosphonoxy-L-proline; and 1-amino-3-phosphonocyclohexane carboxylic acid (cyclohexyl-APB). In the lateral perforant path the diastereoisomeric mixtures of γ-MeAPB and β-MeAPB were the most potent of these L-APB analogues with apparent K_d's of 200 and 650 μM, respectively (L-APB has an apparent K_d = 2.5 μM in this pathway). The γ- and β-methylated derivatives were 10 and 13 times less potent, respectively, as antagonists of medial vs. lateral perforant path synaptic transmission. Alpha- and N-MeAPB were comparatively weak, inhibiting <40% of the response in either pathway at 8 mM. Among the cyclic analogues, cyclohexyl-APB was the most potent, inhibiting 50% of the lateral and 30% of the medial response at 4 mM. The two proline derivatives displayed little antagonistic activity with <20% inhibition of the synaptic response at 4 mM in either pathway. At higher concentrations, the trans-isomer gave evidence of agonist activity. These data suggest that placement of a methyl group at either the γ- or β-position of L-APB is least deleterious to antagonistic activity, while methylation of the α-carbon atom and α-amino group markedly reduces potency. The cyclohexane analogue of L-APB has a weak affinity for receptors mediating perforant path synaptic transmission, but the two proline derivatives are not acceptable ligands for these excitatory amino acid receptors. [Supported by USPHS NS17944].

67.7 DISPLACEMENT OF THE RADIOLIGAND ^{3}H-2-AMINO-4-PHOSPHONO-BUTANOIC ACID (^{3}H-APB) BY L-APB ANALOGUES AND GLUTAMATERGIC AGONISTS. M.B. Robinson, S.L. Crooks*, J.F. Koerner and R.L. Johnson*. Depts. of Biochemistry and Medicinal Chemistry, Univ. of Minnesota, Minneapolis, MN 55455.

A class of excitatory amino acid receptors is distinguished electrophysiologically by the potent and specific antagonism of L-2-amino-4-phosphonobutanoic acid (L-APB). Likewise, a class of binding sites with high affinity for ^{3}H-glutamate is distinguished by displacement of the radioligand by L-APB (K_i = 5 μM) and a requirement for Ca^{++} & Cl^- [Fagg et al., J. Neurosci., 2 (1982) 958]. This binding also was studied with ^{3}H-APB as the radioligand [Butcher et al., Br. J. Pharmacol., 80 (1983) 355; Monaghan et al., Brain Res., 278 (1983) 137]. We measured displacement of ^{3}H-APB from synaptic plasma membranes from rat forebrain by synthetic methylated analogues of L-APB with differing antagonist potencies in the hippocampal perforant path and also by synthetic and naturally-occurring glutamatergic agonists. We verified $CaCl_2$-dependent ^{3}H-APB binding homogeneous by Scatchard analysis (K_d = 3 μM; B_{max} = 110 pmole/mg protein). Binding was strongly displaced by the agonists L-quisqualate, L-glutamate, and L-2-amino-4-(5-tetrazolyl)-butanoic acid (L-glutamate tetrazole) and weakly displaced by kainate and N-acetyl-aspartyl-glutamate (NAAG) (see Table). Kynurenate and (-)-baclofen were also weak displacers. The antagonists DL-2-amino-4-methylphosphinobutanoic acid (DL-AMPB), and γ- and β-methyl-APB displaced ^{3}H-APB binding with K_i's approximately 20-fold lower than the apparent antagonist K_d's obtained electrophysiologically, while α-methyl-APB had only slightly greater affinity as a displacer than as an inhibitor of synaptic transmission. Although the rank-ordering of DL-AMPB, γ-methyl-APB, and β-methyl-APB are the same for antagonism and radioligand displacement, the large differences between apparent K_d's and K_i's suggest that the binding assays measure either a different or an altered receptor from that identified by electrophysiology of the perforant path. [Supported by USPHS NS17944].

TABLE: Displacement of ^{3}H-APB (K_i)

L-quisqualate	0.5 μM	DL-AMPB	4 μM
L-glutamate tetrazole	1 μM	γ-methyl-APB	15 μM
L-glutamate	5 μM	β-methyl-APB	30 μM
kynurenate	2 mM	α-methyl-APB	>2 mM
NAAG	2 mM		

(-)-baclofen & kainate: 15% displacement at 1 mM

67.8 TRITIATED L-ASPARTATE BINDING TO RAT BRAIN SYNAPTIC PLASMA MEMBRANES. J.D. Lane and G.E. Fagg#. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107 USA; and #Friedrich Miescher Institute, CH-4002 Basel, Switzerland.

Although a great many studies of 'glutamate' binding sites have been conducted, very few investigations of aspartate binding have been completed (see review by Foster and Fagg, Brain Res. Rev., in press). The glutamate sites have been subdivided into subclasses according to their affinities for the agonists N-methyl-D-aspartate (NMDA), quisqualate (Q) and kainate (K); with L-alpha-aminophosphonobutyrate (L-APB) posing a possible fourth binding or 'glutamate reuptake' site. NMDA and Q sites are insensitive to ions and enhanced by freeze-thawing; K sites are insensitive to ions and freeze-thawing; APB sites are chloride-dependent, enhanced by calcium, inhibited by sodium and abolished by freeze-thawing. To establish the binding characteristics of L-aspartate, synaptic plasma membranes (SPM's) were prepared from whole brains of adult male rats, using buffers free of sodium, calcium and chloride. Membranes prepared this way contained less than 120 pmol/mg protein residual free aspartate. The binding of L-aspartate to SPM's was characterized according to: protein concentration; pH, temperature and time optima; freeze-thawing sensitivity; the effects of sodium, calcium and chloride ions; and the ability of a variety of agents to displace radiolabelled L-aspartate. L-aspartate binds to a single population of sites, with a Kd of 0.8 uM and Bmax of 30 pmol/mg protein. It was insensitive to chloride and calcium, alone or in combination. Sodium enhanced aspartate binding consistent with a 'reuptake' site. Freeze-thawing enhanced binding, by changing Bmax and not affinity. In decreasing order of potency, the following agents displaced tritiated aspartate in a sodium-independent fashion: L-aspartate, L-glutamate, D-aspartate, D-glutamate, D-alpha-aminoadipic acid, cis-2,3-piperidine dicarboxylic acid. NMDA, APB, Q and DL-2-amino-4-phosphonovaleric acid were not effective displacers, even at high concentrations. These data suggest that there is one aspartate binding site which is separate and distinct from the multiple glutamate binding sites. We are continuing to characterize this aspartate site. (Supported in part by NIH NS-19644 to JDL)

67.9 THE ONTOGENY AND PHYLOGENY OF N-ACETYL-ASPARTYL-GLUTAMATE. K.J. Koller, P.G. Antuono* and J.T. Coyle. The Johns Hopkins Medical School, Dept. Psychiatry, Div. Child Psych., Baltimore, MD 21205.

N-Acetyl-aspartyl-glutamate (NAAG) is an endogenous dipeptide that binds with high affinity to a subpopulation of [^{3}H]-glutamate receptors in brain. In addition, NAAG has an uneven regional brain distribution as well as potent excitatory effects with a pharmacological profile similar to that of the endogenous transmitter in the lateral olfactory tract. In this study, we examined the ontogeny and phylogeny of NAAG using an anion exchange HPLC method.

The development of NAAG levels was studied from 15 days of gestation (DG) to adulthood in the rat. Before birth, NAAG levels were measured in the whole brain; after birth, the brain was dissected into brainstem, midbrain, cerebellum, hippocampus, cortex-striatum, and spinal cord. NAAG levels in whole brain were 30% of adult at 15 DG and rose to 90% of adult by birth. Between 2 and 8 days after birth, there was a marked increase in NAAG levels to 2-3 fold above adult in cortex, hippocampus and midbrain; the levels fell progressively during the subsequent three weeks. This peak in NAAG concentration at eight days after birth suggests that NAAG may be localized in an early maturing neuronal system.

While NAAG was not detected in whole hydra (Hydra littoralis) or in the earthworm (Lumbircus terrestris) nervous system, relatively high levels of 6.84 ± 0.56 and 3.58 ± 1.32 nmol/mg prot were found in the invertebrates sea anemone (Aiptasia palida) and planaria (Phagocata morgani), respectively. The arthropod (Periplanta americana) also had a NAAG level of 1.83 ± 0.19 nmol/mg prot. NAAG was present in the brain of all vertebrates examined except the goldfish (Carasius auratus) (< 0.50 nmol/mg prot). Hagfish (Bttratreus stoutii) brain levels were 2.17 ± 0.15 nmol/mg prot. Frog (Rana pipiens) and chick (Gallus domesticus) brain exhibited high concentrations of NAAG (12.52 ± 0.97 and 12.50 ± 1.00 nmol/mg prot, respectively). For comparison, the adult rat whole brain NAAG concentration was 3.30 ± 0.13 nmol/mg prot. The human cerebral cortex displayed an intermediate level for NAAG (5.02 ± 0.79 nmol/mg prot). These results are consistent with the developmental profile for NAAG in the rat brain since a higher concentration of NAAG is found in the brains of lower vertebrates such as frog and chick than in mammal. In addition, the presence of NAAG in most species examined suggests that NAAG may be important as a neurotransmitter in the nervous system across phylogeny.

67.10 IMMUNOCYTOCHEMICAL LOCALIZATION OF GLUTAMATE DEHYDROGENASE. R.J. Wenthold, K.K. Skaggs* and R.A. Altschuler. Dept. of Neurophysiology, Univ. of Wisconsin, Madison, WI, 53706 and NIH, Bethesda, MD, 20205.

We are studying the immunocytochemical distributions of enzymes known to be involved in the metabolism of the putative neurotransmitters, glutamate and aspartate. Our recent studies have shown that phosphate dependent glutaminase (GLNase) and cytoplasmic aspartate aminotransferase (AAT) are largely neuronal in mammalian brain and, in many cases, are enriched in neurons believed to use an excitatory amino acid as a neurotransmitter. This enrichment may reflect a role for these enzymes in the production of glutamate and aspartate. In the present study we have continued this effort by determining the immunocytochemical distribution of glutamate dehydrogenase (GD). Antibodies were made in rabbits against commercially-supplied (Sigma) GD from bovine liver. Analysis of this preparation by SDS gel electrophoresis showed a major protein band of 55 Kd, corresponding to the subunit of GD, and a minor band of about 40 Kd. The antiserum obtained was capable of inactivating the enzymatic activity of both purified GD and a crude preparation of GD from rat cerebellum. The antiserum was further characterized by immunoblotting. The major immunoreactive species in the purified GD preparation was the 55 Kd subunit, although several minor immunoreactive bands were also present. The major immunoreactive species in a gel of rat cerebellum co-migrated with the 55 Kd subunit. Peptide mapping of the 55 Kd subunit and the 55 Kd immunoreactive band from rat cerebellum showed similar immunoreactive peptide patterns. To eliminate the possibility of contaminating antibodies, we affinity purified antibodies specific for the 55 Kd subunit by using the 55 Kd subunit transferred to nitrocellulose paper as the affinity ligand. Immunocytochemistry was done on 10 micron thick cryostat sections of rat cerebellum, cochlear nucleus and hippocampus after 4% paraformaldehyde fixation. Immunoreactivity was visualized using PAP. GD was localized predominantly in glial structures. This was most obvious in the cerebellum where the major labelled structures were the Bergmann glial cells and their fibers. Astrocytes were also heavily labeled in the granule cell layer, while Purkinje cells were only very lightly labeled. These studies show that GD, like glutamine synthetase, is enriched in glia while GLNase and AAT are enriched in certain neurons.

67.11 PRESUMPTIVE GLUTAMERGIC/ASPARTERGIC (GLU/ASP) CELLS PROJECTING TO THE OLFACTORY CORTEX. T.A. Fuller, F.T. Russchen* and J.L. Price. Depts. of Psychiatry and Anatomy & Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110

Previous electrophysiological and neurochemical studies have indicated that glutamate and/or aspartate are utilized as neurotransmitters in the olfactory cortex, possibly in the axons of the mitral and tufted cells in the olfactory bulb which project to the cortex (Collins, Brain Res., 296: 145). To investigate this, we have used ^{3}H-D-aspartate (D-Asp) as a specific tracer for Glu/Asp projections. D-Asp is taken up by axon terminals, via the high affinity glutamate uptake system, and retrogradely transported to the cell soma, but is not metabolized (Streit, J. Comp. Neurol., 191: 429). Injections of D-Asp were made into the olfactory cortex of rats, and after 20-48 hrs the brains were fixed with 5% glutaraldehyde and processed for autoradiography.

The results show D-Asp is not retrogradely transported to mitral or tufted cells, whether it is injected into the olfactory cortex or into the olfactory bulb, and therefore suggest that these cells are not Glu/Asp. However, pyramidal cells and fiber tracts in many of the olfactory cortical areas are labeled by transport of D-Asp. These match many of the associational systems which have been shown with other tracers to interconnect different parts of the olfactory cortex. The labeled projections include those from: (1) the anterior olfactory nucleus (AON) to the olfactory bulb, the contralateral AON, and the olfactory tubercle (OT), (2) the anterior piriform cortex (PCa) to the AON, OT, and more posterior parts of the cortex, (3) the posterior piriform cortex to the PCa, (4) the nucleus of the lateral olfactory tract to the OT (bilaterally) and (5) the entorhinal cortex to the OT. In addition, injections of D-Asp into the OT also labeled cells in the midline thalamus, the basolateral and basomedial amygdaloid nuclei, and an intercalated nucleus rostral to the amygdala (Ir). Cells in the dorsal endopiriform nucleus and the nucleus of the diagonal band were also labeled in several cases. Labeled cells were not found in the substantia nigra, ventral tegmental area, dorsal raphe and locus coeruleus.

This suggests that many of the neurons which give rise to the associational fibers of the olfactory cortex, as well as other afferents to this cortex, are Glu/Asp. Previous results which have suggested that the mitral and tufted cells of the olfactory bulb are Glu/Asp may have reflected indirect effects on the associational system.

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67.12 DISTRIBUTION OF ASPARTATE N-ACETYLTRANSFERASE ACTIVITY IN RAT BRAIN REGIONS AND SPINAL CORD MF Truckenmiller, †MJ Brownstein, JH Neale, and MAA Namboodiri*, Department of Biology, Georgetown University, Washington, DC 20057; †Laboratory of Cell Biology, National Inst. of Mental Health, NIH, Bethesda, MD 20025.

N-Acetyl aspartate (NAA), which is found exclusively in neural tissues, is formed by the enzymatic acetylation of aspartate in the presence of acetyl CoA. The enzyme, aspartate N-acetyltransferase (ANAT), is highly specific for aspartate in this reaction. Although NAA is present in high concentrations in neural tissue, the role of aspartate acetylation is uncertain. To study this, a sensitive new assay has been developed and used to measure ANAT activity in rat spinal cord and various brain regions.

Tissues were homogenized in 100 mM sodium phosphate buffer pH 6.8 (100 mg wet weight/ml), and 25 ul aliquots were incubated (37°C, 30 min.) in the presence of L-aspartate (5 mM) and 1-[^{14}C]-acetyl CoA (0.5 mM, 2uCi/umol) in a total volume of 50 ul. The reaction was stopped by the addition of 50 ul ethanol. The product, ^{14}C-NAA, was separated from the radiolabeled substrate by TLC on silica gel using a solvent system composed of chloroform, methanol and acetic acid (90:10:50). NAA was visualized on the plates by ultraviolet illumination following spraying with 0.05% ethanolic solution of Morin. The spots corresponding to NAA (Rf = 0.27) were scraped off and the radioactivity determined.

Fifteen brain regions and spinal cord were assayed for ANAT activity. Highest activity was found in medulla (9.7±.8 nmol/min/g tissue). Other regions of high activity, as compared to medulla, include pons, midbrain, cervical spinal cord and thalamus (70-90%). Regions with intermediate activity were cerebral cortex, hypothalamus, cerebellum and pre-optic area of anterior hypothalamus (40-60%). Low activity was found in amygdala, septum, hippocampus, caudate, olfactory bulb and retina (10-40%). No activity was detected in pituitary. The distribution of ANAT activity appears to parallel concentrations of N-acetyl aspartyl-glutamate (NAAG), a neural tissue specific acidic dipeptide, but not that of NAA.

Characterization of this enzyme in the brain and spinal cord, as well as defining its regional and cellular distribution, should prove useful in the future studies of aspartate acetylation and NAAG biosynthesis in neural tissues.

67.13 LOCALIZATION OF GLUTAMATE AND ASPARTATE RELEASING NEURONS IN THE CHICK RETINA. A.M. López-Colomé and F. Somohano*. Centro de Investigaciones en Fisiología Celular, UNAM. 04510, México, D. F. México.

Glutamate (GLU) and aspartate (ASP) are among the most viable excitatory neurotransmitter candidates in the vertebrate retina. Although considerable amount of evidence supports this assumption, the identity of neurons which release these compounds as transmitters is still unclear. In order to try to clarify this point, we lesioned chick retinas by intraocular injection of 6, 60, 120 and 200 nmoles of kainate (KA) for selectively eliminating neuronal types (Morgan & Ingham, 1981), and measured the Ca^{++} -dependent K^{+}-stimulated release of L-^{3}H-GLU and L-^{3}H-ASP as previously described (López-Colomé et al., 1978). As a control, we measured ^{3}H-GABA and ^{14}C-Glycine (GLY) release. Lesion induced by 6 nmoles KA reduced bipolar and amacrine cells causing a concomitant decrease in the release of GLU and ASP (90% and 20% respectively); the release of GLY was abolished and that of GABA was unaffected. 60 nmoles KA decreased bipolars to 1/3 and eliminated amacrines; while ASP release was not modified further, that of GLU was 10% lower and that of GABA was significantly reduced. Treatment with 120 nmoles KA eliminated horizontal cells and further reduced bipolars, inducing an additional 30% decrease in ASP release and supressing that of GABA. In retinas treated with 200 nmoles KA (few bipolars present), neither compound was significantly released. These results together with our previous data on receptor distribution using this model, suggest that while OFF-bipolars could release GLU onto glycinergic amacrines, ON-bipolars could use ASP as the transmitter released to GABAergic amacrines.

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67.14 A PEPTIDE WITH GLUTAMATE LIKE ACTIVITY ISOLATED FROM THE RAT CEREBELLUM. N.S. Nadi* (SPON. E.S. Gershon), Section on Psychogenetics, NIMH, Bethesda, MD 20205

The dicarboxylic amino acid glutamate has been proposed as a major excitatory neurotransmitter in the brain. Recently, a receptor capable of binding ^{3}[H] glutamate has been extensively studied. This receptor has been shown to be located in synaptic membranes. The K_d for the binding of glutamate has been shown to be in the micromolar range. However, recent electrophysiological studies using glutamate antagonists have raised some questions as to whether glutamate is the real transmitter in the "glutamatergic" pathways. With this evidence in mind, and using the glutamate receptor assay as a bioassay we attempted to isolate a factor(s) from the brain which might interfere with glutamate binding. The cerebellum was chosen as the tissue from which the factor(s) were isolated because it contains the granule cells which are interneurons and known to be glutamatergic.

Using perchloric acid extraction and sephadex column filtration, we have isolated a factor from the cerebellum which interacts with the glutamate site. The molecular weight of this compound is estimated to be 1,000. The compound loses its activity when treated with trypsin. The absence of free glutamate in the isolate was determined by thin layer chromotography using the dansyl chloride technique (sensitivity = 10^{-12}M). This compound did not effect ^{3}[H]-quinuclidinyl benzilate, ^{3}[H]-muscimol or ^{3}[H]-dihydroalprenolol binding to brain membranes. Upon stimulation of slices of the cerebellum by K^{+} this compound was released in a Ca^{++} dependent manner. On a per milligram protein basis the distribution of the compound was as follows: cerebellum > hippocampus > olfactory bulb > cortex. We are currently establishing the amino acid composition of the peptide.

Recently, the existence of N-acetylaspartylglutamate having high affinity for the glutamate receptor site has been reported. Our peptide has a different molecular weight (1,000) and a somewhat different distribution (it is highest in the cerebellum). Further studies are required for establishing the intrinsic physiologic roles of these peptides.

EXCITATORY AMINO ACIDS: ELECTROPHYSIOLOGY AND RELEASE

68.1 EFFECT OF ZINC STATUS ON MARKERS OF EXCITATORY AMINO ACID NEUROTRANSMISSION IN HIPPOCAMPUS. E.J. Kasarskis and J.T. Slevin. Dept. of Neurology, Veterans Administration and Univ of Kentucky Medical Centers, Lexington, KY 40536.

Hippocampal mossy fibers contain the highest concentration of Zn in brain where the cation may participate in the process of neurotransmission. Although the transmitter at this synapse is not known with certainty, both aspartate (ASP) and glutamate (GLU) have been proposed. Because previous investigators have suggested that neurotransmission over this pathway is impaired during Zn deficiency, we investigated the effect of both dietary Zn restriction and Zn added in vitro on synaptosomal uptake and binding of GLU and ASP.

Near lethal Zn deficiency was induced by feeding diets containing <1 ppm Zn ad libitum to weanling male rats. Ad libitum and pair-fed controls consumed identical diets supplemented with 50 ppm Zn. After decapitation, the hippocampus was rapidly dissected and homogenized. Synaptosomal fractions were isolated for uptake studies by differential centrifugation and membrane fractions were further prepared by sonication for determination of receptor binding of [3H] L-GLU and [3H] L-ASP in the presence or absence of Mg, Ca, or Zn added in vitro.

Despite the presence of severe Zn deficiency, neither the uptake of GLU or ASP by hippocampal synaptosomes nor postsynaptic binding of these ligands was altered when compared to either control group. Moreover the augmentation of GLU and ASP binding by Ca and Mg, respectively, was not affected by dietary Zn status. The binding of both GLU and ASP however, was markedly inhibited by Zn added in vitro at concentrations less than the endogenous Zn levels present in hippocampus. (IC 50 = 0.131 and 0.050 mM, respectively).

These data demonstrate that the receptor affinities for GLU and ASP can be directly modulated by Zn, which could potentially function as a tonic inhibitor of excitatory synapses. However they do not support the hypothesis that the processes of synaptosomal uptake and postsynaptic binding of GLU and ASP in hippocampus are directly affected by severe dietary Zn deficiency. (Supported by the VA Research Service and BRSG grant No. 2-S07-RR05374)

68.2 **Analogues of Piperazine-2,3-Dicarboxylic Acid Inhibit Excitatory Synaptic Transmission in Rat Hippocampal Slices.** A.H. Ganong, C.W. Cotman, A.W. Jones*, and J.C. Watkins*. Dept. of Psychobiology, Univ. Calif., Irvine, CA 92717 and Dept. of Pharmacology, Univ. of Bristol Medical School, Bristol BS8 1TD, England.

Many of the potent amino acid antagonists which have been developed recently are selective for N-methyl-D-aspartate (NMDA) receptors. There are few antagonists of excitatory neurotransmission at non-NMDA CNS synapses. We now report a new series of analogues of piperazine-2,3-dicarboxylic acid (PzDA) that block excitatory synaptic transmission at non-NMDA synaptic pathways in the hippocampus.

Antagonist compounds were applied by superfusion to submerged rat hippocampal slices. Piperazine-2,3-dicarboxylic acid at 1 mM inhibited Schaffer collateral-commissural synaptic field potentials less than 10%. Other PzDA analogues were more potent as Schaffer-CA1 synaptic blockers. N-Benzoyl-PzDA, N-(m-chlorobenzoyl)PzDA, N-(3',4'-dichlorobenzoyl)PzDA, and N-(o-chlorobenzoyl)PzDA depressed field potentials in that order of potency with 50% depression occurring with 0.4 - 2 mM solutions.

The most potent PzDA derivatives were N-(p-chlorobenzoyl)-PzDA and N-(p-bromobenzoyl)PzDA. Half-maximal inhibition of Schaffer-CA1 responses was near 0.2 mM for these two derivatives. Medial and lateral perforant path responses and mossy fiber synaptic responses were also inhibited by about 50% by 0.05 mM to 0.2 mM solutions of these two compounds.

Focal depolarizations induced by ionophoretic application of excitatory amino acids in stratum radiatum of field CA1 were also antagonized by solutions of N-(p-chlorobenzoyl)PzDA and N-(p-bromobenzoyl)PzDA. These PzDA derivatives applied as 0.2 mM solutions blocked NMDA responses by about 25 - 35% but were less effective against kainate and quisqualate responses.

The similar effect of PzDA analogues against synaptic responses in different hippocampal pathways may indicate that neurotransmission in these pathways is mediated by similar excitatory amino acid receptors. The weak depression of hippocampal pathways by specific NMDA antagonists indicates that the primary synaptic receptor is not of the NMDA class. Although agonist-induced focal potentials and synaptic field potentials are difficult to compare quantitatively, the apparent greater potency of PzDA analogues against synaptic compared with agonist-induced responses suggests the possibility of a postsynaptic receptor type not selectively activated by NMDA, kainate, or quisqualate. (Supported by DAMD 17-83-C-3189 and Medical Research Council, UK)

68.3 INTRACELLULAR STUDIES OF AN APPARENT DESENSITIZATION OF EXCITATORY AMINO ACID RECEPTORS IN HIPPOCAMPAL SLICES. L. Fagni*, M. Baudry and G. Lynch. Center for the Neurobiology of Learning and Memory. U. C. Irvine, CA 92717.

Previous studies from this laboratory have shown that classes of excitatory amino acid receptors can be distinguished on the basis of pharmacological sensitivities and the extent of apparent desensitization observed upon successive application of their respective agonists. The present experiment used intracellular recording in hippocampal slices to reexamine this issue and to extend it to include a cholinergic agonist. The results were obtained from 28 pyramidal cells of field CA1 (mean resting membrane potential (RMP) = -62MV and membrane input resistance (Rm) = 16MΩ). Perfusion of the slices with L-Glutamate (0.5 to 1.0mM), N-methyl aspartate (10μM) and D,L-Homocysteate (50 to 200 μM) for one minute caused a large depolarization, a marked decrease in Rm, and the disappearance of excitatory postsynaptic potentials (EPSPs) washout produced a brief hyperpolarization followed by a return to baseline conditions. Repeated applications of L-glutamate and N-methylaspartate (10 minute intervals) resulted in a decrease in the effect of these aminoacids on the RMP and evoked EPSP, with no change in the between-application RMP, Rm, or evoked EPSPs. In contrast, successive perfusions of homocysteate did not result in significant alteration of its depolarizing effects.

In agreement with earlier reports, carbachol (50-100μM) caused a depolarization and an increase in membrane resistance; upon washout, the membrane potential and resistance returned to pre-drug values without a period of hyperpolarization. Repeated application of carbachol did not produce any evidence of desensitization in any of 5 seperate experiments. The depolarization elicited by potassium (5mM) was also not reduced across several applications and this treatment did not result in any obvious change in the efficacy of L-glutamate.

The absence of desensitization after repeated applications of carbachol, D,L-homocysteic acid, and potassium indicates that the effect is not a general response of hippocampal neurons to successive depolarizations. The desensitization found with l-glutamate and NMDA were not accompanied by generalized changes in membrane properties and did not affect synaptic responses measured between applications; together this evidence strongly supports our previous conclusion that NMA and L-glutamate stimulate receptors other than those used by the endogenous transmitter and that these sites are readily modified by repeated exposure to their agonists (supported by the Air Force Office of Scientific Research grant 82-0116).

68.4 DISTINCT NMDA AND ASPARTATE RECEPTORS ON PIRIFORM NEURONS AND THE EFFECTS OF VARYING Mg++. J. ffrench-Mullen*, N. Hori*, H. Nakanishi* and D. Carpenter, New York State Dept. of Health, Albany, N.Y. 12201.

Pyramidal neurons in rat piriform cortex brain slices are excited by N-methyl-DL-aspartate (NMDA), aspartate (Asp), glutamate (Glu) and quisqualate (Quis). Asp and Glu are considered to be mixed agonists, acting at aspartate- or glutamate-preferring receptors at which NMDA and Quis respectively are specific agonists. We have examined receptors for these substances using extra- and intracellular recordings with bath perfusion of anatagonists.

Neurons were excited in order of apparent potency NMDA> Quis>Glu>Asp when agonists were either bath perfused or ionophoresed. When recorded intracellularly the responses to NMDA, Asp and Glu were associated with an apparent conductance decrease while that to Quis was associated with a conductance increase. The NMDA, Asp and Glu response amplitudes increased with depolarization; the Quis response amplitude increased with hyperpolarization. These observations suggest that in this preparation Glu and Quis do not act at a common receptor. When amino phosphonovaleric acid (APV), a specific antagonist of the NMDA receptor, was perfused, responses to NMDA and Asp were blocked but at very different concentrations. APV blocked NMDA responses at 10^{-6} M, but responses to Asp were unaffected at both 10^{-6} and 10^{-5}M. At 10^{-4} M both responses were blocked. On consideration of the relative potencies these observations suggest that NMDA and Asp activate distinct receptors. High Mg^{++} blocks responses to NMDA and Asp presumably through a blockade of the associated ion channel (Nowak et al. Nature 307:462: 1984). We compared the Mg^{++} dependence of the responses by lowering Mg^{++} concentration. The NMDA and Asp responses were dramatically potentiated in Mg^{++}-free or 25% control Mg^{++} solutions. The potentiation of the NMDA was much greater than that to Asp. These observations imply that these receptors are associated with ion channels which are maintained in a state of partial channel blockade physiologic levels of Mg^{++}.

Our present results are consistent with the conclusion that the receptors for the excitatory amino acids in piriform cortex are different from those reported at some other sites particularly in that NMDA and Quis receptors are both distinct from those for Asp and Glu. The conductance changes and effects of low Mg^{++} suggest that the Asp and NMDA receptors are associated with an ionophore which is partially blocked by Mg^{++} at physiological concentrations.

68.5 EVIDENCE THAT L-PROLINE SELECTIVELY DEPOLARIZES PYRAMIDAL CELLS IN THE RAT HIPPOCAMPAL SLICE. B. Ault* and J. V. Nadler. Dept. Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

L-Proline has been examined in several studies as a possible L-glutamate antagonist. In the hippocampal slice proline reportedly excites CA1 pyramidal cells and inhibits their synaptic responses, possibly through blockade of glutamate receptors (Van Harreveld, A. & Strumwasser, F., Neuroscience, 6, 2495 (1981)). To evaluate this possibility, we examined the effect of proline on excitatory transmission and conduction at several sites in the rat hippocampal slice.

Just-maximal population spikes were recorded in the appropriate cell body layer after stimulation of Schaffer collateral-commissural fibers in areas CA1 and CA3, alvear fibers in area CA1, mossy fibers in area CA3 and perforant path fibers in the fascia dentata. Orthodronic stimulation of Schaffer collateral-commissural or mossy fibers during superfusion with 2-8 mM proline initially evoked a complex of 2-6 population spikes rather than a single spike. When the stimulus in proline-containing medium evoked more than two population spikes, continued superfusion with proline decreased population spike amplitude. Concentrations of proline within this range also depressed the amplitude of antidromically-evoked population spikes in area CA1. Qualitatively similar results were obtained by superfusing the slice with the excitatory amino acid L-aspartate, although at 2-4 times lower concentrations. In contrast to its depression of pyramidal cell firing, concentrations of proline as high as 10 mM little affected the amplitude of population spikes recorded in the dentate granule cell layer during perforant path stimulation. Aspartate, on the other hand, inhibited the orthodromically-evoked firing of granule cells almost as potently as pyramidal cell firing.

These results suggest that proline, like aspartate, inhibits synaptic responses of hippocampal pyramidal cells through a postsynaptic depolarizing action. Unlike aspartate, however, proline appears to lack excitatory activity on dentate granule cells. Our results do not support the suggestion that proline acts as a glutamate antagonist. (Supported by NIH grant NS 16064.)

68.6 ATTENUATION OF EPILEPTIFORM BURST FIRING IN THE RAT HIPPOCAMPAL SLICE BY ANTAGONISTS OF N-METHYL-D-ASPARTATE RECEPTORS. Mary A. Hynes* and Raymond Dingledine. Dept. Pharmacology and Neurobiology Curriculum, Univ. North Carolina, Chapel Hill, N.C. 27514.

Of the excitatory amino acid receptors thought to exist on hippocampal pyramidal cells, the NMDA receptor may be the best characterized. Activation of this receptor by N-methyl-aspartate (NMA) turns on a voltage- and calcium-dependent cation conductance, and selective receptor antagonists have been described. The NMDA receptor might be expected to be engaged normally under conditions in which synaptically activated calcium spikes are produced in pyramidal cells. We examined the effect of NMDA receptor antagonists in one of these conditions, epileptiform burst firing induced in the CA1 region by bicuculline (10-100 μM).

In extracellular recordings of population spike bursts evoked by stimulation in stratum radiatum, low concentrations of DL-2-amino-5-phosphonobutyric acid (APV, 1-10 μM) selectively reduced burst duration without affecting the amplitude of the first population spike. Much higher APV concentrations (100-1000 μM) were needed to reduce the amplitude of the first population spike in a burst. All effects were reversible upon washing. Selectivity was further shown by the lack of effect of 100 μM APV on input-output curves formed by plotting the population spike as a function of the field EPSP, or the field EPSP as a function of the fiber volley. The effects of gamma-D-glutamylglycine and DL-diaminopimelic acid were similar but less selective than that of APV. In intracellular recordings APV (10-100 μM) reduced the number of spikes in an evoked burst and decreased the duration of the depolarizing wave underlying the burst. No consistent effects on membrane potential or input resistance were noted. Furthermore, APV had no effect on repetitive firing induced by a depolarizing current pulse. In the presence of 1 μM tetrodotoxin, APV markedly antagonized depolarizations evoked by iontophoresis of NMA and blocked NMA-evoked calcium spikes without affecting calcium spikes produced by a large depolarizing current pulse. The most parsimonius interpretation of the data is that APV attenuated bursts by blocking synaptic receptors. Before it can be concluded that NMDA receptor activation contributes to epileptogenesis, additional tests are needed of the selectivity of these antagonists towards the various acidic amino acid receptors. Supported by NS-17771 and MH-14277.

68.7 GLUTAMATE ACTIVATED SINGLE-CHANNEL EVENTS ON HIPPOCAMPAL PYRAMIDAL CELLS. Alan R. Kay*, Robert B. Clark* and Robert K.S. Wong (SPON: D. C. Eaton). Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

There is much evidence to suggest that glutamate acts as an excitatory neurotransmitter at central nervous system synapses. Application of glutamate or one of its analogues to central neurons, produces a depolarization whose time course and underlying conductance change depend both on the brain region and the type of analogue. Verification of glutamate or one of its analogues as a transmitter in the brain is difficult because (1) the pharmacological evidence points to the existence of at least three distinct types of glutamate receptors and (2) there is a lack of physiological data, at the receptor level, demonstrating the specificity of antagonists for these glutamate actions.

In this study we have undertaken to investigate whether the different classes of glutamate receptors may be distinguished using the techniques of patch clamping. Within the hippocampus, glutamate is presumed to be the transmitter at both the perforant pathway input and the recurrent synapses between CA2-CA3 pyramidal cells. Application of glutamate antagonists including gamma-d-glutamylglycine suppresses synchronized discharge in the CA2-CA3 region (Miles et al., 1984). We have carried out our experiments using dissociated pyramidal cells from the hippocampus of adult guinea pigs by the method of Numann et. al. (1981) in which papain (15 mg/ml) was used to affect the dissociation. Single channel currents were recorded from whole-cell patches in the presence of glutamate (50 μM). Recordings gave evidence of at least two conductance states of about 25 and 42 pS all with a reversal potential of approximately 5 mV.

(Supported by NS 18464).

68.8 EXCITATORY AMINO ACIDS DIRECTLY DEPOLARIZE RAT BRAIN ASTROCYTES IN PRIMARY CULTURE. C.L. Bowman* and H.K. Kimelberg (SPON: R.S. Bourke). Division of Neurosurgery, Albany Medical College, Albany, New York

L-glutamic (glu) and L-aspartic (asp) acid are considered to be major excitatory amino acid transmitters in the mammalian central nervous system (CNS). They appear to activate at least three different receptors which are preferentially stimulated by kainic acid (KA), n-methyl-d-aspartate (NMDA) or quisqualate (QA). These receptors are thought to be located exclusively on excitable membranes since their stimulation causes neuronal depolarization and excitation and since electrophysiologically unresponsive cells, presumably glia in cat cerebral cortex in vivo (1) or glial cells in explant cultures from rabbit cerebellum (2) did not appear to respond to ionophoresed L-glu. More recent work in mixed neuronal-glia explant cultures from rat spinal cord has revealed that glia are depolarized by bath application of glu or asp but this response was attributed to increased $[K^+]_o$ due to K^+ released from neighboring excited neurons (3,4). However, glu depolarized glial cells in a deaxonated optic nerve preparation from Necturus (5). Since astroglia can be grown in a relatively homogeneous form in primary monolayer cultures in the virtual absence of neurons, we decided to re-investigate the effects of L-glu and L-asp on the resting membrane potentials of astrocytes. We found that identified astrocytes exhibit a marked depolarization (19 ± 7mV, 48 cells) in response to bath application of 10^{-4}M L-glu. L- or D-asp and KA (10^{-4}M) also depolarized the cells. Cells that were depolarized by glu also contained glial fibrillary acidic protein (GFAP), a marker considered specific for astrocytes. None or minimal changes (< 4mV) in the resting membrane potential were found in response to the other glutamate agonists (NMDA or QA), the inhibitory amino acids (GABA or glycine), or amino acids D-glutamate, taurine or L-glutamine.

(Supported by grant NS 19492 from NINCDS).

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68.9 EFFECTS OF ANTAGONISTS OF AMINO ACID TRANSMITTERS ON CORTICALLY EVOKED EPSPs AND IPSPs IN CAT CAUDATE NEURONS. P.L. Herrling. Wander Research Institute (a Sandoz Research Unit), P.O.Box 2747, CH-3001 Berne, Switzerland.

To explore the nature of the transmitters involved in cortically evoked EPSP-IPSP sequences in caudate neurons, intracellular recordings were made from these cells in halothane-anaesthetized cats during simultaneous stimulation of the anterior sigmoid gyrus and iontophoretic application of drugs.

The clearest effect of the competitive GABA-antagonist bicuculline-methochloride (BMC) was to increase both the amplitude and the duration of cortically evoked EPSPs and to abolish the early part of the following IPSP in those cells where a hyperpolarization was visible.

2-amino-7-phosphonoheptanoic acid (AP-7), a potent and selective antagonist of N-methyl-D-aspartate (NMDA) receptors, antagonized the excitatory effects of NMDA, but not those of glutamate (GLU) or quisqualate (QS). Even large iontophoretic currents of AP-7, i.e. fivefold those needed to antagonize effects of locally applied NMDA, did not visibly affect cortically evoked synaptic potentials. Furthermore, repetitive cortical stimulation at 50 Hz did not produce the typical bursting firing pattern usually elicited by NMDA, but a firing pattern similar to the one induced by QS (Herrling et al., J. Physiol. 339: 207, 1983). The tryptophane-metabolite kynurenic acid (KYAC) antagonized excitations induced by NMDA>GLU~QS~kainate (KA) in this approximate order of potency and also reversibly inhibited cortically evoked EPSPs, but at higher currents than those needed to affect amino acid induced excitations. However, firing elicited by intracellular current injections were not inhibited.

These results suggest that the amplitude of cortically evoked EPSPs is regulated by a GABA-mediated IPSP and that these synaptic excitations are probably mediated by QS- or KA-type receptors and not by NMDA-receptors.

68.10 PK 26124 (2-AMINO-6-TRIFLUOROMETHOXY BENZOTHIAZOLE). A POSSIBLE GLUTAMATE ANTAGONIST. J. Bénavidès*, J. Mizoule*, A. Uzan, C. Guérémy* and G. Le Fur. PHARMUKA Laboratoires, Groupe RHONE POULENC SANTE, 35, quai du Moulin de Cage, 92231 Gennevilliers, France.

PK 26124 is a new anticonvulsant drug with an atypical pharmacological spectrum. It differs from benzodiazepines and other agents enhancing the GABAergic neurotransmission in which it is more potent against convulsant agents acting through an enhancement of excitatory aminoacid neurotransmission. Moreover it potently antagonizes convulsions elicited by intracerebellum-ventricular injections of glutamate. Biochemical studies demonstrate that PK 26124 can decrease the cerebellar cGMP levels in the "in vivo" but not in the "in vitro" conditions. In cerebellar slices PK 26124 antagonizes the increase in cGMP levels elicited by l-glutamate, but not those elicited by l-aspartate, l-cystein sulfinate, N-methyl-dl-aspartate or kainate. In the "in vivo" conditions PK 26124 also antagonizes the cGMP increases originated by glutamate. Interestingly enough PK 26124 can "in vivo" antagonize the cGMP levels increases elicited by pharmacological enhancement of glutamate neurotransmission at doses where it does not antagonize the effect of GABA neurotransmission blockers. As cGMP levels reflect the activity of the cerebellar Purkinje cells the PK 26124 anticonvulsant properties might be due to an inhibition of the glutamaergic neurotransmission. An additional support of the antiglutamate properties of PK 26124 comes from the strong decrease of acetylcholine turnover elicited by this compound in striatum and olfactory tuberculi, since it might be due to an inhibition of the tonic stimulation of the cholinergic neurons by a glutamaergic pathway. Moreover PK 26124 also antagonizes the stimulation by N-methyl-dl-aspartate of the acetylcholine release from striatum and olfactory tuberculum slices. The potency of PK 26124 in this test (IC 50 ≃ 20 μM) compares favourably with most of the known antagonists.

68.11 PHARMACOLOGY OF CHEMICAL TRANSMISSION AT COCHLEAR NERVE SYNAPSES IN THE AVIAN COCHLEAR NUCLEUS. E.F. Nemeth, H. Jackson and T.N. Parks. Dept. of Anatomy, Univ. of Utah School of Medicine, Salt Lake City, UT 84132.

Synaptic transmission in the avian cochlear nucleus (nuc. magnocellularis, NM) is selectively inhibited by non-N-methyl-D-aspartate (non-NMDA) receptor antagonists (Neurosci. Lett. 40:39-44, 1983). The present experiments further define the pharmacological characteristics of transmission in this system.

Synaptic transmission in NM was assessed by recording field potentials evoked by direct electrical stimulation of the cochlear nerve in vitro. Selective NMDA receptor antagonists, in this case DL-a-aminosuberate and DL-2-amino-3-phosphonopropionate (5mM each), failed to inhibit transmission. In contrast, kynurenic acid, which blocks responses mediated by both NMDA and non-NMDA receptors, completely and reversibly blocked transmission (IC_{50}=1mM). Dipicolinic acid was the only other of several kynurenines tested which selectively blocked responses in NM. Like kynurenic acid, dipicolinic lacks receptor specificity and both differ from streptomycin, which preferentially blocks responses to the non-NMDA receptor agonist quisqualate. Streptomycin (2.5 mM) completely inhibited evoked responses in NM. Most of the inhibitory effects of streptomycin appeared to result from postsynaptic actions because inhibition was only partially overcome by increasing the Ca concentration three-fold.

Selective NMDA receptor agonists, such as aspartate (10 mM), NMDA (5 mM), and quinolinic acid (5 mM), failed to affect transmission whereas the non-NMDA agonists quisqualate (3.5 mM) and kainate (10 uM) completely suppressed evoked responses. Likewise, DL-a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), which seems to be a specific agonist at receptors activated by glutamate, is a potent inhibitor of transmission (IC_{50}=25-50 uM). Results obtained with both agonists and antagonists could be taken as evidence favoring glutamate as the cochlear nerve transmitter. This view, however, is inconsistent with the much greater potency of kainate relative to quisqualate. Moreover, glutamate had little or no effect on evoked transmission even at 10 mM. Discrepant observations such as these suggest that the avian cochlear nerve transmitter is a compound that acts in NM by non-NMDA receptors particularly sensitive to kainate.

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68.12 GLUTAMATE NEUROSECRETION IN LS AND SS MICE: CORRELATION OF STIMULATED RELEASE AND VESICULAR CONTENT IN BRAIN REGIONS. J. A. Ruth and J. K. Disbrow*, School of Pharmacy, University of Colorado, Boulder, CO 80309.

In an effort to correlate levels of L-glutamate neurosecretion with levels in synaptic vesicles, we have examined differences in K^+-stimulated, Ca^{++}-dependent glutamate release in several brain regions, and the size of the osmotically-sensitive glutamate pool in synaptic vesicles of long-sleep (LS) and short-sleep (SS) mice, lines selected for difference in sleep time response to acute ethanol administration. In slices of cortex and striatum, the 30mM K^+-stimulated release of endogenous glutamate was 36% and 45% higher, respectively, in SS than in LS mice. Basal efflux of glutamate did not differ between the lines. The Ca^{++}-antagonists La^{+3} (10^{-3}M) and verapamil (5 X 10^{-5} M) reduced K^+-stimulated release by more than 85% without altering basal efflux.

Mouse brain synaptic vesicles suspended in a tartrate medium displayed saturable accumulation of ^{3}H-L-glutamate at 37°C (LS, Km 1.87 X 10^{-4} M; SS, Km 0.53 X 10^{-4} M). The accumulation was stable, temperature sensitive, osmotically labile, and partially ATP-dependent. ^{3}H-L-glutamate accumulation in vesicles from SS mice was nearly twice that observed in vesicles from LS mice at several loading concentrations. The vesicular content of endogenous glutamate in vesicles from SS mice was 35% greater than in vesicles from LS mice.

The demonstration of an osmotically sensitive vesicular pool of L-glutamate in LS and SS mouse brain tissue suggests that this pool may exist *in vivo*, and may play a role in glutamate neurosecretion.

68.13 THE EFFECT OF CHOLINERGIC AGENTS ON THE RELEASE OF ^{3}H-D-ASPARTATE FROM RAT HIPPOCAMPAL SLICES. R. D. Schwarz, T. A. Pugsley, C. J. Spencer*, and A. A. Bernabei*. Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

Specific excitatory neuronal pathways in the hippocampus have been identified which use glutamate (Glu) and/or aspartate (Asp) as their neurotransmitter substance. A measure of the functional output of these neurons is the estimation of Ca^{++}-dependent ^{3}H-Glu/Asp release. Since there is a well defined cholinergic input from the septum to the hippocampus, the following experiments were designed to examine the effect of cholinergic agents on the release of ^{3}H-D-Asp from rat hippocampal slices. ^{3}H-D-Asp has previously been shown to be a valid marker for measuring release from Glu/Asp neurons.

Rat hippocampi were sliced (0.3 x 0.3 mm) and then incubated with ^{3}H-D-Asp for 20 min. at 37°C in Krebs-Ringer Hepes buffered medium. After washing 3x, 10-15 mg of tissue were incubated for 15 min. in normal medium or medium with elevated K^+ in the presence or absence of test compound. The reaction was terminated with tissue and medium being separated by rapid centrifugation.

It was shown that K^+-induced release of ^{3}H-D-Asp was Ca^{++}-dependent and controlled by the activation of a kainate (KA)-type of presynaptic amino acid receptor; thus adding further evidence to a transmitter function for Glu/Asp (Fed. Proc. 43:772, 1984). The present findings indicated that the muscarinic agonist, oxotremorine (Oxo), markedly decreased K^+-stimulated ^{3}H-D-Asp release at 10^{-5} - 10^{-4}M. Atropine blocked this decrease while having no effect on release by itself. The effect of Oxo was seen at low levels of K^+-stimulation, (40 mM), in contrast to our earlier obervations that L-Glu and KA decreased release only at high K^+ concentrations (60 mM). The nicotinic agonist, 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP), increased the release of ^{3}H-D-Asp and was atropine insensitive. Raising endogenous levels of Ach with the cholinesterase inhibitor, physostigmine, produced an increase in ^{3}H-D-Asp release.

In conclusion, cholinergic agents appeared to significantly control the release of ^{3}H-D-asp from rat hippocampal slices. Muscarinic agonists decreased release, while nicotinic agonists increased release. Thus, cholinergic control of neurons releasing Glu/Asp may ultimately be a function of nicotinic and muscarinic receptor distribution and number.

68.14 ORNITHINE AS A PRECURSOR OF NEUROTRANSMITTER GLUTAMATE: *IN VIVO* LABELING STUDIES. J. T. Wroblewski*, W. D. Blaker and J. L. Meek. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

We have previously shown that ornithine aminotransferase (OAT) may catalyze glutamate formation in nerve terminals. The rat hippocampal-septal glutamateric projection was selected as a model because inhibition of OAT by septal injection of L-canaline decreases local glutamate content. Since an acute lesion of the hippocampal-septal glutamatergic afferents changes the action of L-canaline, the neuronal location of the change is suggested.

In the present work, we studied the *in vivo* incorporation of label from ^{3}H-ornithine (ORN) and ^{14}C-α-ketoglutarate (α-KG), the two substrates of OAT, into septal amino acids. These labeled precursors were injected intraventricularly and the rats were sacrificed by head-focussed microwave irradiation at various times. The amino acids were extracted, analyzed by HPLC and the radioactivity was measured in effluent fractions.

Radioactivity from ^{3}H-ORN was incorporated into glutamate, glutamine, GABA and proline, accounting for >90% of the extracted radioactivity. Labeled ORN decreased with a half life of ~13 min. The labeling of glutamate reached a maximum at 3 min post-injection, while glutamine and proline were more slowly labeled. After ~30 min, the radioactivity in the amino acids decreased with half lives of 10-20 min. ^{14}C-α-KG labeled glutamate, glutamine and GABA, but not proline. Glutamate was labeled much faster than with ^{3}H-ORN, while GABA was labeled more slowly. The kinetics of glutamine labeling was similar to that seen with ^{3}H-ORN. These results demonstrate the existence in the brain of pathways which can convert ORN and α-KG into glutamate, glutamine and GABA.

The role of OAT in the above labeling was studied by the intraseptal injection of L-canaline prior to the administration of the radioactive precursors. L-canaline reduced the labeling of glutamate, glutamine and proline from ^{3}H-ORN by 6-9 fold. The labeling from ^{14}C-α-KG was affected to a much smaller extent.

These results indicate that the formation of glutamate from ORN *in vivo* proceeds mainly via OAT, whereas its formation from α-KG proceeds predominantly through other pathways. Since OAT inhibition seems to affect both the maintenance of the nerve terminal pool of glutamate and the conversion of ORN to glutamate, it seems possible that ORN may be a precursor of neurotransmitter glutamate.

68.15 DEPOLARIZATION INDUCED RELEASE OF ENDOGENOUS SULFUR CONTAINING AMINOACIDS IN RAT BRAIN SLICES.
K.Q.Do*, P.Streit*, M.Wolfensberger* and M. Cuénod. Brain Research Institute, University of Zurich, CH-8029 Zurich, Switzerland.

An HPLC method has been applied to the investigation of the release of highly polar substances. Slices of various rat brain regions (cortex, hippocampus, striatum, meso-diencephalon, cerebellum, pons and medulla, spinal cord) were superfused with oxygenated Earl's bicarbonate buffered salt solution. The depolarization was induced on one hand by raising the $[K^+]$ to 50mM either in the presence of 2mM Ca^{++} and 1mM Mg^{++} or of 0.1mM Ca^{++} and 10mM Mg^{++} or, on the other hand, by addition of veratrine (33ug/ml). The collected fractions were lyophilized, derivatized with DABITC (4-dimethylamino-azobenzene-4'-isothiocyanate) and analysed on a reversed-phase column. Substances more polar than Glu and Asp, among them sulfur containing aa (cysteic acid or cysteine sulfinic acid, homocysteic acid or homocysteine sulfinic acid) were increased by K^+depolarization in a Ca^{++}-dependent manner or under veratrine stimulation. At rest, these compounds are present in the superfusate in amounts 10 to 50 times smaller than that of the major aa. K^+depolarization increased their rate by a factor of 2 to 4. This release was high in neocortex, hippocampus and medulla and low in striatum and spinal cord. The release of endogenous sulfur containing aa supports the proposal, made by others (Curtis, D.R. and Watkins, J.C., J. Neurochem., 6:117-141, 1960; Recasens, M., Varga, V., Nanopoulos, D., Saadoun, F., Vincendon, G. and Benavides, J., Brain Research, 239: 153-173, 1982; Iwata, H., Yamagami, S. and Baba, A., J. Neurochem., 38: 1275-1279, 1982), that they play a role as neurotransmitters.

68.16 QUANTITATIVE ANALYSIS OF FREE AMINO ACIDS IN BIOLOGICAL SAMPLES BY GAS-LIQUID CHROMAOTGRAPHY OF THEIR N(O,S)-PENTAFLUOROBENZOYL ISOBUTYL ESTERS. J. M. Yeung*, G. B. Baker, and R. T. Coutts*. Neurochemical Research Unit, Dept. of Psychiatry and Faculty of Pharmacy and Pharmaceutical Sciences, Univ. of Alberta, Edmonton, Canada, T6G 2G3.

A sensitive, rapid and reliable gas chromatographic method for simultaneous quantitative separation of 23 free amino acids has been developed. The success is attributable to the development of mild and quantitative micro derivatization reactions which can convert hydroxyl, thiol, and primary and secondary amines to electron-capturing electrophores using pentafluorobenzoyl chloride with picogram (10^{-12} g) sensitivity. Rat brains were homogenized in 10 volumes of a mixture of methanol: 6N aqueous HCl (9:1 v/v). After centrifugation, a 10 µL aliquot of the supernatant was dried and taken up with 1 drop concentrated HCl in 1 mL isobutanol and heated. After cooling, the isobutanol-HCl was evaporated under a stream of nitrogen. Then 5 µL of pentafluorobenzoyl chloride in 1 mL chloroform was added, followed by 1 mL of aqueous saturated sodium bicarbonate. After mixing, the aqueous layer was aspirated off and the organic layer evaporated under a stream of nitrogen. The residue was taken up with 300 µL decane and washed with 1 mL distilled water. A 0.2-1 µL aliquot of the decane phase was injected on a GC equipped with a 25 m 5% phenylmethyl silicon-fused capillary column and an electron-capture detector. GC conditions: carrier gas, helium, 20 p.s.i.; makeup gas, argon/methane, 90/10 at 35 mL/min; oven program: initial time, 0.5 min, initial temperature, 170°C, level 1 program rate, 10°C/ min, final value, 230°C, final time, 0.1 min; level 2 program rate, 8°C/min, final value, 330°C, final time, 10 min; injection port temperature, 200°C; detector temperature, 350°C. Amino acids were conveniently analyzed in 1 mg or less of brain tissue. All amino acid derivatives were characterized by gas chromatography/mass spectrometry.

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CATECHOLAMINES: RECEPTORS II

69.1 **THERMODYNAMIC ANALYSIS OF AGONIST INTERACTIONS WITH THE β-ADRENERGIC RECEPTORS.** Margarita L. Contreras*, Barry B. Wolfe, and Perry B. Molinoff, Dept. of Pharmacology, University of Pennsylvania, Philadelphia, PA.

The interactions of agonists and antagonists with membrane-bound and digitonin-solubilized β-adrenergic receptors prepared from L6 myoblasts were examined at five temperatures from 10° to 30°C. The binding of antagonists to membrane-bound and soluble receptors was generally temperature insensitive. In contrast, agonists were involved in temperature-sensitive reactions. In the absence of GTP, binding of agonists to membrane-bound receptors was described by Hill coefficients of less than one. Inhibition curves for agonists were mathematically analyzed to estimate affinity constants for high (K_H) and low (K_L) affinity states of agonist binding. K_H values markedly decreased with decreasing temperature. K_L values decreased to a smaller extent with decreasing temperature. The K_D values for binding of agonists in the presence of GTP changed in a manner similar to the change in the K_L values for agonist binding. The binding of agonists to digitonin-solubilized receptors was unaffected by GTP and was similar to the binding of agonists to membrane-bound receptors in the presence of GTP. Thermodynamically, binding of antagonists was entropy-driven. The energetics of the low-affinity component of agonist binding to membrane-bound receptors was similar to the thermodynamic properties for the binding of agonists to soluble receptors. These reactions were described by changes in entropy and enthalpy that were negative relative to the thermodynamic parameters for binding of antagonists. These thermodynamic changes are consistent with an agonist-induced conformational change in the receptor. The high-affinity component of agonist binding, which is thought to represent formation of the ternary complex, was associated with larger negative changes in entropy and enthalpy than was seen for the low-affinity state of agonist binding. Thus, the interactions between the receptor and the guanine nucleotide binding protein (N_s) are driven by enthalpy which compensates for a large negative (energetically unfavorable) change in entropy. The changes in entropy and enthalpy for formation of the high-affinity state of agonist binding correlated with the efficacy of the agonist to activate adenylate cyclase. The extent of interactions between the receptor and N_s may be intrinsically involved in determining the efficacy of a ligand. (Supported by the NIH NS 18479-03, GM 31155 and AHA)

69.2 AUTORADIOGRAPHIC STUDY OF BETA-1 ADRENOCEPTOR DEVELOPMENT IN THE MOUSE FOREBRAIN. A.M. Goffinet, L.M. Hemmendinger & V.S. Caviness, Jr. Univ. of Louvain, B-1200 Brussels, Belgium, and E.K. Shriver Center, Waltham, Ma. 02254

The development of beta-1 adrenoceptors (beta-R) in the mouse forebrain, from embryonic day 14 (E14) to adulthood (P30), has been studied by autoradiographic visualization of I-125 iodocyanopindolol binding sites (ICYP; Engel et al. Naunyn Schmiedebergs Arch. Pharm. 317:277-285, 1981). Blanks were generated by incubation with propranolol and beta-2 adrenergic binding was blocked with zinterol.

At E14-15, beta-R binding sites are found in the ganglionic eminence, in the lateral part of the basal forebrain and in the pyriform cortex. Low concentrations are also seen in the neocortical subplate. By E16-17, beta-R binding sites are concentrated uniformly in the striatum, laterobasal forebrain and pyriform cortex. Within the neocortex labeling is strong in a band coextensive with the cortical subplate but minimal or absent in the cortical plate itself.

In neonates, inhomogeneities are apparent in the pattern of beta-R labeling in the basal ganglia, where the pallidum is more heavily labeled than the striatum. Beta-R binding sites are distributed through the full width of the neocortex but are still more abundant in the subplate than in the cortical plate. By P4, with the emergence of the definitive neocortical laminar pattern, the supragranular layers are for the first time more heavily labeled than the infragranular layers.

From P10, the adult pattern of beta-R distribution is established. Beta-R concentration is highest in the pallidum, high in the striatum and neocortical laminae I,II and III, and moderate in neocortical layer VI and in the pyriform cortex. The concentration of beta-R is low in neocortical layers IV and V as well as in the septal areas. No binding is observed within fiber bundles.

These observations demonstrate that beta-1 adrenoceptors, like opiate receptors (Kent et al. Dev. Br. Res. 2:487-504, 1982), develop early in the forebrain. Biochemical studies in rodents have demonstrated the presence of these receptors no earlier than the postnatal period. Beta-R appear concurrently and are codistributed in the developing cortex with the noradrenergic projection arising in the locus coeruleus. The functional significance of the early development of this system is unknown. Supported by NIH Grants F05 TWO 3375 and NS12005.

69.3 CIRCADIAN RHYTHM IN α_1-ADRENERGIC RECEPTORS IN DISCRETE BRAIN REGIONS. Marian S. Kafka and Marco A. Benedito*. NIMH, Bethesda, MD 20205.

Specific binding of [^{3}H]-prazosin to membranes from discrete regions of the rat brain was used to investigate whether there were circadian rhythms in the α_1-adrenergic receptor. Two groups of rats, entrained to a light:dark cycle (lights on from 7AM to 7 PM) for 18-21 days, were orbitally enucleated 3 days apart. 2-3 days after enucleation, 12 rats from a group were sacrificed every 4 hours over a 24-hour period. In each group, the brains of 12 rats, removed, sliced, frozen and stored at -20°C, were dissected on dry ice into discrete regions. The regions from 3 rats were pooled and kept frozen for the measurement of specific binding. The data from the two groups, not statistically different from one another, were pooled.

There was a circadian rhythm in α_1-adrenergic receptor binding in 12 of 13 regions measured: the olfactory bulb, frontal, cingulate, piriform, parietal, temporal, and occipital cortex, the hypothalamus, hippocampus, pons-medulla, caudate-putamen, and the thalamus-septum. The cerebellum was arhythmic.

Alpha$_1$-receptors can stimulate the production of both cAMP and phosphatidyl inositol. Moreover, a circadian rhythm in the α_1-receptor has been shown to induce a circadian rhythm in NE-stimulated cAMP production in cortical slices (Neurosci Abst 8: 660, 1982). Perhaps through cAMP, phosphatidyl inositol, or other second messengers, a circadian rhythm in α_1-receptors alters adrenergic transmission and modulates the body's responses to environmental events across the day.

69.4 [^{3}H]RX781094: AUTORADIOGRAPHIC EVIDENCE FOR AN INTERACTION AT ALPHA-2 BINDING SITES IN THE RAT BRAIN. A. Myers, S.B. Goehring* and J.R. Unnerstall, Dept. Neurosci., Johns Hopkins Univ. Sch/Med, Balto., MD 21205

RX781094 is an imidazoline analog that has been shown to be a potent and selective antagonist of physiologic responses mediated through alpha-2 adrenergic receptors (Doxey et al., Br. J. Pharmacol. 78:489, 1983; Hannah et al., NS Arch. Pharmacol. 322:221, 1983). The tritiated compound ([^{3}H]RX) has been utilized to label alpha-2 binding sites in brain homogenates (Howlett et al., Br. J. Pharmacol. 74:294P, 1981; Pimoule et al., Eur. J. Pharmacol. 95:79, 1983). In this report, we will present data from receptor autoradiographic experiments which indicate that [^{3}H]RX labels alpha-2 binding sites in intact tissue sections of the rat brain.

[^{3}H]RX (23.5 Ci/mmol) was prepared by tritium-exchange. The conditions utilized to label binding sites in 8 um sections of the rat brain were determined in preliminary biochemical experiments on slide-mounted tissue sections. For autoradiography, sections were incubated in Krebs PO_4 buffer containing 2 nM [^{3}H]RX. Tritium-sensitive film was exposed to the labeled sections for 12 weeks. Serial sections were labeled with the partial agonist [^{3}H]para-aminoclonidine ([^{3}H]PAC, 25 nM). Nonspecific binding was determined in the presence of 10 uM phentolamine.

[^{3}H]RX labeled a single class of high-affinity binding sites in the intact brain sections (K_D = 2.4 $\pm$ 0.3nM, B_{max} = 46.9 $\pm$ 4.2 fmoles/2-10 um striatal sections). Displacement of [^{3}H]RX binding by various compounds revealed that this ligand has a pharmacology appropriate to the alpha-2 receptor. The distribution of binding sites in the rat brain labeled with [^{3}H]RX (44% occupancy) was identical to that seen when using [^{3}H]PAC under conditions which saturate the high-affinity agonist site and label over 50% of the low-affinity sites. High levels of [^{3}H]RX binding were seen in the septum; neo-, insular and entorhinal cortex; bed nucleus of the stria terminalis; amygdala; dorsal and medial thalamus; medial hypothalamus; locus coeruleus; dorsal-medial medulla; and dorsal and lateral horns of the spinal cord.

These data indicate that [^{3}H]RX labels the alpha-2 receptor and suggest that this compound can be a useful antagonist ligand for the biochemical and anatomical analysis of this receptor. (Supported by USPHS grants MH25951, DA00266, MH00053 & and the McKnight Foundation. [^{3}H]RX was a gift from Reckitt & Colman, Great Britain.)

69.5 [^{3}H]-PRAZOSIN BINDING SITES IN RAT NEOCORTEX: ROLE OF DISULFIDE AND SULFHYDRIL GROUPS. **R. Brière*, L. Grondin* and T.A. Reader.** Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec H3C 3J7, Canada.

The tritiated alpha-1 antagonist Prazosin ([^{3}H]-PRZ) binds specifically and with high affinity to postsynaptic adrenoceptors, in membrane preparations from cerebral cortex. Since adrenoceptors are of protein nature, it was of interest to investigate the possible role of disulfide (-SS-) and sulfhydril (-SH) groups in the binding of [^{3}H]-PRZ. Pretreatments of the membrane preparations with the disulfide reactives DL-Dithiothreitol (DTT), L-Dithiothreitol (L-DTT), Dithioerythritol (DTE) or 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB), alone or in combination with the alkylating agent N-Methylmaleimide (NMM), decreased specific [^{3}H]-PRZ binding, with only minor changes in the non-specific counts. Saturation experiments with [^{3}H]-PRZ performed after preincubating the membranes with the reagents, showed that after DTE and DTT treatments the affinity decreased, as revealed by increases in the dissociation constants (K_d 25°C), but with no changes in the maximum binding capacity (B_{max}). The alkylating agent NMM decreased both the affinity and the binding capacity, unless it followed a first treatment with DTT (DTT + NMM), in which case the effects on specific binding were only on the K_d and similar to those observed with DTT alone. The agent DTNB reduced the affinity as much as DTT, but in addition decreased B_{max}, probably due to its oxydizing properties. Finally, protection experiments were performed by incubating the membrane preparations first with the ligand and thereafter, once equilibrium was reached, the binding was perturbated by adding the reagents L-DTT or NMM. These perturbation experiments indicate that it is the "active" binding site of the receptor protein which is affected by these reagents. Based on the present experimental results, it can now be proposed that the active binding site for [^{3}H]-PRZ requires the integrity of both -SS- and -SH groups.

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69.6 ALPHA$_1$-ADRENERGIC RECEPTORS IN RAT CNS: CORRELATION BETWEEN AUTORADIOGRAPHY AND MEMBRANE BINDING OF [^{125}I]-HEAT. L.S. Jones, G.D. Miller*, L.L. Gauger*, and J.N. Davis, Neur. Res. Lab., VA Med. Cent., Durham. NC 27705 and Dept. of Med. (Neur.) and Pharm., Duke Univ., Durham, NC 27710.

We have recently used [^{125}I]-HEAT to visualize alpha$_1$-adrenergic receptors with in vitro autoradiography (Jones et al., Eur. J. Pharm. '83). The results show that the receptors are present in a distinct, heterogeneous distribution that largely reflects the known noradrenergic innervation of the CNS. Based on these results, eleven regions were dissected from rat brain and binding studies on the homogenized membranes prepared from each region were performed. The number of binding sites and the dissociation constants were calculated for each region and the results compared to the relative optical densities that were measured in the corresponding regions of autoradiographs.

The results indicate that, where the autoradiographs showed homogeneous binding, the correlation with the membrane binding results was very good. The thalamus, for example, has high, homogeneous binding, and this is the area with the highest B_{max} (120 $\pm$ 7 fmol/mg protein) as measured by membrane binding. Also, regions with homogeneously low levels of binding, such as the quadrigeminal plate, contain a correspondingly low number of receptors in the membrane binding work. However, in much of the brain, the alpha$_1$-adrenergic receptors are heterogeneously distributed. The cerebellum has moderate binding in the molecular layer, but virtually no binding in the granular layer; the membrane binding results can only reflect some average of these varying receptor densities. This is also true for the olfactory bulb, where the external plexiform layer has the highest autoradiographic density seen in the entire brain, yet the binding studies showed only average numbers of receptors overall, and for the frontoparietal cortex, where there is dense laminar binding in layers Va and Vc that cannot be detected in the membrane binding studies.

This work demonstrates the value of in vitro autoradiography with [^{125}I]-HEAT for localizing the anatomical distribution of alpha$_1$-adrenergic receptors. This work also shows that the autoradiographic results are corroborated by the membrane binding studies, with the anatomical specificity of the one complementing the quantitative precision of the other.

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69.7 DIFFERENTIAL AUTORADIOGRAPHIC DISTRIBUTIONS OF THE α_2-ADRENOCEPTOR LIGANDS [^{3}H]RAUWOLSCINE AND [^{3}H]CLONIDINE IN RODENT BRAIN. C.L. Boyajian*, R.P. Burgoon*, D.B. Sternberg, S.E. Loughlin, and F.M. Leslie. Department of Pharmacology, University of California, Irvine, CA 92717.

Rauwolscine has previously been classified as a selective α_2-adrenoceptor antagonist (Perry, D.B. and U'Prichard, D.C., Eur. J. Pharmacol., 76:461). We have thus utilized [^{3}H]rauwolscine to determine the autoradiographic distribution of α_2-adrenoceptors in mouse and rat brain tissue sections. Cryostat sections (20μm) were incubated in [^{3}H]rauwolscine (0.5nM), in the absence and presence of phentolamine (1 μM) to determine specific binding. Radioligand binding was then visualized by exposure of tissue sections to tritium sensitive film. Highest autoradiographic grain densities were observed in the olfactory nuclei, olfactory tubercles, caudate-putamen, nucleus accumbens, and posterolateral nucleus of the amygdala. This distribution did not correspond closely with known regions of noradrenergic innervation. We have thus examined the localization of [^{3}H]-clonidine (1.5nM), an α_2-adrenoceptor agonist. The binding of this radioligand did correspond well with the terminations of noradrenergic fibers. These areas included the olfactory bulbs and nuclei, septum, hippocampus, amygdala, entorhinal cortex, and locus coeruleus-subcoeruleus. The distinct distributions of the two α_2-adrenoceptor ligands were largely non-overlapping, such that the localization pattern of [^{3}H]clonidine was not merely a subset of that of [^{3}H]rauwolscine. Two different hypotheses may explain this differential localization. Since the pattern of [^{3}H]rauwolscine binding parallels that of the dopamine system, this ligand may have higher affinity for dopamine receptors than for α_2-adrenoceptors. This is consistent with recent evidence suggesting that rauwolscine possesses antidopaminergic activity (Scatton, B. et al., Eur. J. Pharmacol., 86:427, 1983). Alternatively, [^{3}H]rauwolscine and [^{3}H]-clonidine may differentially localize antagonist and agonist states, respectively, of the α_2-adrenoceptor. These alternative hypotheses are currently being examined.

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69.8 **HUMAN LOCUS COERULEUS AUTORADIOGRAPHY: α_2-ADRENERGIC AND OPIATE RECEPTORS.** G.N. Ko, J.R. Unnerstall*, H.H. Holcomb, J.E. Kleinman and M.J. Kuhar*. National Institute of Mental Health, Wash., D.C. and Bethesda, Md.; and *Johns Hopkins University School of Medicine, Baltimore, Md.

In this report we have examined α_2-adrenergic and opiate receptors utilizing an in vitro autoradiographic technique in 18 post mortem brains. Unfixed pontine material was obtained from the Washington D.C. Medical Examiner's Office, cut and mounted on brass microtome chucks with brain paste, and in the cryostat, coronal sections of 16 micron thickness were thaw mounted on subbed microscope slides and allowed to air dry at room temperature before being stored. [^{3}H]para-aminoclonidine (PAC, 40 Ci/mmol) was used to label α_2-adrenergic binding sites in the mounted tissue sections (Unnerstall et al., Brain Res. Rev., in press). Briefly, sections were preincubated for 30 minutes at room temperature in Krebs-phosphate buffer (pH 7.4 at 25°C) containing 100 μM Na^+-GTP. Adjacent sections were then washed for 10 minutes at room temperature in the incubation buffer alone (0.17 M Tris HCl, pH 7.6 at 25°C plus 10 mM $MgCl_2$). Sections were then incubated for 60 minutes at room temperature in buffer containing 0.25 nM [^{3}H]PAC, 25 nM [^{3}H]PAC and 0.70 nM [^{3}H]dihydromorphine (70 Ci/mmole), the former in order to assess both the high and low affinity α_2 sites and the latter for opiate receptor sites. Non-specific binding was assessed utilizing the above ligands in the presence of 10-5 M phentolamine and 10^{-6}M levelorphan, respectively. After a 10 minute wash at 4°C in incubation buffer and a rinse in cold deionized water, the sections were dried under a stream of cooled dried air. Standard curves were constructed from 16 micron sections of brain paste containing varying concentrations of [^{3}H]ornithine so as to allow for conversion of optical density measurements to receptor concentrations. 'Labeled tissue was apposed to tritium sensitive film, and after exposure and development of films, they were scanned by an automated densitometric system allowing each image to be digitized and stored (Goochee et al., Ann. Neurol., 7:359, 1980). These images will be presented along with receptor quantification.

The present study simply corroborates pharmacology which has been known in the animal literature and extends the relevance of this knowledge to the human brain. Hypernoradrenergic states have been found in anxiety, the opiate withdrawal syndrome, depression, schizophrenia and Alzheimer's disease. The quantification of locus coeruleus receptors by this technique should allow for direct assessment of the possibility that these hypernoradrenergic conditions are the result of changes in presynaptic autoreceptor binding site numbers, or that antidepressant medications alter presynaptic receptor numbers.

69.9 BINDING STUDIES OF α_2- ADRENERGIC RECEPTORS IN CULTURED CEREBROVASCULAR SMOOTH MUSCLE CELLS. B. Wroblewska*, M. Spatz and J. Bembry* (SPON: W. D. Lust). Lab. of Neuropathology and Neuroanatomical Sciences, National Institutes of Health, Bethesda, Maryland 20205.

Previous studies concerned with characterization of adrenergic receptors linked to adenylate cyclase (AC in cerebrovascular smooth muscle cell cultures demonstrated the existence of β_2- and α_1- but an absence of α_2- type adrenergic receptors coupled to AC (Wroblewska, Spatz, Merkel and Bembry, Life Sci. 34: 783, 1984). However, both α_1- and α_2-type adrenergic receptors were shown to mediate the central and peripheral vascular contraction. Therefore, we investigated the possibility of α_2- adrenergic receptors' presence by binding studies using ^{3}H clonidine (α_2- adrenergic agonist) as a ligand.

The confluent cerebrovascular smooth muscle cell cultures utilized for these studies were derived from dissociated cerebral microvessels of weanling rats (Spatz, Dobson and Bembry Brain Res. 280: 387, 1983). The bindings sites of ^{3}H clonidine were determined in homogenates of cultured smooth muscles with and without withdrawal of serum used in the feeding solution. The ^{3}H clonidine [spec. act. 66.8 Ci/mmol] binding assays were performed in 50mM Tris-HCl buffer pH 7.6 at 25°C for 30 min., filtered under vacuum through Watman GF/B filters and washed with ice-cold buffer (U' Prichard et al. Mol. Pharm. 13: 454, 1977).

Specific binding sites of ^{3}H clonidine were defined as the excess over blanks taken in the presence of 1μM "cold" clonidine. For the competitive studies differenct concentration of various adrenergic agonists and antagonists were used to displace binding with 4nM ^{3}H-clonidine. The rank order of potency for α- adrenergic agonists and antagonists was clonidine > phentolamine = yohimbine >> phenylylephrine >> prazosin. The IC_{50} for the investigated displacers were: 25nM, 300 nM, 1μM and 9mM respectively. Competition curve produced by competing "cold" for ^{3}H-clonidine (4nM) showed a biphasic pattern indicative of multiple binding sites. Besides, the Scotchard analysis of saturation curve (concentration of ^{3}H-clonidine ranged from 6μM to .3 nM) and dissociation rate were characteristic of multiple population of the α_2- adrenergic binding sites in cultured smooth muscle cells. Thus, the existence of α_2-type adrenergic receptors not linked to AC activity observed in the cerebrovascular smooth muscle cells strongly suggest that their reactivity which is mediated by these receptors might be associated with Ca++ fluxes.

69.10 ALPHA-ADRENERGIC RECEPTOR REGULATION OF BRAIN MEMBRANE Ca^{++}/Mg^{++} ATPase. V. Gandhi* and D.H. Ross. (SPON: S.L. Dalterio). Division of Molecular Pharmacology, The University of Texas Health Science Center, San Antonio, TX 78284.

Ca^{++} translocation in nerve terminals appears to be under multiple regulator influences. Three to five buffer mechanisms exist to influence intracellular Ca^{++}, while the protein calmodulin regulates both intracellular Ca^{++} buffering and Ca^{++}/Mg^{++} ATPase. Mobilization of intracellular Ca^{++} is of major importance for the maintenance of synaptic transmission. Ca^{++}_i is required for a variety of enzymes, phosphorylation and transmitter release. Since α-adrenergic receptors have been implicated in the rise of cytosolic Ca^{++} in liver plasma membrane vesicles and hepatocytes, it was of interest to examine the possibility that adrenergic agents may similarly affect Ca^{++} translocation in brain plasma membranes. We have chosen to examine the agents clonidine, yohimbine, and the transmitter norepinephrine (NE) on Ca^{++}/Mg^{++} ATPase in nerve ending fractions (P_2) isolated from cortex (CX), hippocampus (HP) and cerebellum (CB). NE produced a significant concentration-dependent decrease in enzyme activity in P_2 fractions from CB, CX and HP. Significant saturable inhibition was seen at 10 (24%) and 50 (45%) μM. Clonidine (10 - 100 M) produced inhibition of enzyme activity in both CX and CB, with the CX most sensitive. Yohimbine (5 - 100 μM) produced a slight stimulation (not significant) at higher concentrations. Yohimbine significantly antagonized the inhibition seen with clonidine (10 - 100 μM) when used in equimolar concentrations. The results suggest that presynaptic α receptors may be mediating their autoregulation of transmitter release by preventing the adequate translocation of Ca^{++} from the nerve ending. Rises in cytosolic Ca^{++} may be expected to increase K^+ conductance leading to neuronal inhibition and reduced transmitter release.

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69.11 SUPPRESSION OF ADRENERGIC RESPONSES IN FOOD DEPRIVATION BY POST-RECEPTOR MECHANISMS. C. M. Kuhn, M. K. McMillian, G. E. Evoniuk and S. M. Schanberg. Dept. of Pharmacology, Duke Univ. Med. Ctr., Durham, N. C. 27710.

We have shown previously that 48 hours of food deprivation (FD) of preweanling of young adult rats suppresses liver and heart ornithine decarboxylase (ODC) induction by alpha adrenergic agonists. Beta adrenergic responses are suppressed in heart at all ages, but only in preweanling liver. In the present studies, we demonstrate that the pattern of receptor changes differs from this particular index of responsivity.

FD (48 hours) decreased liver ODC induction by the alpha agonist phenylephrine (PHE) in 16 and 30 day old rats, but potentiated PHE in 90 day old rats. In contrast, liver alpha-1 receptor number as determined by binding of 3H-prazosin was decreased at all ages except 16, which was unchanged. Isoproterenol (ISO) effects on ODC were decreased on day 16, unaffected on day 30 and potentiated at day 90. Liver beta receptors, as evaluated by binding of 125I-pindolol, were increased to varying degrees at all ages. Liver ODC induction by dexamethasone and aminophylline was unaffected except on day 90, when these responses were potentiated. Heart ODC responses to PHE and ISO decreased at all ages after FD. Heart alpha and beta receptors decreased on day 16 and 30, but responsivity changes observed on day 90 were not accompanied by changes in receptor number.

Preliminary studies suggest that carbohydrate may be an important component in regulation of adrenergic responses, as refeeding with carbohydrate alone reversed these changes at all ages.

These findings further demonstrate that nutrition affects adrenergic receptor function. While post-receptor mechanisms seem to mediate these effects altered receptor number is thought to mediate phosphorylase responses in FD rats. These results suggest that a complex interplay of different subcellular mechanisms changes the profile of adrenergic response during FD (and other physiologic states).

69.12 PENETRATION OF BETA-ADRENERGIC AGONISTS AND ANTAGONISTS INTO BRAIN. D.J. Brunswick* and P. Conway, VA Medical Center and Dept. of Psychiatry, Univ. of Pennsylvania, Phila, PA 19104.

There is interest in knowing whether β-adrenergic antagonists or agonists can enter the brain to interact with central β-adrenergic receptors. Previously, we found that after the in vivo administration of (-)^{125}I-iodopindolol (IPIN) to rats, radioactivity measured in different areas of the brain had the characteristics expected if IPIN was binding to β-adrenergic receptors (i.e., stereoselectivity, pharmacological specificity, regional localization, etc.). Consequently, evaluation of the ability of systemically administered β-agonists or antagonists to displace the binding of IPIN in brain would provide an assessment of whether the drugs penetrated into brain and interacted with β-receptors.

Approximately 2uCi IPIN was injected intravenously into male Sprague-Dawley rats (200-250g) five minutes after the injection of either unlabelled β-antagonist, agonist or saline. Two hours later, radioactivity was measured in cortex, cerebellum and brainstem. Data are expressed as the ratio of the radioactive concentration in the cortex or cerebellum to that in the brainstem, since only a small proportion of the radioactivity observed in the brainstem is to β-adrenergic receptors. Thus, variations in these ratios reflect variations in receptor occupancy for cortex or cerebellum. The following drugs caused a significant decrease in the ratio, with the figure in parentheses representing the lowest dose tested that had a significant effect: a) Antagonists - alprenolol (0.1mg/Kg in cortex, 0.03mg/Kg in cerebellum), betaxolol (1mg/Kg in cortex, 30mg/Kg in cerebellum), butoxamine (30mg/Kg in cerebellum, no effect in cortex); b) Agonists - clenbuterol (1.0mg/Kg for cortex, 0.5mg/Kg for cerebellum), prenalterol (6mg/Kg). The following drugs caused no displacement of radioactivity at any dose tested: a) Antagonists - practolol (up to 30mg/Kg) or atenolol (up to 30mg/Kg); b) Agonists - salbutamol (up to 30mg/Kg), salmefamol (up to 30mg/Kg) or dobutamine (up to 10mg/Kg). Drugs that produced no displacement of radioactivity 2 hrs after their administration also did not reduce the concentration of radioactivity when measured 30 min after their administration. These data indicate that measurement of IPIN binding in vivo can provide a measure of the ability of drugs to penetrate into brain and to interact with central β-adrenergic receptors. (Supported by Research Funds from the Veterans Administration and USPHS MH 36761.)

69.13 CONSEQUENCES OF DSP4 ADMINISTRATION ON NORADRENERGIC RECEPTORS ON RAT CORTICAL AND HIPPOCAMPAL MEMBRANES. N.R. Zahniser, R.P. Yasuda*, G.R. Weiner* and T.V. Dunwiddie. Dept. Pharmacology, Univ. Colorado Sch. of Med., Denver, CO 80262.

Partial depletions of rat hippocampal norepinephrine (NE; 73% of control) produced by the noradrenergic neurotoxin DSP4 do not result in either increases in the number of beta-adrenergic receptors or in electrophysiological supersensitivity to beta-adrenergic receptor agonists (Dunwiddie et al., Brain Res. 269:311, 1983). In contrast, we now report that following more complete depletions of NE (>90%), selective increases in the numbers of beta-, but not of alpha-, adrenergic receptors occur in the brain. One week after the administration of DSP4 (50 mg/kg; i.p.), NE levels were decreased from 580±29 to 48±10 ng/g tissue in the cerebral cortex and from 840±75 to 51±8.6 ng/g tissue in the hippocampus, while the levels of dopamine remained unchanged in the striatum (control: 9.8±0.93; DSP4: 10±0.48 ug/g tissue). Accompanying the decreased levels of NE, there were significant increases in the numbers of beta-adrenergic receptors as measured with [125I]-cyanopindolol in membranes prepared from both cortex (control: 61±1.4; DSP4: 74±1.5 fmol/mg protein) and hippocampus (control: 39±1.3; DSP4: 48±1.7 fmol/mg protein). The affinities of these receptors were not altered (cortex control: 2.8±0.20; DSP4: 3.0±0.24; hippocampus control: 5.8±1.1; DSP4: 5.8±0.81 pM). Despite the >90% depletion of NE in these same tissues, however, no changes in the properties of either alpha-1 or alpha-2 adrenergic receptors were detected. Alpha-1 receptors were quantitated using [125I]-BE-2254, and alpha-2 receptors were measured with [3H]-rauwolscine. Other investigators (Zigmond and Stricker, Experientia 36:436, 1980; Hefti et al., Pharmacol. Biochem. Behav. 12:185, 1980) have concluded that 6-hydroxydopamine induces functional postsynaptic changes in the nigrostriatal system only after losses of >80% striatal dopamine content. Our results with DSP4 lesions support a similar conclusion for the beta-adrenergic receptor system in the hippocampus.

This work was supported by grants NS 09199 and AG 04418 (N.R.Z.), GM 07635 (R.P.Y.) and DA 02702 and VA 394463116 (T.V.D.).

69.14 PATTERNS OF CHANGE IN PLASMA PROLACTIN AND α-MSH AND IN ANTERIOR PITUITARY DOPAMINE AND β-ADRENERGIC RECEPTORS FOLLOWING MEDIAN EMINENCE LESIONS IN THE RAT. S.L. Petrovic*, J.C. Bedran De Castro*, O. Khorram* and S.M. McCann (SPON: R. Rosenberg). Department of Physiology, Univ of Tex Hlth Sci Ctr at Dallas, Dallas, Texas 75235

We have measured plasma prolactin (Prl) and α-MSH in male rats subjected to bilateral electrolytic lesions of the median eminence (MEL), and also measured the content of dopamine and of beta-adrenergic receptors in anterior pituitary membranes from animals given MEL 1-14 days prior to sacrifice, or in corresponding sham-lesioned animals. Plasma Prl increased to about three times the level of sham-lesioned animals in 4-8 days following MEL. The lesions also led to an initial depletion of D2-dopamine receptors (days 1-2). However, their density per unit membrane protein increased 4-14 days after MEL, in accord with Libertun et al. (Endocrinology 107, 1905), while decreasing in terms of D2 complement per whole anterior, or per unit of tissue wet weight. α-MSH in plasma had increased to 4-5 times the control levels by 2 days after MEL, and remained essentially unchanged to day 14. β2-adrenergic receptors increased significantly at day 1 and even more at days 2-4, as expressed per either the membrane protein or the unit tissue weight. At 7-14 days after MEL, their density was elevated per unit of membrane protein, but not in overall terms. There was a significant correlation of β-receptor levels with plasma Prl and α-MSH while there was a lesser correlation of dopamine receptor density with these hormones. The elevations in plasma Prl and α-MSH after MEL are probably the result of removal of an inhibitory hypothalamic control. The greater initial rise in α-MSH as compared to Prl after MEL may be related to a greater capacity of the MSH-producing cells than the lactotrophs to increase rapidly their synthesis of MSH as well as its release following the lesions. The increased concentration of receptors in membranes following lesions is presumably an up-regulation as a result of loss of input of dopamine, norepinephrine, and epinephrine following the lesions. Supported by NIH Grant AM-10073 and HD-09988.

69.15 LOCUS COERULEUS α_2-RECEPTOR SENSITIVITY ASSESSED IN TWO DIFFERENT RAT STRAINS: ELECTROPHYSIOLOGICAL AND BIOCHEMICAL CORRELATES. G. Vantini*, J.M. Stolk*, D.C. U'Prichard and E.D. French (SPON: A.M. Wagman). Maryland Psychiatric Res. Center, Nova Pharmaceutical Corporation, Baltimore, MD, 21228

Several lines of evidence suggest that α_2-receptors located in the locus coeruleus (LC) play an important role in controlling the physiological activity of this noradrenergic cell group. It has been suggested that these receptors could mediate the effect of: (a) epinephrine-containing LC afferents; (b) norepinephrine-containing terminals from recurrent axon collaterals of LC neurons; or (c) local noradrenergic autoregulatory dendro-dendritic synapses within the LC. In partial support of hypothesis (a) we recently reported that epinephrine content and PNMT activity in the medulla-pons of Fisher 344 (F344) inbred rats is from 3 to 8 fold higher than that of the Buffalo (Buf) inbred strain, and that these strain-dependent differences in adrenergic neurons are reciprocally related to α-receptor density (Science 221:1297,1983). Moreover, in brain areas devoid of epinephrine no differences in α-receptor number could be found. These data suggest that α-receptors in medulla-pons might be regulated by epinephrine. Since the large difference in α_2-receptors in the medulla-pons of the two strains may be reflected by proportional differences in LC α_2-sites, we evaluated the electrophysiological effects of clonidine, an α_2-agonist, on LC neuronal activity in F344 and Buf rats.

Rats (220-260g) were anesthetized and prepared for extracellular single unit recording within the LC. Clonidine was injected through a jugular catheter. Presumptive LC neurons having a positive-negative action potential with rates ranging from 0.5-4 spikes/sec were found 5.4-6.2 mm below the skull surface; all were found to respond to a noxious stimulus with a short increase in rate followed by a longer period of inactivity. Cumulative dose-response determinations were used to calculate the clonidine IC_{50} for each cell tested. Cells were accepted only after histological verification of the presence of an ejected dye spot within the LC nucleus.

For a given basal firing rate, clonidine more effectively decreased the firing rate of LC neurons of Buf rats compared to its effects in the F344 strain. In both strains there was also an inverse relationship between basal activity and sensitivity to the α_2-agonist. These data coupled with our biochemical findings strongly suggest that epinephrine may play a major role in regulating LC α_2-receptor density. (Supported by NIMH Grant MH32842; JMS holds RSDA MH00018)

69.16 ADRENERGIC RECEPTOR REGULATION IN HYPERINNERVATED NEURONS. Jerome Sutin and Kenneth P. Minneman. Depts. of Anatomy and Pharmacology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

Following neonatal 6-OHDA treatment the motor trigeminal nucleus of the rat shows increased norepinephrine content due to an increased number of aminergic varicosities. This noradrenergic hyperinnervation results in a marked facilitation of the masseteric reflex due to an increase in the amplitude of the EPSP in motoneurons produced by muscle afferent fibers from the mesencephalic trigeminal nucleus. Although the cerebellar cortex receives its noradrenergic innervation from a different group of aminergic neurons, it also becomes hyperinnervated following neurotoxin treatment. We compared the adaptive changes in alpha-1 and beta adrenergic receptors in these two hyperinnervated structures, one composed of cholinergic post-synaptic neurons and the other with GABAergic output cells. Tissue punches from the motor trigeminal nucleus and cerebellar cortex samples were taken from untreated control and littermate neonatal 6-OHDA treated Sprague-Dawley rats. Tissue samples from both sides of the brain were pooled. An aliquot was used to measure NE by HPLC and amperometric detection. The remainder of the sample was used to measure receptor density using ^{125}I-Iodocyanopindolol or ^{125}I-BE 2254 as radioligands. Although NE was increased 195% in the motor trigeminal nucleus there was only a 15% reduction in alpha-1, and 18% decrease in beta-adrenergic receptor density in hyperinnervated animals. The cerebellar cortex showed a 165% increase in NE, but no change in alpha-1 and beta adrenergic receptor density. In the neurotoxin treated animals, NE was depleted in the cerebral cortex and receptor density was increased by 20 to 50%, depending on the region. In a noradrenergic hyperinnervated cranial motor nucleus or the cerebellum there is only a small, or no, down regulation of adrenergic receptors. The lack of a major adaptive change in receptors after a marked increase in transmitter release sites contrasts to the regulatory response following NE denervation. Since physiological studies provide evidence for a tonic hyperpolarization in NE hyperinnervated motor V cells (Vornov and Sutin, Soc. Neurosci. Abst. 1983) it is unlikely that the absence of down regulation is due to reduced activity in adrenergic axons. Supported by NIH grant #NS 14778 and a grant from the Scottish Rite Schizophrenia Research Program.

CATECHOLAMINES: RECEPTORS III

70.1 **DOPAMINE RECEPTORS IN HUMAN RETINA**
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D-2 receptors in human retina were characterized using radioligand binding assays with (^{3}H)-spiroperidol. Nonspecific binding was measured in the presence of 2 μM (+)-butaclamol. At 37^{o}C, the binding of (^{3}H)-spiroperidol was rapid, with an association rate (k_1) of 4.0 X 10^8 $M^{-1}min^{-1}$ and reversible, with a dissociation rate (k_{-1}) of 0.04 min^{-1}. Scatchard analysis of the equilibrium binding of (^{3}H)-spiroperidol (0.01-2 nM) resulted in linear plots and yielded a Kd of 91 pM and a Bmax of 1500 fmol/mg protein. The Kd values determined at equilibrium and by kinetic analysis were in good agreement. Studies of the inhibition of the binding of (^{3}H)-spiroperidol (0.2-0.3 nM) were performed with a number of competing ligands, including domperidone, sulpiride, fluphenazine and (±)-butaclamol. The agonists dopamine and N-propylnorapomorphine were studied in the presence of GTP. In parallel experiments, (+)-butaclamol was approximately 1000 fold more potent than the (-) stereoisomer. The inhibition curve for dopamine was shifted to the right and the Hill coefficient was increased by the addition of 300 μM GTP. This effect was agonist specific and suggests that some of the receptors are coupled to stimulation or inhibition of the enzyme adenylate cyclase. The inhibition curves for most of the competing ligands had Hill coefficients between 0.6 and 0.8, even in the presence of GTP, suggesting that subtypes of the D-2 receptor are present in the retina. Nonlinear regression analysis of untransformed data, using the PROPHET computer system, revealed that these shallow inhibition curves were best explained by the presence of two populations of binding sites; 40% of the sites having a high affinity for dopamine and domperidone and the remaining 60% having a lower affinity for these ligands. The larger population of sites had a higher affinity for sulpiride, fluphenazine and N-propylnorapomorphine. It is not likely that either of these classes of sites represents serotonin receptors since the S-2 antagonist ketanserin had a low affinity for both classes of sites. The characteristics of D-1 receptors in the retina were also investigated using radioligand binding assays with (^{3}H)-α-flupenthixol (0.1-10 nM). Nonspecific binding was measured in the presence of 1 μM fluphenazine. Scatchard analysis of the binding of (^{3}H)-α-flupenthixol resulted in linear plots and yielded a Kd of 2.4 nM and a Bmax of 6000 fmol/mg protein. Since (^{3}H)-α-flupenthixol labels both D-1 and D-2 receptors, the density of D-1 receptors was approximately 4500 fmol/mg protein and the ratio of D-1 to D-2 receptors was 3:1. Thus, the retina appears to be a promising tissue in which to study human dopamine receptors and their subtypes. (Supported by NS 07272, MH 14654, and NS 18591)

70.2 STUDIES OF DOPAMINE RECEPTORS (DAR-1) IN BOVINE RETINA MEMBRANES. E.T. Suen* and S.T. Crooke* (SPON: B. Ho). Dept. of Molecular Pharmacology, Smith, Kline and French Labs, Philadelphia, PA 19101.

The adenylate cyclase linked dopamine receptors (DAR-1) were studied in bovine retina membranes. Two benzodiazepine compounds, SKF 38393 and SKF 82526 were previously reported to be DAR-1 selective agonists which induce renal vasodilation in anesthetized dogs. Both compounds stimulate adenylate cyclase activity with stereospecificity (D-isomer < < L-isomer) in bovine retina membranes, and SKF D82526 is two orders of magnitude more potent than dopamine. SKF 82526- or SKF 38393-stimulated adenylate cyclase activity is inhibited by cis-flupentixol, piflutixol and (+)-butaclamol but not by (-)-butaclamol, phentolamine or propranolol. These results suggest that the increase of adenylate cyclase activity is due to the stimulation of dopamine receptors.

Radioligand binding studies show that [^{3}H]-SKF 82526 labels dopamine receptors with high affinity (K_d 3-5 nM). The specific binding is reversible, stereospecific and saturable (B_{max} 400-450 fmol/mg protein). Piflutixol, (+)-butaclamol and SKF D38393 inhibit [^{3}H]-SKF 82526 binding with IC_{50} values of 2.0×10^{-8}M, 3.1×10^{-8}M, and 3.5×10^{-7}M, respectively. Domperidone and (-)-sulpiride which are specific antagonists of a different dopamine receptor subtype (DAR-2) do not inhibit specific binding with concentration up to 10^{-6}M. Furthermore, SCH 23390, a selective DAR-1 antagonist, was shown to inhibit both dopamine-stimulated adenylate cyclase and [^{3}H]-SKF 82526 binding. These results suggest that the bovine retina DAR-1 labeled by [^{3}H]- SKF-82526 are similar to the dopamine receptors located in striatum and parathyroid gland. This may be a useful model system to study the function and regulation of these receptors.

70.3 [^{125}I]SPIPERONE: BINDING TO D_2 DOPAMINE RECEPTORS IN STRIATAL HOMOGENATES. R.J. Pelchat, A.L. Gundlach, B.L. Largent and S.H. Snyder. Dept. of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

[^{125}I]-Labeled ligands provide unique advantages over their [^{3}H]-labeled counterparts, in terms of increased sensitivity and economy of tissue and reagents. [^{3}H]Spiperone is well characterized as a high affinity radioligand for D_2 dopamine receptors (Seeman, P., Pharmacol. Rev., 32:229, 1980). This report describes the pharmacological characteristics of [^{125}I]spiperone binding to homogenates of rat striatum.

In drug competition studies 25-100 pM [^{125}I]spiperone and various concentrations of drug were incubated with 0.5-1.0 mg wet wt tissue in a final volume of 0.5 ml Tris pH 8.0, 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$ buffer for 10 min at 37°C. Samples were filtered on glass-fiber filters presoaked in 0.5% polyethylenimine and 10 μM spiperone. This treatment substantially reduced binding of [^{125}I]spiperone to the filter and totally eliminated any specific binding (binding displaceable in the presence of 400 nM (+)butaclamol) of ^{125}I-spiperone to the filter. Nonspecific binding was defined as that remaining in the presence of 400 nM (+)butaclamol. Specific binding was routinely 40-60 percent of total binding.

Saturation experiments revealed that [^{125}I]spiperone (25-1500 pM) labels a single population of high affinity sites with an apparent K_D of 340 pM and a B_{max} of 36 pmol/g wet wt (Hill coefficient = 0.99). Stereoselectivity of binding is seen for the isomers of butaclamol with the (+) isomer being 1000 x more potent than the (-) isomer.

Dopamine antagonists such as haloperidol, chlorpromazine, pimozide and sulpiride potently inhibited [^{125}I]spiperone binding (K_i's 1-50 nM). Agonists such as apomorphine, RU24926 and lergotrile are also potent inhibitors of binding (K_i's ~ 100 nM). However, serotonergic antagonists such as ketanserin and cinanserin and putative D_1 selective ligands such as SKF38393 and SCH23390 are relatively weak competitors for [^{125}I]spiperone binding (K_i's 1- > 10 μM).

These studies reveal similarities of drug potencies at [^{125}I]- and [^{3}H]-spiperone labeled sites in striatum and suggest that [^{125}I]spiperone is a sensitive and useful ligand for labeling D_2 dopamine receptors.

70.4 EFFECT OF REPEATED ADMINISTRATION OF APOMORPHINE AND BROMOCRIPTINE ON CIRCLING AND ^{3}H-SPIROPERIDOL BINDING IN RATS WITH A UNILATERAL LESION OF THE NIGROSTRIATAL PATHWAY. C. Rouillard*, P. Deshaies*, P.J. Bédard, R. Boucher, P. Falardeau* and T. Di Paolo. Lab. Neurobiology, Dept. Anatomy and Dept of Molecular Endocrinology, Univ. Laval, Québec, Canada, G1K 7P4.

The effect of repeated administration of apomorphine 0.35mg/kg s.c. or bromocriptine 10mg/kg I.P. was studied behaviorally (circling) and biochemically (^{3}H-Spiroperidol binding) in rats bearing a unilateral lesion of the nigrostriatal pathway performed with 6-OHDA. Starting at least a month after surgery, both dopamine agonists were administered eight times to different groups, the injections being separated by forty eight hours. A progressive and significant increase in contraversive circling was seen with both drugs. Similar groups were run in paralell and received only the first and last injection of agonist. No increase in circling was seen. In rats with a lesion of the entopeduncular nucleus no increase in ipsiversive circling was seen after eight injections of apomorphine. Such lesions involve the output system of the striopallidum and denervation supersensitivity is not expected. All animals were sacrificed three days after the last dose of agonist and ^{3}H-Spiroperidol binding to crude striatal membrane preparations was studied.

Apomorphine had little effect on binding in the intact striatum but on the side of the 6-OHDA lesion there was a significant increase in Bmax in animals having been exposed to eight versus two injections of apomorphine. In bromocriptine treated rats on the other hand the main findings was a significant reduction in Bmax on the intact side in the animals exposed to eight injections. The present finding therefore suggest that although both agonists induced an increase in contraversive circling, they do so by different mechanisms, apomorphine apparently further increasing the sensitivity of dopamine receptors on the denervated side and bromocriptine on the contrary "desensitizing" the intact side. (Supported by MRC of Canada).

70.5 NIGROSTRIATAL STIMULATION INCREASES IN VIVO [H-3] SPIPERONE BINDING. D.C. Chugani*, J.R. Barrio* and M.E. Phelps. Departments of Pharmacology and Radiological Sciences, Division of Biophysics, UCLA School of Medicine, Los Angeles, CA 90024.

The quantitative measurement of neuroreceptors in humans with PET and high affinity ligands requires an understanding of those critical variables determining in vivo ligand deposition. In order to determine whether endogenous dopamine can compete with [H-3]spiperone ([H-3]SP) binding in vivo in rat striatum, we performed unilateral nigrostriatal stimulations using parameters which have been reported to increase striatal dopamine release and looked for left/right differences in striatal accumulation of [H-3]SP. Bipolar electrodes were stereotaxically implanted bilaterally in substantia nigra compacta (AP 3.1 mm, Lateral 2.0 mm, Vertical - 7.2 mm, incisor bar - 2.4 mm) one week prior to experiments. Unilateral stimulations (400 μAmps, 30 Hz, 0.5 msec duration) were begun 10 min prior to [H-3]SP (250 μCi/kg) bolus intravenous injection and continued for 70 min. Rats were sacrificed 1 hr after the injection, and the brains were rapidly removed. Left and right striata were dissected, homogenized in 10 volumes ethanol, and 50 μl aliquots were counted. Electrode placements were confirmed histologically. Stimulation produced ipsilateral turning in rats with proper electrode placement which continued for the entire experiment but decreased in intensity with time. In rats which displayed ipsilateral turning, an increased [H-3]SP accumulation (ipsilateral striatum: 324±1.4 dpm/mg±SEM; contralaterial striatum: 286±3.2 dpm/mg±SEM, n = 3) on the side of the stimulation was observed. These results do not confirm our hypothesis that endogenous dopamine can compete with [H-3]SP bindng in striatum. However, these results demonstrate that increasing the firing rate of substantia nigra compacta neurons can alter the accumulation of [H-3]SP in striatum.

70.6 THIORIDAZINE IS LESS POTENT THAN ITS METABOLITES AT RAT STRIATAL DOPAMINE RECEPTORS. D. M. Niedzwiecki*, L.X. Cubeddu and R.B. Mailman. Departments of Pharmacology and Psychiatry and the Biological Sciences Research Center, University of North Carolina School of Medicine, Chapel Hill, N.C. 27514.

The relative in vitro potencies of the phenothiazine antipsychotic thioridazine (THD) and two of its major metabolites, thioridazine-2-sulfoxide [mesoridazine (MES)] and thioridazine-2-sulfone [sulforidazine (SUL)] at rabbit striatal dopamine receptors were estimated. We determined how these drugs affected the binding of [^{3}H]-spiperone to crude striatal membrane preparations (B_{max}=13 pmol/g tissue; K_d=0.13 nM). THD, MES, and SUL appeared to bind competitively to the [^{3}H]-spiperone labelled sites. The IC_{50} for THD was 51.4 nM. Both metabolites were significantly more potent than the parent compound in the binding assay (MES 8 fold; SUL 20 fold). The comparative potencies of these compounds was determined on dopamine (DA) receptors which modulate the electrically-evoked release of [^{3}H]-DA and [^{14}C]-acetylcholine (ACh) from slices of rabbit striatum. Apomorphine [APO (30 nM)] inhibited DA overflow by 70% and ACh overflow by 53%. The inhibitory effects of APO on DA overflow were competitively antagonized by THD, MES, and SUL (IC_{50}s: THD 130 nM; MES 14 nM; SUL 6 nM). Endogenous DA also inhibited DA release. The DA neuronal uptake inhibitor nomifensine (10 μM) enhanced the inhibitory effect of endogenous DA on transmitter release. THD, MES, or SUL reversed this inhibition in a competitive fashion (IC_{50}s: THD 363 nM; MES 46 nM; SUL 15 nM). MES and SUL were equipotent in antagonzing the inhibitory effect of APO and of endogenous DA on ACh overflow (IC_{50}s: MES 17 nM, SUL 25 nM vs. APO; MES 48 nM, SUL 54 nM vs. endogenous DA). THD, however, was at least 70 times less potent than its metabolites in both paradigms. Independently of whether we tested the effects of the three drugs at displacing [^{3}H]-spiperone, at blocking presynaptic DA receptors modulating DA release, or at post-synaptic receptors modulating ACh release, THD was always less potent than its metabolites. These results suggest that the metabolites may play an important role in the actions of thioridazine.

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70.7 DIFFERENTIAL INACTIVATION OF SOLUBILIZED DOPAMINE (DA) RECEPTOR SUBTYPES: EFFECT ON MODULATION BY GTP.
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We have investigated the effects of the irreversible DA antagonist N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (Hamblin and Creese, Life Sci. 32:2247-2255, 1983) on the binding of ^{3}H-spiroperidol (^{3}H-Spi) to CHAPSO solubilized striatal DA receptors. The administration of EEDQ to rats (6 mg/kg, i.p. 24 hrs prior to decapitation of the animals) results in a 75-85% decrease in specific ^{3}H-Spi binding to solubilized DA receptors. Pretreatment of the rats with the D-2 DA antagonist (+)sulpiride (200 mg/kg 3 hrs prior to EEDQ) protects approximately 80%, while pretreatment with the D-1 antagonist SCH 23390 (3 mg/kg, i.p. 30 min prior to EEDQ) only protects approximately 20% of the solubilized DA receptors from inactivation by EEDQ. Thus, pretreatment with (+)sulpiride selectively protects the D-2, while SCH 23390 selectively protects the D-1 DA receptors from inactivation by EEDQ. To determine whether the modulation of DA receptor binding by GTP is associated with the D-2 solubilized DA receptors, we investigated the effects of the nucleotide on the inhibitory potency of apomorphine (Apo) in displacing the binding of ^{3}H-Spi. The affinity of Apo in displacing ^{3}H-Spi from solubilized DA receptors was decreased by GTP to the same extent in the untreated as in the (+)sulpiride plus EEDQ treated animals. These results suggest that the GTP binding protein is still linked with the D-2 DA receptors in the CHAPSO solubilized preparations. Studies supported by Grants NIMH 02717 and NINCDS 06801.

70.8 LATERAL-TO-MEDIAL GRADIENT OF DOPAMINE (D-2) RECEPTORS IN THE STRIATUM: ANALYSIS AND FURTHER CHARACTERIZATION.
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In previous work from this laboratory, the quantification of brain receptor autoradiographs has been improved by using a computerized image analyzer to "linearize" a digitized image, so that the gray value of each picture element (pixel) is a linear function of the fmoles of ^{3}H-ligand bound/mg tissue protein (Altar et al., J. Neurosci. Meth., In Press). This procedure has revealed a previously unsuspected lateral-to-medial gradient of D-2 dopamine (DA) receptors in the rat caudate-putamen (CP; idem). The present study was undertaken to characterize further this gradient and to begin analyzing the basis for it.

Adjacent coronal sections of the rat forebrain were incubated with various concentrations of ^{3}H-spiroperodol or ^{3}H-spiroperidol plus 1 μM (+)butaclamol, and sections were exposed for 3 wks to ^{3}H-sensitive film to produce autoradiographs of total and nonspecific binding, respectively. The image processor digitized and averaged 64 successive video frames, and the gray value of each pixel was converted to a linear function of ^{3}H-spiroperidol concentration, using a calibration curve obtained from ^{3}H-containing standards. Saturation analysis revealed a significant 2-fold lateral-to-medial CP gradient in the density (B_{max}) of D-2 sites, while no dorsoventral gradient was observed. No regional variations in the affinity (K_D) for the radioligand were found. We confirmed this approximate 2-fold difference in the density of D-2 sites between lateral and medial caudate-putamen using saturation analysis of ^{3}H-spiroperidol binding to synaptic membranes derived from the medial or lateral regions of the CP.

Regional variations in D-2 density do not correspond to differences in the concentration of DA or its metabolites in these same regions. Using HPLC to measure DA, HVA, and DOPAC in dissected striatal regions, we found a ventral-to-dorsal gradient, but no lateral-to-medial differences. Thus, the DA receptor of the D-2 class is not distributed in precise register with the density of DA innervation of the caudate putamen.
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70.9 STRIATAL DOPAMINE TRANSMISSION: EFFECTS OF SELECTIVE BLOCKADE OF D-1 RECEPTORS BY SCH 23390.
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Much of the knowledge on striatal dopamine (DA) receptor function derives from the study of behavioural, electrophysiological and biochemical responses to the acute administration of antipsychotic drugs. Thus, haloperidol and other neuroleptics cause catalepsy, accelerate the firing rate of substantia nigra DA (SN-DA)neurons , enhance DA synthesis and activate soluble tyrosine hydroxylase (TH) activity. Although the majority of these drugs can block both D-1 and D-2 receptors, these responses are generally considered to result from the blockade of striatal D-2 sites, located either pre or postsynaptically. Accordingly, sulpiride, a selective D-2 receptor antagonist, activates SN-DA neurons and soluble TH activity, like classical antipsychotics. In the present study, we investigated whether selective blockade of D-1 receptors induced by SCH 23390 could elicit the same effects on DA transmission as haloperidol. In rats, the intraperitoneal injection of SCH 23390 caused catalepsy of dose-dependent intensity and duration, with maximal effect at 5 mg/kg. At this dose, SCH 23390 elicited a marked increase of the firing rate of SN-DA neurons, observed with a single unit recording method. This increase (approximately 100%) was of the same magnitude as that produced by 2 mg/kg of haloperidol, which failed to enhance SCH 23390-induced stimulation. Contrary to haloperidol, SCH 23390 failed to increase the affinity of soluble TH for the pteridine cofactor and caused only a modest increase (30%) in L-DOPA accumulation following NSD 1015. These results indicate that the blockade of postsynaptic D-1 receptors can generate behavioural and electrophysiological responses similar to those elicited by classic neuroleptics. The failure of SCH 23390 to fully activate DA synthesis despite the increased firing rate of SN-DA neurons suggests that the presynaptic D-2 mediated inhibitory control on DA synthesis remains operative. Such presynaptic control seems to prevail over the effect of enhanced firing.

70.10 D-2 DOPAMINE RECEPTOR MEDIATED INHIBITION OF ADENYLATE CYCLASE ACTIVITY IN RAT STRIATUM.
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Striatal membranes contain two classes of dopamine (DA) receptors, named D-1 and D-2. Stimulation of D-1 receptors is associated with enhanced adenylate cyclase (ac) activity, whereas D-2 receptors are considered to play no role in the regulation of striatal ac. The present study indicates that activation of D-2 receptors is linked to the inhibition of ac in rat striatum. To prevent D-1 activation of ac, striatal synaptic membranes were incubated in the presence of 0.1 μM SCH 23390, a selective blocker of D-1 receptors. The reaction mixture also contained 100 mM NaCl and 50 μM GTP. Under these conditions, DA inhibited striatal ac in a concentration-dependent manner (IC_{50}=3.5 μM). The DA inhibition was completely antagonized by 5 μM l-sulpiride, a selective D-2 receptor blocker. Like DA, (-)-apomorphine and (-)-propylnorapomorphine inhibited ac activity with IC_{50} values of 0.2 and 0.04 μM, respectively. Maximal inhibition elicited by the different DA agonists corrisponded to a 17-22% decrease of basal enzyme activity. In the absence of SCH 23390, a similar degree of inhibition was induced by Ly 171555, a selective D-2 agonist, with an IC_{50} of 3.5 μM. The inhibitory effect of Ly-171555 was observed only at concentrations of GTP>1 μM and was enhanced by NaCl. In the absence of SCH 23390, and in the presence of increasing concentrations of GTP, DA maximally stimulated striatal ac at 1 μM GTP. At higher concentrations of the nucleotide, the DA-stimulated enzyme activity decreased. This decline was antagonized by l-sulpiride (5 μM) but not by d-sulpiride. Thus, at concentrations of GTP higher than 1 μM, the net response of ac to DA appears to be the result of a balance between D-1 stimulation and D-2 inhibition of the enzyme activity. Blockade of D-1 receptors discloses the D-2 mediated inhibition, while blockade of D-2 receptors potentiates the D-1 stimulation of ac. As already shown for other inhibitory transmitters, the D-2 mediated inhibition of striatal ac was associated with the stimulation of a membrane-bound high affinity GTPase.

70.11 PROPERTIES OF SULPIRIDE DISPLACEABLE ^{3}H-SPIPERONE BINDING IN NUCLEUS ACCUMBENS AFTER CHRONIC TREATMENT WITH ANTIPSYCHOTIC DRUGS. R. Strong, F.J. White, M.M. Voigt, G. Wood and R.Y. Wang. Depts. of Pharmacol. and Int. Med., St. Louis Univ. Sch. of Med., and VA Med. Ctr., St. Louis, MO 63125.

Several studies have shown that ^{3}H-spiperone binds to more than one type of receptor. Recently, it was reported that ^{3}H-spiperone labels a single class of receptors in rat neostriatum if S-sulpiride is used to define specific binding. That class of receptors was shown to be identical to sites labelled by ^{3}H-sulpiride (Zahniser et al., JPET 227, 592-599, 1983) and resemble the D_2 dopamine (DA) receptor. In the present work, we used S-sulpiride displaceable ^{3}H-spiperone binding to study the properties of this receptor site in the nucleus accumbens (NAc). Furthermore, we compared the effects of chronic treatment with "classical" (Haloperidol, HAL) and "atypical" (clozapine, CLZ) antipsychotic drugs (APD's) on this class of receptors in both NAc and neostriatum (STM).

The STM and NAc were dissected on ice from brains of Sprague Dawley rats. Membranes were prepared with a Tekmar Tissuemizer, centrifuged and washed three times. Tissue was incubated for 30 min. at 37°C in the presence of ten concentrations of ^{3}H-spiperone (0.008-4 nM) and, in half of the tubes, 10 µM S-sulpiride.

Saturation data from NAc were consistent with a single site model with B_{max}=263 fmoles/ng prot and K_D=70 pM. This is consistent with the results of Zahniser et al. using striatal tissue.

We have shown previously that classical and atypical APD's exert differential effects on A9 and A10 dopamine neurons, after chronic administration (White and Wang, Science, 271, 1054-1057, 1983). Classical APD's decreased the number of spontaneously active DA neurons in both A9 and A10 while atypical drugs selectively affected A10 DA neurons. To correlate the differential effects of HAL and CLZ on A9 and A10 DA systems with APD-induced DA receptor supersensitivity, rats were treated for 4 weeks with saline, HAL or CLZ and binding was assessed in NAc and STM using six concentrations of ^{3}H-spiperone (0.12-1nM)±10 µM S-sulpiride. Scatchard analysis revealed a 30-50% increase in B_{max} in both NAc and STM after HAL but not CLZ. There was no alteration in K_D in either region by chronic treatment. (Supported by USPHS grants MH-34424, MH-38794, MH-00378, MH-08886, the Scottish Rite Schizophrenic Research Program N.M.J., USA and the Veterans Administration.)

70.12 THE CLOSE RELATIONSHIP BETWEEN PRE- AND POST-SYNAPTIC DOPAMINE (DA) RECEPTORS AS DEMONSTRATED BY DOPAMINERGIC AGONISTS FROM TWO HOMOLOGUE SERIES

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Recent work from our laboratory on S(-)-3-(3-hydroxyphenyl)-N-n-propylpiperidine (S(-)-3-PPP)[1] has revealed an interesting relationship between pre- and postsynaptic DA receptors. The results from these studies were rationalized by Carlsson[2] in terms of differences in the balance between affinity and intrinsic activity of this compound on these receptors. Furthermore Carlsson states the possibility that the difference between these receptor types might be a matter of receptor conformation, differently developed from the same origin due to different receptor occupancy during a certain period of time.

The present work describes how the same conclusion of a close relationship between the pre- and the postsynaptic DA receptors can be made from a structure-activity point of view by using two homologue series of isomeric, monophenolic trans-1,2,3,4,4a,5,6,10b-octahydrobenzo(f)quinolines (trans-7-OH- and 9-OH-OHB(f)Q). The compounds were tested pharmacologically for their effects on pre- and postsynaptic DA receptors, using biochemical and behavioral methods.

The results reveal that the same basic dopaminergic structure can exhibit a continuously increasing postsynaptic stimulation by increasing the lipophilic interaction in a defined area of the receptor, as represented by a DA receptor model recently presented by Wikström[3].

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70.13 CENTRAL DOPAMINERGIC PROPERTIES OF THE ENANTIOMERS OF CIS-5-HYDROXY-1-METHYL-2-(DI-N-PROPYLAMINO)TETRALIN

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The central dopaminergic (DA) actions of the (+)- and (-)- enantiomers of cis-5-hydroxy-1-methyl-2-(di-n-propylamino)teralin,(UH-242), have been investigated using biochemical and behavioural models. The (-)-enantiomer dose dependently decreased the DA synthesis rate (DOPA formation) and DOPAC/HVA levels in rat brain. This was also the case when animals were pretreated with reserpine or γ-butyrolactone (GBL). Furthermore, the (-)-enantiomer produced biphasic changes in the locomotor activity i.e. reduction and stimulation at low and high doses, respectively. The drug was also able to antagonise reserpine-induced akinesia.

In contrast, the (+)-enantiomer dose dependently increased DOPA formation and DOPAC/HVA levels. Although the drug failed to influence the GBL-induced increase in DOPA formation, it antagonised the action of apomorphine. In the behavioural models, the (+)-enantiomer clearly reduced apomorphine and d-amphetamine-induced hyperactivity. Surprisingly, the locomotor activity of non-pretreated rats was only slightly reduced at very high doses of the drug.

In summary, these results indicate that the (-)-enantiomer has a classical DA agonist profile i.e. stimulating both DA auto- and postsynaptic receptors. In contrast, the (+)-enantiomer possesses antagonist properties at these sites, although its profile appears to differ from classical DA antagonists.

70.14 CENTRAL DOPAMINERGIC ACTIONS OF (+)- AND (-)-3-PPP IN RELATION TO PREVIOUS SYNAPTIC ACTIVITY.

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Recently, we have detailed the neurochemical and behavioural properties of the novel dopaminergic agent 3-(3-hydroxyphenyl)-N-n-propylpiperidine, 3-PPP, and its enantiomers (for rev. see Hjorth, Acta Physiol. Scand., suppl. 517, 1, 1983). While (+)-3-PPP appears to act as a full dopaminergic agonist in most experimental paradigms, the action of its (-)-counterpart is more complex. Thus, (-)-3-PPP displays either full agonist, partial agonist or weak antagonist effects depending on the test model applied. Carlsson (J. Neural Transm., 57, 309, 1983) has recently proposed that the degree of previous agonist occupancy determines dopamine (DA) receptor responsiveness, and thereby in part the intrinsic activity of agents interacting with the receptors. In an attempt to gain further insight into these issues we have studied the effects of (+)- and (-)-3-PPP (and apomorphine) in vivo upon central DA-receptor (autoreceptor) mediated synthesis control at different time intervals after disruption of synaptic activity by means of GBL or reserpine.

Although potency-wise differing, (+)-3-PPP and apomorphine showed full agonist intrinsic activity (IA=100) in all experimental conditions tried. (-)-3-PPP on the other hand, exhibited only partial agonist IA (=60-70) after short-term interruption of synaptic DA transmission. However, with intermediate-term reserpine pretreatment (-)-3-PPP was shifted to a full agonist at central DA-synthesis controlling autoreceptors - its IA (=90-100) approaching that of (+)-3-PPP and apomorphine. Prolonging the interruption of synaptic activity also slightly, though consistently, increased the DA synthesis-reducing potency for all three compounds. The findings indicate that although already highly responsive, the sensitivity of DA autoreceptors can be further enhanced, thus suggesting their likely being influenced by an, albeit low, endogenous tone under physiological conditions.

The data support the proposal by Carlsson (1983) that the IA of compounds like (-)-3-PPP is a possible function of the adaptive state of the DA receptors involved, in turn related to previous agonist occupancy. The 3-PPP enantiomers provide a unique array of tools to explore central DA receptor responsiveness under normal, physiological as well as under conditions involving short- or long-term alterations in synaptic activity.

Supported by "Magnus Bergvalls Stiftelse", "Åke Wibergs Stiftelse and the Swedish MRC (grant no. 155). D.C. was the recipient of a fellowship from the S.E.R.C. (U.K.).

70.15 **INTERACTIONS OF PARTIAL AGONISTS WITH β-ADRENERGIC RECEPTORS OF C6 GLIOMA CELLS.** **K.A. Neve, D.A. Barrett and P.B. Molinoff, Dept. of Pharmacology, Univ. of Pennsylvania, Sch. of Medicine, Phila., PA 19104.**

Agonists at β-adrenergic receptors stimulate adenylate cyclase activity, and the consequent increase in cAMP levels is responsible for many of the physiological effects of these agents. There are, however, compounds that have intrinsic sympathomimetic activity, but do not stimulate adenylate cyclase. Thus, pindolol and celiprolol, drugs with intrinsic sympathomimetic activity, fail to elevate adenylate cyclase activity in membranes prepared from various tissues, including L6 myoblasts and human lymphocytes (Mol. Pharm. 24:398-408, 1983; unpublished). The interactions of agonists and antagonists with β-adrenergic receptors on the BU1 subclone of C6 glioma cells were studied. These cells have been reported to have both β-1 and β-2 receptors (Mol. Pharm. 20:463-469, 1981). Scatchard analysis of the binding of ^{125}I-iodopindolol (^{125}I-IPIN) to membranes resulted in linear plots with Kd and Bmax values of approximately 80 pM and 85 fmoles/mg protein, respectively. Nonspecific binding, defined in the presence of 50 uM (-)-isoproterenol, was low, less than 10% of total binding at a concentration of the radioligand close to the Kd. Inhibition of the binding of ^{125}I-IPIN by agonists and antagonists was analyzed by nonlinear regression analysis, using the PROPHET computer system. The presence of both β-1 and β-2 receptors in C6 cells was confirmed using the antagonists ICI 89.406 (β-1 selective) and ICI 118.551 (β-2 selective). Inhibition of ^{125}I-IPIN binding by either drug indicated that β-1 receptors comprise approximately 60% of the adrenergic receptors on these cells. Analysis of the inhibition curves for isoproterenol and pindolol showed that the β-1 and β-2 receptors had similar affinities for these ligands. In contrast, inhibition curves for zinterol, a β-2-selective ligand, and celiprolol, a β-1-selective ligand, both indicated the presence of 2 sites. GTP (100 uM) had little effect on inhibition of binding by pindolol and celiprolol, but markedly altered inhibition by the agonists isoproterenol and zinterol. The dose-response curves for the latter two drugs were shifted to the right by GTP, and the Hill coefficients were concomitantly increased. These results are consistent with the conclusion that the agonist activity of pindolol and celiprolol is not mediated by changes in adenylate cyclase activity. Incubation of C6 cells in the presence of isoproterenol for 24 h induced a pronounced decrease in the density of β-adrenergic receptors. Pindolol and celiprolol also decreased the density of receptors, although the magnitude of the decrease was less than that caused by isoproterenol. (NS 18479)

70.16 EFFECT OF AN ANALOG OF LUTEINIZING HORMONE-RELEASING HORMONE ON DOPAMINE RECEPTORS IN PROLACTIN-SECRETING TUMORS 7315a AND MtTW15. T. Di Paolo, P. Falardeau* and M. Daigle*, Department of Molecular Endocrinology, Laval University Hospital Center, Ste-Foy, Québec G1V 4G2, Canada.

We investigated the effects of [Des-Gly^{10}]-[D-Tryp^{6}, Pro^{9}-ethylamide]-LHRH, an agonistic analog of luteinizing hormone-releasing hormone (LHRH) on the growth of the prolactin-secreting tumor 7315a in female Buffalo rats and MtTW15 in female Wistar-Furth rats. These tumors, which are estrogen-dependent, contain dopamine receptors indistinguishable from the dopamine receptors characterized in the normal anterior pituitary gland. However, unlike the normal response of the anterior pituitary gland, the 7315a and MtTW15 cells are refractory to dopaminergic inhibition of prolactin release. Chronic administration of [Des-Gly^{10}]-[D-Tryp^{6}, Pro^{9}-ethylamide]-LHRH with a dose of 2 μg/day or 10 μg/day starting the day after the inoculation with the tumor inhibited the growth of the pituitary tumors. The Buffalo rats were sacrificed twenty-four days and the Wistar-Furth forty-four days after inoculation. The tumor, ovary and uterus weights were significantly reduced in treated animals. Plasma prolactin and estradiol concentrations were lower in rats treated with the LHRH analog (at 10 μg/day). The density of dopamine receptors (expressed per mg of tissue or per mg of protein) as assessed by [^{3}H]-spiperone binding is significantly increased in the tumor of treated Buffalo (260%) and Wistar-Furth (170%) rats compared to the tumor of animals receiving the vehicle alone while the affinity is unchanged. However, the number of dopamine receptors per tumor is not significantly changed with the LHRH analog treatment. The mechanism by which chronic administration of an agonistic LHRH analog can inhibit the growth of estrogen-dependent pituitary tumor 7315a and MtTW15 in rats appears to be related to a suppression of sex steroid levels. Dopamine receptor density is increased in the tumors with this treatment indicating that the inhibiting effect of the LHRH analog may also involve dopamine receptors. (Supported by the National Cancer Institute of Canada).

70.17 THE FUNCTIONAL STATE OF THE DOPAMINE RECEPTOR IN THE ANTERIOR PITUITARY IS THE HIGH-AFFINITY FORM. P. Falardeau*, T. Di Paolo, F. Labrie*, S. George, M. Watanabe* and P. Seeman (Spon: G. Pelletier), Department of Molecular Endocrinology, Laval University Hospital Center, Ste-Foy, Quebec G1V 4G2, Canada and Department of Pharmacology, University of Toronto, Toronto M5S 1A8, Ontario, Canada.

This study was done in order to determine whether it was the high-affinity state or the low-affinity state of the dopamine receptor which mediated the inhibition of release of prolactin by dopamine agonists. 30 dopaminergic agonists and 30 antagonists were tested for their potencies to inhibit the binding of ^{3}H-spiperone to porcine anterior pituitary tissue, and for their potencies to affect the release of prolactin from rat anterior pituitary cells in culture. Prolactin release-inhibiting activity was evaluated in vitro after a 4-hour incubation of a drug with rat anterior pituitary cells in primary culture; prolactin in the medium was then measured by a specific radioimmunoassay. All agonists (except bromocryptine, ergocryptine and dehydroergocryptine) inhibited ^{3}H-spiperone binding in two phases: one phase occurred at very low concentrations (representing the high-affinity state of the dopamine receptor, D_2^{high}) and the other phase occurred at high concentrations of agonist (D_2^{low}). The dissociation constants (K) for each drug at each state were derived by computer, using the programme LIGAND. It was observed that the agonists K values for the high-affinity state were approximately identical to those drug concentrations affecting prolactin release; the K values for D_2^{low} were about 2 orders higher. ^{3}H-apomorphine was also shown to bind to the high-affinity state of the dopamine receptor in the anterior pituitary and K values for agonists and antagonists are also approximately identical to those affecting prolactin release. These data suggest that the high-affinity state of the anterior pituitary dopamine receptor is the functional state which mediates the inhibition of prolactin release.

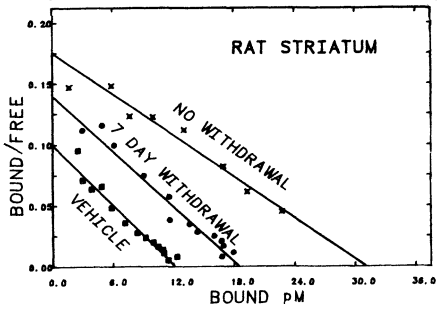
70.18 SELECTIVE BLOCKADE OF D-1 RECEPTORS BY SCH23390 EVIDENTIATES SEDATION AND SLEEP INDUCED BY HIGH DOSES OF APOMORPHINE. M.L. Porceddu*, M. Collu*, G. Mereu*, M. Serra*, E. Ongini*, G. Biggio and G.L. GESSA*, Institute of Biology, Chair of Pharmacology and Institute of Pharmacology, University of Cagliari, Italy.

Apomorphine and other dopamine receptor stimulants have a biphasic effect on behaviour in rodents: low doses produce sedation and decrease locomotor activity, high doses produce hyperactivity and stereotypy (Fog, R., Psychopharmacology, 14:299 1969; Di Chiara et al., Nature 264:564,1976). In rats, such behavioural changes are associated with consistent changes in the EEG pattern, in that low doses of apomorphine produce a marked synchronization while high doses produce EEG activation (Kafi, S. and Gaillard, J.M., Europ. J. Pharmacol., 38, 357,1976). A high dose of apomorphine (1 mg/kg s.c.) produced stereotypy associated with EEG desynchronization. At the dose of 1 mg/kg i.p., SCH 23390 decreased motor activity but failed to alter the EEG pattern. The administration of either the low or high dose of apomorphine to SCH 23390-treated rats elicited a marked sedative response associated with EEG synchronization. The EEG synchronization produced by apomorphine (50 μg/kg) in SCH 23390-treated rats was prevented by (-)sulpiride (25 mg/kg i.p.), a D-2 receptor blocker. It is concluded that, by preventing the excitatory response to apomorphine, SCH 23390 evidentiates the existence of a population of dopamine receptors mediating sedation and sleep.

70.PO DOPAMINE D_2 RECEPTOR DENSITY INCREASES MARKEDLY, USING CONSTANT INFUSION OF HALOPERIDOL BY OSMOTIC PUMP.
D. Grigoriadis*, S.R. George, M. Watanabe*, and P. Seeman. Dept. of Pharmacology, University of Toronto, CANADA, M5S 1A8.

The density of striatal D_2 dopamine receptors generally increases by 40% following long-term treatment with neuroleptics. Differences in the literature on the magnitude of this increase arise from the various conditions used (dose of neuroleptic; routeof administration; drug withdrawal time).

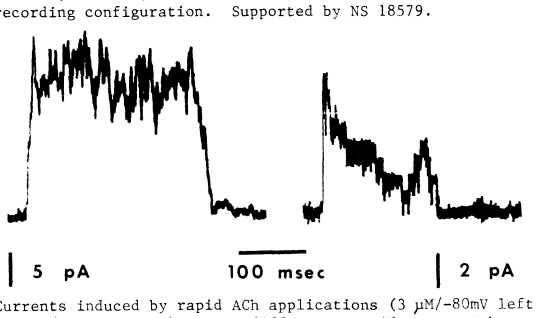
Using the constant infusion method, we now find a much greater increase in density with 100 µg/Kg/hr haloperidol. Under general anaesthesia, male Sprague-Dawley rats were implanted s.c. with an osmotic pump (Alzet) containing haloperidol (in 0.4M tartaric acid) or vehicle alone. The pumps were removed after 21 days. The Fig. shows that chronic haloperidol treatment had no effect on the affinity of the receptors for ^{3}H-spiperone. The vehicle-treated animals had a receptor density of 13 pM. The density measured after 21 days of treatment (no withdrawal) was 31 pM, a 130% increase over a vehicle-treated group. Seven days after removal of the pumps, the density was 18 pM which represents the typical 40% increase in receptor density generally seen by others. Thus, a constant small infusion of haloperidol maximally increases the receptor density when no withdrawal period is allowed. Agonist competition of ^{3}H-spiperone binding revealed the same proportion of high- and low-affinity states of D_2 in treated and untreated striata.
(Supported by Ontario Mental Health Foundation and MRC).



MEMBRANE BIOPHYSICS II

71.1 IMPROVED "CONCENTRATION CLAMP" FOR USE WITH MEMBRANE PATCHES
RS Brett, JP Dilger*, PR Adams. Depts of Neurobiology and Behavior and Anesthesiology, SUNY at Stony Brook, NY 11794.

Techniques for perfusion of membrane patches with solutions of varying composition differ in simplicity, speed of application and wash-off, and degree to which the remaining cells in culture are exposed to test solution. Yellen (Nature 296:357) brought excised patches near the end of a tube from which test solution flowed. Although simple, this method does not offer rapid changes between solutions and may contaminate the remaining cells. Fenwick et al. (J Physiol 331:599) brought patches near a hole in a tube through which test solution flowed. Abrupt occlusion of the distal tube flushed test solution over the patch. Application was fast (perhaps 100 ms), but wash-off slow (2 s). We introduced an "isolated flow" technique in which an excised patch was inserted into a hole in a tube (Biophys J 45:386a). A 3-way valve was used to switch between control and test solutions. Application and wash-off were fast (100 ms). We have now modified this method using a pinch valve (Neptune Research Inc., Maplewood, NJ 07040) and a "Y"-shaped conduit. The hole lies 4 mm downstream from the junction; the solution velocity within the tubing is 100 cm/s. This method yields very rapid concentration changes (10 ms; see figure) and avoids contamination of other cells by the test solution. It lends itself well to precisely timed repetitive applications for ensemble variance measurements. Unlike other methods, however, it cannot be used in the whole-cell recording configuration. Supported by NS 18579.



Currents induced by rapid ACh applications (3 µM/-80mV left, 100 µM/-50 mV right) to two different outside-out patches from BC3H1 cells.

71.2 A SIMPLE METHOD FOR MAKING INTRACELLULAR ION-SENSITIVE MICROELECTRODES FOR USE WITH EXCITABLE MAMMALIAN CELLS.
W.G. Carlini, M. Borrelli* and B.R. Ransom. Dept. Neurol., Stanford Univ. Sch. of Med., Stanford, CA 94305.

We have developed a quick and simple method for making intracellular ion-sensitive microelectrodes. Double-barreled glass stock containing filaments (O.D.-1.0 mm, I.D.-0.5 mm) was cut into 88 mm lengths, one barrel on each end was broken back by 5 mm and the prepared blank was pulled to a tip diameter of < 0.5 µm. The shank of the long barrel was injected with about 1 µl of tri-N-butylchlorosilane in CCl_4 (concentration varied between 0.5 to 1.5%, depending on tip size and type of ion exchange resin). The silane filled pipette was placed on a 280°C hot plate for 5 min. A fine glass needle (tip about 5 µm drawn by hand from hematocrit capillaries) was used to add about 1 µl of exchange resin to the silanized barrel. An appropriate reference solution was added to the short barrel and the electrode was put aside until no bubbles remained in the resin, at which time an appropriate backing solution was added to the resin barrel. To reduce the resistance of the resin barrel, a fine microelectrode tip filled with backing solution was introduced into the shank to within 100 µm of the tip. Silver chlorided wires were led from each barrel to a $10^{15}\,\Omega$ input impedance electrometer. The time necessary to manufacture several electrodes was generally not more than 30 min. The resistance of the ion-sensing barrel was dependent upon the type of resin used; K^+ having the least resistance (about $14\times10^9\,\Omega$) and Na^+ the most (> $50\times10^9\,\Omega$). K^+-sensitive microelectrodes have been tested extensively thus far. About 75% of these were sensitive and responsive enough (i.e. slope for 10-fold change in $[K^+]_o$ > 45 mV and half response times < 100 msec, respectively) for intracellular use. Using such electrodes we have measured intracellular K^+ activities in a variety of excitable mammalian cells maintained in culture including murine spinal neurons (108 ± 20 mM), murine DRG cells (124 ± 9 mM), human neuroblastoma (104 ± 17 mM), and murine skeletal muscle (99 ± 11 mM). The method described here for making intracellular ion-sensitive electrodes is reliable and quite simple. The concentration and heat parameters necessary to produce highly reliable K^+ electrodes have been completely explored and we are currently in the process of determining the precise silane concentration, duration of heating, etc. necessary to make reliable electrodes sensitive to Ca^{++}, Cl^-, Na^+ and H^+. Supported by NIH grants NS 15589 and NS 00473 from the NINCDS and MSTP support to WGC.

71.3 MONITORING MEMBRANE POTENTIALS IN NEUROBLASTOMA X GLIOMA NG-108 CELLS WITH A FLUORESCENT DYE. D. Cavalla*, W. J. Wojcik and N. H. Neff. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

Undifferentiated NG-108-15 cells accumulated the fluorescent cyanine dye DiO-C_5-(3) to a degree dependent on their membrane potential. With a dye concentration of 25 nM, the fluorescence level increased with hyperpolarizing agents and decreased with depolarizing agents; the new equilibrium levels were established within 10 min. Thus 1 μM of valinomycin (a potassium ionophore) increased fluorescence by 24%. Potassium reversed this effect (i.e. depolarized) in a dose-dependent manner. The calcium ionophore A23187 (1 μM) increased fluorescence by 3.5%. Lasalocid (X-537A) is another calcium ionophore but is not so selective: potassium and sodium are also transported. At a concentration of 10 μM, this agent elicited a rapid, 20% increase in fluorescence, followed by a return to the base level within 20 min. Gramicidin (1 μg/ml), a channel-forming quasi-ionophore, decreased fluorescence by 15%. Veratridine (50 μM) which opens sodium channels, had no effect on fluorescence with these cells which have been previously reported to lack such channels. None of these agents had any effect on fluorescence after lysis of the cells by sonication. No noticeable toxic effects of the dye on the cells were observed.

71.4 NON-UNIFORM DISTRIBUTION OF SPECIFIC MEMBRANE RESISTIVITY IN CULTURED MOUSE VENTRAL HORN NEURONS. P.B.Guthrie and G.L.Westbrook. Lab Developmental Neurobiology, NICHD/NIH, Bethesda, MD 20205.

The passive electrical structure of a neuron influences the processing of synaptic inputs, and is influenced in turn by the location and activity of those synaptic inputs. We have combined frequency-domain (A.C.) analysis with morphological reconstruction and compartmental modeling to determine the spatial distribution of passive membrane properties.

Whole-cell patch voltage recordings (KCl patch solution, $pCa^{++}=8$) were made from mouse ventral horn neurons after 14-21d in culture. The recording medium contained 1uM TTX to suppress electrical activity; however, some spontaneous low amplitude synaptic activity was still present. The complex impedance of the neuron (0.1-500 Hz) was measured using a computer generated, multi-component sinusoidal stimulus current; small stimulus currents (0.02-0.2nA P-P) minimized changes in voltage-dependent channel activation during stimulation. At rest potential, input impedances ranged from 30 to 150 megohms. Neurons were subsequently filled with Lucifer Yellow, fixed and photographed for morphological reconstruction.

The simulation program NEUROS was used for compartmental modeling of the reconstructed neurons. To reduce lumping errors inherent in compartmental modeling, a large number (200-500) of short (usually<0.05 length constant) compartments were used.

None of the neurons (n=8) could be adequately modeled using uniform membrane properties. In all cases, the fit of the model was best with a high membrane resistivity at the soma, which decreased towards the distal dendritic tips. The somal resistivity was up to eight times that of the distal membrane. Similar results were obtained with a patch solution $pCa^{++}=7$, suggesting that internal perfusion of the soma by EGTA from the patch electrode did not account for the non-uniformity.

We are currently investigating several possible explanations for this observed resistivity gradient, including: 1) spontaneously releasing synaptic terminals on the distal processes; 2)high concentrations of open, voltage-sensitive channels in the distal processes; and 3)a high degree of membrane infolding in the distal processes, increasing the true membrane surface area without affecting the light-microscopic measurement of surface area.

71.5 A CABLE MODEL REPRESENTING MAUTHNER'S CELL AS A SPINDLE. W. D. Crank* (SPON: M. Wood). Div. Neurobiology, Dept. Physiology, SUNY, Buffalo, NY 14214.

To conveniently calculate passive voltage spread on a fusiform cell without grossly misrepresenting the cell's shape, a cable model for spindles has been developed. Defining radius, r, of a figure of revolution about an axis, x, by $r(x) = (\text{constant})\cdot(p^2 - x^2)$ defines a spindle between $x = \pm p$. With such a shape assigned to the cable in one-dimensional core conductor cable theory, a partial differential equation for passive voltage spread along a spindle cable has been derived. The solution has temporal behavior given by exponential functions and spatial behavior given by Legendre polynomial derived functions, which are easy to evaluate. Therefore, computation of spindle voltages is straightforward.

With certain qualifications, the goldfish Mauthner cell can be represented as a spindle. The lateral dendrite is represented by one limb of the spindle; the ventral dendrite is represented by the other limb; and the soma is represented by the thick central region. Fine dendrites which branch from this spindle are neglected, as is the axon. Using physical dimensions, membrane specific capacitance, and specific resistances appropriate for the Mauthner cell, the spindle solutions for $t\cdot\exp(-a\cdot t)$ current inputs have been evaluated on an Apple II plus microcomputer. The voltage magnitudes and waveforms computed for Mauthner spindles are compared to magnitudes and waveforms computed for comparable cylinders. Differences between the two arise from the greater opposition to current flow imposed by the narrowed cable near the spindle terminations.

This cable model adds the spindle to the brief list of cell shapes having convenient solutions for passive voltage spread.

Supported by NIH Grant #EY03470.

71.6 CALCIUM INJECTION IN GIANT NEURON OF APLYSIA CAUSES TRIPHASIC, PROLONGED CURRENT RESPONSE. D.V. Lewis, C. Evans* and W.A. Wilson*. Depts. of Pharmacol. and Peds., Duke University Medical Center, Durham, NC 27710.

Intracellular injections of calcium into neurons have been used to help determine the effects of physiological calcium influx on membrane conductances. Early reports suggested a monophasic effect of calcium, i.e. the activation of a calcium dependant outward potassium current (Meech, R.W., Comp.Biochem.Physiol., 42A:493, 1972). More recent studies suggest a biphasic response consisting of an early transient inward current followed by a prolonged outward potassium current (Hofmeier, G. and Lux, H.D., Pflugers Arch. 391:242, 1981). Here we will describe a triphasic membrane current response to intracellular calcium. We have iontophoresed calcium into the giant, ordinarily silent or non-pacemaking neuron R2 of Aplysia californica. With the neuron held in voltage clamp at resting potential (-40 to -50 mV) the response to calcium injection can be quite complex.

The majority (two-thirds) of neurons respond to iontophoresis of intracellular calcium with a triphasic, outward, inward and finally outward current response. The initial, phase I, outward current rises during the iontophoresis and then falls to pre-iontophoretic baseline within 5-10 sec after termination of calcium injection. The current then becomes inward with the peak of the phase II inward current occurring 10-20 sec after cessation of calcium injection. Next there is a very slow outward shift of the membrane current to the peak of the phase III, or slow outward phase, occurring 50 to 100 sec after the cessation of calcium influx. Often the current does not return to baseline for several minutes. Preliminary experiments suggest that the phase I current reverses at -50 to -60 mV and phase II at -35 mV, reversal potential for phase III has not yet been determined. Preliminary results also suggest phase II current is insensitive to removal of extracellular sodium or calcium but is blocked by manganese. Experiments are in progress to further characterize these currents. These phase I outward and phase II inward currents may correspond to the previously described calcium activated potassium current and calcium activated inward current respectively (Hofmeier, G. and Lux, H.D., Ibid). The phase III current could represent a novel prolonged effect of calcium on membrane channels, a prolonged phase of the usual calcium activated potassium current, or a manifestation of the previously described ultra slow outward potassium current found in R2 (Zbicz, K.L., and Wilson, W.A., J.Pharmacol.Exp.Ther. 217:222, 1981).

71.7 THEORETICAL DEPENDENCE OF TETANIC HYPERPOLARIZATION ON THE INTEGRITY OF A PUMP CONDUCTANCE IN THE PRESENCE OF CYANIDE. Gordon M. Schoepfle, J. T Tarvin*, and R. M. Martin. Neurosciences Program, Dept. of Psychiatry, University of Alabama Med. Ctr., Birmingham, AL 35294 and Dept. of Physics, University of Mississippi, Oxford, MS 38677.

An ATP driven ion translocation mechanism operating under equilibrium conditions generates an electromotive force E_p which may be formulated as

$$E_p = 3(RT/F)\ln[(Na)_o/(Na)_i] - 2(RT/F)\ln[(K)_o/(K)_i] + [\Delta H_o - T\Delta S_o^o]/F + (RT/F)\ln[1/(H_2O)] + (RT/F)\ln[x^2/(a_o - x)] \quad \text{---------- (1)}$$

(Schoepfle et al., Bull. Math. Biol.,45:1013, 1983). Here a_o represents the maximum level of ATP and x, the corresponding level of either (ADP) or (P_i) after attainment of equilibrium. All concentration dependent terms are introduced as mole fractions. The change in membrane potential ΔV_m at the end of a tetanic interval is

$$\Delta V_m = -(RT/F)(g_{Na} + 3g_p)\Delta(Na)_i/[G(Na)_i] + (RT/F)(g_K - 2g_p)\Delta(Na)_i/[G(K)_i] \quad \text{-------- (2)}$$

where $[(Na)_i + (K)_i]$ is constant and G is $[g_{Na} + g_K + g_{Cl} + g_p)$. Here g_p is a pump conductance. $\Delta(Na)_i$ represents sodium loading. Equation (2) follows from the vanishing of total current density J_m such that on neglecting the CdV_m/dt term

$$J_m = g_{Na}(V_m - E_{Na}) + g_K(V_m - E_K) + g_{Cl}(V_m - E_{Cl}) + g_p(V_m - E_p) = 0 \quad \text{---------- (3)}$$

If now in the steady state designated by (3), the term a_o and hence x assume very small values, the equilibrium represented by (1) may still be valid. However, in the presence of cyanide, a_o may eventually fall to such an extent as to preclude the possibility of an equilibrium within the translocation mechanism proper. In such an event, ion translocation ceases, g_p vanishes, and equation (1) becomes invalid. The g_p terms of (2) and (3) disappear, thereby reducing the magnitude of ΔV_m.

71.8 **SODIUM-23 NMR STUDIES OF SODIUM TRANSPORT IN MOUSE NEUROBLASTOMA CELLS.** **N.R. Shochet, C.S. Springer Jr., and I. Spector*, Departments of Chemistry and Anatomical Sciences(*), State University of New York at Stony Brook.**

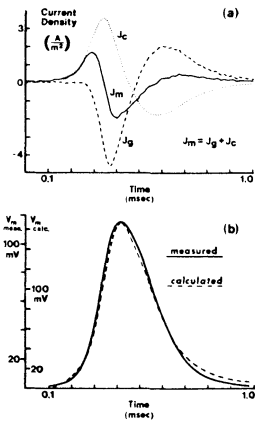
Among the techniques presently available to detect changes in cytosolic sodium ($[Na]_i$), sodium-23 NMR spectroscopy has the advantages of using the naturally abundant stable sodium isotope, being non-invasive and allowing the separation of the $[Na]_i$ signal from that of the extracellular sodium ($[Na]_o$) with the use of shift reagents. This technique presents the unique opportunity to continuously measure changes in $[Na]_i$ resulting from entry through different transport systems and to relate this entry to different cellular functions. The complexity and heterogeneity of nervous tissue constitute major obstacles for the application of the NMR technique to the study of Na transport in such preparations. The low $[Na]_i$ is another obstacle in attempts to detect chenges in $[Na]_i$ by NMR under physiological conditions. To overcome these problems we are using clonal lines derived from the C-1300 mouse neuroblastoma. These lines provide a suitable preparation for NMR studies because large quantities of homogeneous cells necessary for such studies can readily be obtained, and the growth characteristics of these cells can be manipulated under controlled conditions.

The initial experiments reported here were performed at 79.4 MHz using cells of the clonal line N1E-115. 10^8-10^9 cells/ml were suspended in a 10mm O.D. NMR tube in a physiological medium. The separation of the $[Na]_i$ resonance from that of the $[Na]_o$ was achieved by the addition of the membrane impermeable, anionic shift reagent Dy(III)-bis-tripolyphosphate to the medium and spectra were generated by accumulating 128 free induction decays with an acquisition time of 256 msec each. The $[Na]_i$ peak area reached a value of 5-10% of that of the total observed Na, and it increased slightly when the cells were treated with batrachotoxin + scorpion toxin, neurotoxins which cause persistant activation of the voltage-gated Na channel. While the results demonstrate the feasibility of studying ion transport in nerve cells with NMR, the small intensity of the $[Na]_i$ resonance under our experimental conditions did not allow a rigorous analysis of time dependent changes in $[Na]_i$. Current efforts are aimed at improving the quantification of this resonance, e.g., by the use of spectral supression pulse sequences.

71.9 EXTRACELLULAR MAGNETIC MEASUREMENTS TO DETERMINE THE TRANSMEMBRANE ACTION POTENTIAL AND THE MEMBRANE CONDUCTION CURRENT IN A SINGLE GIANT AXON[1]. B.J. Roth* and J.P. Wikswo, Jr., Dept. of Physics, Vanderbilt Univ., Nashville, TN 37235.

We have developed a volume conductor model to calculate the transmembrane action potential V_m of a nerve axon from measurements of the extracellular magnetic field B_θ. V_m is related to B_θ by Maxwell's and Laplace's equations, whose solution in cylindrical coordinates contains Bessel functions and Fourier integrals, which can be calculated using the FFT. The form of the solution allows the forward calculation of B_θ from V_m, and, more importantly, the inverse calculation of V_m from B_θ. The measured magnetic field can also be used to calculate other quantities, such as the total (J_m), capacitive (J_c), and conduction (J_g) current densities in the membrane, as shown in (a) of the figure.

A crayfish medial giant axon was used to test the model. V_m was measured with a glass microelectrode, and B_θ was recorded by threading the nerve through a wire-wound ferrite toroid connected to a special low-noise amplifier. A comparison of theory and experiment is shown in (b). The difference in the scales is due soley to uncertainty in the intracellular conductivity and the axon radius; the model has no free parameters. Sources of systematic error, such as the averaging of B_θ over the toroid cross-section, the finite size of the bath, the effects of the nerve bundle on the signal from the single axon, and the effect of the toroid on the external current were either negligible or were included in the model.



This study demonstrates tnat extracellular magnetic measurements are useful in studying single axons and nerve bundles, particularly when microelectrodes are impractical due to axon size, specimen motion, or the need for a recording that is stable without adjustment for many hours.

[1]Supported by NINCDS, ONR and the Vanderbilt Research Council.

71.10 RELATIVE PERMEABILITIES OF GAP JUNCTIONS TO SYMMETRICAL TETRA-ALKYLAMMONIUM IONS. V. Verselis*, D.C. Spray, R.L. White*, and M.V.L. Bennett. (SPON: P.Brink) Dept. Neuroscience, Albert Einstein Coll. Med., Bronx, N.Y. 10461.

The passage of a series of spherical cations of the same charge but differing size is being examined between amphibian blastomeres. Previous evidence indicates that electrical and molecular coupling in the preparation are both mediated by voltage dependent gap junctions that close in the presence of moderate transjunctional voltages of either sign. To determine concentrations, ion sensitive electrodes were used filled with classic K sensitive liquid ion exchange resin (Corning 477317) which displayed a high selectivity over K for the symmetrical tetraalkylammonium ions, methyl (TMA), ethyl (TEA), propyl (TPA) and butyl (TBA), and a useful range of 0.01 to 100 mM. The ion sensitive electrodes had a second KCl-filled barrel for measuring the reference voltage and were calibrated in standard solutions of alkylammonium ions in 100mM KCl before and after each experiment. Junctional conductance (g_j) was calculated simultaneously by passing current through a second electrode in each cell, determining input and transfer resistances, and solving the pi-tee transform. Junctional permeability can be calculated from cell volumes and change in concentration over time and correlated with junctional conductance. One cell of a coupled pair was rapidly injected with the probe molecule of interest through a fifth electrode that was briefly inserted into the cell. The permeant probes then equilibrated in the two cells. Both TMA and TEA were permeant, but TPA and TBA were not. The time constant of equilibration was as short as 10 min and was shorter for higher junctional conductances and for TMA as compared to TEA. The ionic diameters of TMA, TEA, TPA and TBA are 6.6, 9.3, 11.8 and 13Å respectively. The restricted permeability to TPA and TBA and the relatively greater permeability of TMA as compared to TEA implies that the effective channel diameter is quite accurately defined by these molecules at a value between 9 and 12 Å.

72.1 A MODEL OF THE TONOTOPIC ORGANIZATION OF THE VENTRAL NUCLEUS (V) OF THE MEDIAL GENICULATE BODY AND LATERAL PART OF THE POSTERIOR GROUP OF THALAMIC NUCLEI (Po) IN THE CAT. T.J. Imig and A. Morel*. Department of Physiology, University of Kansas Medical Center, Kansas City, KS 66103.

Best frequencies and minimum response latencies of single units and clusters of units to tone burst stimulation were recorded at 100 μm intervals along vertical electrode penetrations through V and Po of eight barbiturate anesthetized cats. V and Po were identified physiologically as regions containing narrowly-tuned, short-latency (<40 ms) responses, and a tonotopic organization. Best frequencies obtained from several electrode penetrations in the same transverse plane in one brain were used to construct a two dimensional best frequency map. Maps were obtained at different caudorostral levels through V and Po. A three dimensional model of the tonotopic organization of V and Po was constructed from the maps. In V, the tonotopic organization consists of a planar component located rostrally, laterally, and dorsally, and a concentric component located caudally, medially, and ventrally. Low frequencies are represented laterally, and high frequencies are represented medially within the planar component. Within the concentric component, low frequencies are represented in a central column oriented more or less horizontally, which passes through a hole in the middle frequency representation. The representations of a "single" frequency in both the planar and concentric components of the model are contiguous. The planar and concentric components correspond to pars lateralis and pars ovoidea, respectively (D.K. Morest, J. Anat. 99:143-160, 1965). Within Po, a "single" frequency is represented as an irregularly shaped lamina oriented roughly parallel to the transverse plane. High frequencies are represented caudally, and lower frequencies are represented more rostrally within the nucleus. The high frequency representations of V and Po are contiguous. The three dimensional model is consistent with two dimensional tonotopic maps, and the three dimensional organization of arrays of neurons in V and Po labeled by injections of HRP into low, middle, and high frequency representations in auditory cortex. Together, V and Po comprise the tonotopic division of the auditory thalamus in the cat. Supported by NS17220 from NINCDS, and BRSG S07 RR 05373 from NIH.

72.2 NEURONS IN THE TONOTOPIC THALAMUS OF THE CAT ARE TOPOGRAPHICALLY ORGANIZED WITH RESPECT TO THEIR TARGET FIELDS IN AUDITORY CORTEX. A. Morel* and T.J. Imig. (SPON: J.L. Voogt). Department of Physiology, University of Kansas Medical Center, Kansas City, KS 66103.

Projections from the tonotopically organized nuclei of the thalamus (ventral nucleus, V, and lateral posterior complex, Po) upon fields A, AI, P and VP of cat auditory cortex were investigated using retrograde transport tracing techniques. Twenty-one barbiturate anesthetized cats were used for these experiments. Best frequency maps obtained in each brain served as guides for placement of the injections. One tracer (HRP), or two different tracers (HRP and either tritiated bovin serum albumin or nuclear yellow) were injected in the middle frequency representation of one or two separate fields, respectively. Arrays of labeled neurons in the thalamus were related to the cytoarchitecture and shapes of arrays of neurons labeled after HRP injections in the middle frequency representation of all four fields in one experiment. In eight cats, the injection of HRP in the cortex was followed by best frequency mapping of thalamic regions containing labeled cells.

Injections in field VP label neurons in most caudal part of the ventral nucleus, a region where isofrequency contours parallel the ventrolateral border of the MGB. Neurons projecting to field P are also found in the caudal pole of V, but in addition are located in the middle part of the nucleus where labeled cells form a flexed array paralleling the isofrequency contours. Injections in AI label neurons in the middle and rostral parts of V, and in caudal Po. In rostral V, labeled neurons and isofrequency contours are oriented vertically. Field A receives a sparse projection from rostral V and a dense projection from Po that extends further rostrally than the region projecting to AI. In conclusion, there is a caudal to rostral topographic organization of the projections from V-Po to the four fields: VP receives its strongest input from caudal extreme of V, P from its caudal half, AI from its rostral half, and field A, from Po.

Supported by NS17220 (NINCDS) and BRSG S07 RP 05373 from NIH.

72.3 CODING OF NARROW FREQUENCY-MODULATED (FM) RAMPS BY SINGLE NEURONS IN CAT AUDITORY CORTEX. D.P. Phillips*, J.R. Mendelson, and M.S. Cynader (SPON: D.E. Mitchell). Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1

Single neurons in cat primary auditory cortex (AI) display narrow tuning to the frequency of constant-intensity tone stimuli. We have examined the responses of AI cells to continuous tones in which were embedded linear frequency-modulated (FM) ramps of a fixed 2.0 kHz excursion. The center frequency of the ramp was varied in and around a neuron's response area, and the cell's preference for the direction of FM was quantified by comparing its peak firing rates for oppositely-directed, coextensive FM ramps. All data were obtained from anesthetized, muscle-relaxed cats to which tonal stimuli were presented using sealed stimulating systems incorporating calibrated probe microphone assemblies.

AI neurons responded to short FM ramps only if the frequency range covered by the ramp encompassed at least part of a neuron's response area. In addition, the FM stimuli were effective only if the direction of frequency change was towards a cell's best frequency. Thus, preferential responding to upward-directed ramps was limited to those ramps whose frequency excursions invaded the response area from the low frequency side, while sensitivity to down-directed ramps was restricted to those invading the response area from the high frequency side. The strength of a neuron's preference for direction of FM was strictly associated with the gradient of the cell's spike-count-vs-frequency function over the frequency range covered by the ramp. The timing of spike discharges within an FM ramp was explicable in terms of the cell's latent periods for pure tones and the time after the ramp's onset that its instantaneous frequency invaded the response area. These data indicate that sensitivity to the direction of short FM ramps is governed by neural processes local to a cell's response area.

72.4 RESPONSE PATTERNS OF SINGLE AUDITORY CORTICAL NEURONS TO TONE SEQUENCES T. McKenna, D.M. Diamond, J. Pearson, and N.M. Weinberger. Center for the Neurobiology of Learning and Memory, and Dept. of Psychobiology, Univ. of Calif. Irvine, Irvine CA 92717.

The perception of acoustic sequences is an essential feature of acoustic communication. Human perception or recognition of a sequence of tones can be based on attributes such as tonal order, timing, temporal gaps or grouping by similarity. While tone responsiveness is a ubiquitous property of central auditory neurons, there exists little data on cortical neural responses to tone sequences. Based on the psychophysical results one might expect that neural responses to tone sequences would be dependent on the order and timing of the sequence. Furthermore, we previously demonstrated that the discharge of visual cortical neurons in response to sequences of oriented bars exhibited a strong dependence on the rate and order of presentation, and sensitivity to the absence of omitted stimuli (Pearson, et.al.Soc.Neurosci.1983). In order to test the generality of such sensory cortical phenomena we have investigated the responses of single neurons in the primary and secondary auditory cortices of the unanesthetized, neuromuscular blocked cat, to sequences of tones. Sequences of five isointensity tones of different frequencies are employed, all tones of equal duration equally spaced with the entire sequence repeated at the rate of .1 to 1 Hz. for 25 repetitions. Permutations of the tone sequence were presented in which the tone order was altered, a tone omitted, or the tone duration and/or tone spacing was changed. One or more of the following effects were observed in most of the neurons sampled: (1) there was a "response" to an omitted stimulus, that is, discharges occurred during the time that a tone would have been present for a complete tone sequence; (2) there were significant changes (increase ,decrease, or both) in the responses to the remaining tones when a tone was omitted; this effect could be seen for some tones which preceded and/or followed the omitted tone; (3) the tone responses depended on the order of presentation of the tones; (4) the preceding effects were dependent on the duration of the inter-tone interval. These effects are difficult to explain in terms of discharge responses to isolated tones, demostrating that auditory cortical neurons are sensitive to tone pattern. Supported by Univ. Cal. Focused Research Program in Cooperative Brain Function, and a grant from the Monsanto Company.

72.5 PLASTICITY OF FREQUENCY TUNING OF SINGLE NEURONS IN AUDITORY CORTEX DURING LEARNING. N.M. Weinberger, D.M. Diamond and T. McKenna. Center for the Neurobiology of Learning and Memory and Dept. of Psychobiology, University of California, Irvine, Irvine, CA 92717

Numerous studies of classical and instrumental conditioning have demonstrated that the evoked responses of neurons in sensory cortex change during learning. Consistent with earlier multiple unit studies, we previously recorded from single neurons in auditory cortex and found that most developed discharge plasticity during learning (Behav. Neurosci., 98:171-188; 189-210, 1984). In this report, we address the issue of the functional significance of the evoked plasticity which develops as the meaning of sounds changes during learning. Our working hypothesis is that discharge plasticity to an acoustic signal represents changes in the sensory response properties of auditory cortical neurons. This "retuning" is viewed as a dynamic process in which the discharges of cortical neurons convey aspects of the meaning as well as of the physical parameters of sounds.

Single unit activity was recorded in chronically prepared cats under neuromuscular blockade to ensure stimulus constancy. The training stimuli were a tone (CS) and electrodermal stimulation (US); pupillary dilation to the CS served as the indicator of learning. Frequency tuning was determined by presenting isointensity tones for a range of frequencies before and after a sensitization phase (CS/US unpaired) to control for non-associative effects, and following conditioning (CS/US paired) and extinction (CS alone).

Evoked activity to the CS frequency was changed as a function of CS/US pairing, as reported previously. Of particular importance, frequency tuning was also altered by conditioning. This effect was associative because frequency tuning was not changed by sensitization procedures. Further, alterations in tuning induced by conditioning were reversed by extinction.

These data indicate that aspects of the receptive field properties of auditory cortical neurons, such as frequency tuning, can be modified by learning.

Supported by a grant from the Monsanto Co.

72.6 **AN AUDITORY REPRESENTATION OF THE BIRD'S OWN SONG IN THE ADULT HVc OF WHITE-CROWNED SPARROWS.** D. Margoliash, Division of Biology, Caltech, Pasadena, CA 91125.

An intriguing component of song learning is the requirement that vocal feedback be intact, but only during development. Neurons in HVc, a telencephalic nucleus necessary for song production, have been shown to have auditory responses. A small subset of auditory neurons in HVc, song-specific neurons, respond only to sequences of phrases and complex artificial stimuli mimicking sequences of phrases from the individual's own song, suggesting that their response properties are modified by experience. The use of a complex search stimulus however, potentially introduces a bias while sampling from the population of auditory neurons in HVc. To evaluate this caveat, I investigated the auditory response properties of HVc single units and multiunit clusters.

The present data indicate that the population of auditory neurons in HVc are optimally stimulated by the bird's own song. This was assessed with a variety of tests: comparison against the responses to conspecific songs, forward versus backward song, amplitude and frequency shifting, and comparison against responses to tone and noise bursts. Each individual's song has its own idiosyncratic morphology; HVc auditory neurons are often selective for these components. Single units typically respond optimally to stimuli that have particular acoustic parameters--especially absolute frequency, frequency modulation, and duration--that are found in various phrases of the individual's song. For multiunit clusters, the individual's song is typically the most effective stimulus. Even intradialect songs elicit considerably weaker responses. Presenting the bird's own song in reverse, or presenting tone or noise bursts with the same amplitude modulation as song, significantly reduces the response strength. Shifting the song up or down in frequency diminishes the response strength monotonically, while varying the amplitude over a range of 40 dB only marginally varies the response strength. Different recording sites respond maximally to different phrases of song, although a map of song has not been observed.

The existence of an auditory representation of the bird's own song must be the result of modification of response properties during ontogeny. Song does not require vocal feedback for its maintenance, however, suggesting that its representation in the adult plays a role in conspecific song recognition.

72.7 **CORRELATION AMONG SPIKE TRAINS IN CAT'S AUDITORY CORTEX (AI) DURING PRESENTATION OF THREE TONE SEQUENCES.** **I.E.Espinosa* and G.L.Gerstein, Univ. of Pennsylvania, Dept. of Physiology, Philadelphia, PA 19104.**

Ablation studies in cats have shown that AI is needed for the discrimination of simple tone sequences. Reported single neuron response properties do not account for such discrimination. These experiments examine the possible role of neuronal assemblies in the representation of melody.

Recordings of 10-15 neurons (simultaneously and separably) were made in AI of sedated cats. A wire bundle electrode was stereotactically placed along a tangential path (Gerstein, G.L. et al., IEEE Trans. SMC-13:668, 1983). Stimuli consisted of three tone sequences (all tones near average best frequency (BF) of the recorded group of neurons). Later, each recorded pair of neurons was analyzed by cross correlation methods. Activity and the analysis were separated according to which of the six possible melody sequences had been presented. Interpretation of the correlograms allowed the usual inferences of neuronal connectivity and its variation (Dickson, J. and G.L.Gerstein, J. Neurophysiol. 37:1239, 1974).

In one group of 10 AI neurons (45 pairs), correlograms taken during spontaneous conditions showed the signatures of both direct neural connections and shared input. Correlograms from 10 neuron pairs varied with variation of the tone sequence; these pairs involved only shared input. In about half of these stimulus sequence dependent pairs, the correlograms showed an increase in a broad central peak (shared input) compared to the spontaneous situation ONLY for some ONE of the six tonal sequences. In some cases all other tonal sequences reduced or even eliminated the broad central peak seen in the spontaneous condition. The known geometry of the electrode bundle suggests that neurons in the pairs with selectivity for tonal sequence are physically close. Some other pairs with neurons at a greater distance (>500um) also showed variation of correlogram with stimulus variation. However the changes were more complicated and included the effects of stimulus coordination. No "simple" selection of tonal sequence was noted for these pairs. These experiments suggest that both assembly and solo properties of AI neurons may be involved in discrimination of tonal sequences.

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72.8 MULTINEURON ANALYSIS SHOWS SPATIAL TUNING NOT FOUND IN SINGLE UNIT RESPONSES. M. J. Bloom, and G. L. Gerstein. Depts. of Anesthesia and Physiology, Univ. of Penna. Philadelphia, PA 19104

Ablation studies have shown that auditory cortex (AI) is needed for cats to localize sounds (Jenkins and Merzenich, Soc. for Neurosci. Abstracts. p.392, 1981 and in press), but response fields of single cells have only been described as hemifield or at best selective for the acoustic axis of the pinna (Middlebrooks and Pettigrew, J. Neurosci. 1:107, 1981).

We examined changes in neuronal interactions which may be involved in the cortical representation of acoustic space. Different positions of the sound source were simulated by presenting dichotic tone pips (near the characteristic frequency of the neurons) with different interaural amplitudes and/or time-delays.

A bundle of seven 25um-tungsten wires was inserted tangentially into AI of adult cats using stereotaxic coordinates. After surgical recovery, cats were restrained with a head-bolt and sedated to sit quietly with earphones inserted into the pinnae. The electrode bundle was advanced until stimulus-driven neural activity was found. Details of the data acquisition and analysis methods are reported in Gerstein et al, IEEE Trans. SMC-13 p668, 1983.

Six bundle penetrations each yielded recordings of simultaneous extracellular spike trains from 10-16 neurons. 15% of 136 neuron pairs, studied by cross-correlation analysis, showed variation of firing synchrony dependent on "position" of the stimulus. Responses of the individual neurons (measured by PST histograms) did not show such tuning. The resolution of the tuning is approximately 20-30 degrees of azimuth. The tuning usually varies depending on the method used to simulate stimulus position.

Since single cells do not appear to code for sound position, we believe these changes in correlation of firing provide evidence that sound location is coded as a property of the ENSEMBLE activity of groups of neurons.

(Supported by NIH NS05606 and the Systems Development Foundation.)

72.9 THE EFFECTS OF AUDITORY CORTICAL LESIONS ON MINIMUM AUDIBLE ANGLES FOR SOUND LOCALIZATION BY THE FERRET. G.L. Kavanagh* and J.B. Kelly. Dept. of Psychology, Carleton University, Ottawa, Canada, K1S 5B6.

Ferrets with either unilateral or bilateral lesions of auditory cortex were tested for their ability to localize sounds in the horizontal plane. A two-choice testing procedure was employed which permitted independent assessment of minimum audible angles around 0, -60 and +60 degrees azimuth. Large bilateral lesions which destroyed the entire projection area of the medial geniculate body resulted in severe deficits in both midline and hemifield test situations. Animals with more restricted bilateral lesions of auditory cortex were capable of some degree of localization around the midline position, but were totally incapable of localization in either left or right lateral fields. For example, animals with bilateral lesions restricted mainly to the primary auditory cortex (A1) showed very little impairment in localization around the midline, but still had severe deficits in the lateral fields. With the most restricted bilateral lesion, the minimum audible angle around 0 degrees was near normal, while performance in both left and right hemifields was not above the level expected by chance even at the largest angle of speaker separation (60 degrees).

Unilateral lesions resulted in impaired performance in the field contralateral to the damaged hemisphere, but did not disrupt localization around either midline or ipsilateral positions. Pronounced contralateral deficits were seen following either large lesions or lesions restricted mainly to the primary auditory field. Even after unilateral damage restricted mainly to A1, performance in the contralateral field was near the level expected by chance at the largest angle of speaker separation (60 degrees).

This work was supported by Grant 7654 from the Natural Sciences and Engineering Research Council of Canada.

72.10 THE EFFECTS OF BILATERAL AUDITORY CORTICAL LESIONS ON PURE TONE SOUND LOCALIZATION BY THE RAT. J.B. Kelly and G.L. Kavanagh* (SPON: E.W. Peterson). Dept. of Psychology, Carleton University, Ottawa, Canada, K1S 5B6.

Bilateral lesions of auditory cortex in cats, dogs, monkeys and ferrets result in severe deficits in the ability to localize sounds in space. Equivalent lesions in the albino rat, however, fail to produce severe deficits in sound localization (Kelly, 1980, J. Neurophysiol. 44: 1161). Even following complete destruction of the auditory thalamocortical projection, rats are still capable of localizing clicks and noise stimuli. Because the peripheral cues and central mechanisms for sound localization are different for different frequencies, we have considered the possibility that impairments in localization by rats have been overlooked by using relatively broadband stimuli. Therefore, we have re-examined the issue of localization ability following cortical ablation using pure tones. Animals were tested in a two-choice situation which required a spatial response to a brief auditory stimulus. The stimuli employed were tones of 2, 4, 8, 16 and 32 kHz, 60 milliseconds in duration with 20 millisecond rise and fall times, presented as single bursts at the beginning of each trial. The sound pressure levels were adjusted to 40 dB above the rat's absolute threshold for each frequency. Testing was conducted with loudspeakers placed at -30 and +30 degrees azimuth for a total separation of 60 degrees. Performance levels were assessed before and after bilateral ablation of auditory cortex. The results showed only minor reductions in performance with no indication of a selective impairment at any of the frequencies tested. These results support our previous conclusion that rats are still capable of sound localization following complete bilateral destruction of auditory cortex.

This work was supported by Grant 7654 from the Natural Sciences and Engineering Research Council of Canada.

72.11 MORPHOMETRY OF DEVELOPING AUDITORY CORTEX: THE 3-DIMENSIONAL BRANCHING STRUCTURE OF SPINE FREE NONPYRAMIDAL NEURONS IN THE RABBIT. N. T. McMullen, B. Goldberger*and E. M. Glaser Dept. of Physiology, Univ. of Maryland School of Medicine, Baltimore, MD 21201

The large spine-free neuron (SFNP) is the most common nonpyramidal cell type in the rabbit auditory cortex. Its dendrites exhibit a highly significant pia-white matter orientation and a significant dorsal-ventral orientation in the tangential plane (McMullen et al, JCN, 222, 1984). We have now examined the postnatal development of lamina III/IV SFNP cells using the Golgi Cox Nissl method and computer microscopy. New Zealand White rabbits (N=48) were analyzed at birth (day 0), 3, 6, 9, 12, 15, 21, 30 and 60 days of age. Twenty SFNP neurons were digitized at each age from 200-400 um thick sections. At birth, SFNP neurons are extremely immature and have attained only 34% of their adult soma area. Soma area increases linearly until d-15 when it is 94% of the adult value. Soma area is at adult level by d-30. The number of primary dendrites, 72% of adult values at birth, increases to 122% between days 6-9 and decreases to the mean adult value of 8 dendrites by d-12. The number of dendritic branches, 68% of adult values at birth, increases to 170% from day 6 to 15 and is still at 121% at d-30. Total dendritic length is only 11% of adult values at birth and increases linearly to d-9 when it is 45% of adult values. Between days 9-15, dendritic length doubles to 90% of adult length. Total dendrite length is at the adult level by d-30. During rapid dendritic growth (days 9-15), the somata and dendrites become covered with spine-like processes. Spine density peaks at d-15 and recedes until the cells are spine free by d-30. Spatial analyses revealed that a highly significant vertical (pia-wm) orientation of dendrites is present at birth. Dendritic growth along the pia-white matter axis is not symmetric: from d0-3, the largest amount of dendrite projects toward the white matter while from d6-15, the largest amount of dendrite projects toward the pia. Supernumerary branches present during d6-15 are due to the elaboration of pially-directed dendrites. Due to the loss of excess branches and the growth of dendrites toward the white matter, this vertical asymmetry of dendrites is much reduced by d-21. The adult vertical orientation of dendrites is achieved by d-30. The various features of growth exhibited by these developing interneurons may reflect the ingrowth of afferent fibers and the maturation of local synaptic circuitry. (Supported by NIH grant NS17861 to NTM).

72.12 TOPOGRAPHIC ORGANIZATIONS RELATED TO BINAURAL AND MONAURAL RESPONSE CATEGORIES IN CAT PRIMARY AUDITORY CORTEX (AI). R.E. Kettner and R.A. Reale*. Dept. of Neurophysiology and Waisman Center, Univ. of Wisconsin, Madison, WI 53706

Binaural and aural dominance columns were systematically defined within AI by analyzing neural responses to a wider range of stimulus intensities and interaural intensity differences than previously examined. Binaural columns were classified as either summation, suppression or mixed. The mixed category consisted of those responses which were classified as summation using stimuli with threshold intensity levels, but which were further classified as suppression at higher intensity levels. Individual maps of AI suggested that mixed binaural columns aggregated together and were spatially separate from similar aggregations of summation and suppression columns. Furthermore, among different animals the topographic distributions of different binaural columns appeared to occupy unique regions within AI. To obtain a measure of this apparent consistency, the percentage occurrence of different binaural columns was calculated as a function of distance measured along isofrequency lines crossing the dorsal-to-ventral extent of AI. The percentage of mixed binaural columns was highest at the ventral extreme of AI and monotonically decreased at successively more dorsal locations. The distribution of suppression columns was multimodal but also exhibited a maxima at the ventral extreme of AI and a minima at the dorsal extreme. A multimodal distribution for summation columns showed a minima at the ventral margin of AI and a maxima at its dorsal extreme.

Aural dominance columns, defined using monaural stimuli, indicate whether stimulation delivered to one ear or the other was more effective in eliciting neural discharges. Contralateral dominance columns were most common but showed an unexpected maxima in their distribution at the ventral margin of AI. Ipsilateral dominant and primarily binaural responses were less numerous but each distribution exhibited a single maxima at the dorsal margin of AI. Equidominant responses, by comparison, were evenly distributed. Taken together, these results suggest that AI may be organized with respect to both monaural and binaural response classes in a consistent spatial pattern which is most clearly distinguished near the dorsal and ventral borders of AI. (HD-03352, BNS 7912939, NS06887)

72.13 SPATIAL ORIENTATION OF BASAL DENDRITES OF LAMINA III/IV PYRAMIDAL NEURONS IN RABBIT AUDITORY CORTEX. E. M. GLASER and N. T. McMULLEN. Dept. of Physiology, University of Maryland School of Medicine, Baltimore, Maryland 21201.

We have analyzed the basal dendrite system of pyramidal neurons from lamina III/IV of electrophysiologically localized primary auditory cortex of adult rabbits (N=3). Tissue was processed using the Golgi Cox Nissl method and examined quantitatively with an image combining computer microscope. Pyramidal neurons are the most common cell type in lamina III/IV and account for 87% of the impregnated neurons in this sensory cortex. 60 pyramidal neurons were selected from the middle 100 um of the 300 um thick Golgi-Nissl sections and digitized in their entirety. We found a wide variety of pyramidal cell subclasses to be present. Their cross sectional soma areas ranged from 149 to 346 um^2. Occasionally, pyramidal cells with soma areas as large as 600 um^2 were encountered. The average soma area for the digitized cells was 226 um^2. This value is approximately 60% of the soma areas of the large spine free nonpyramidal cells also present in these layers. The pyramidal neurons have an average of 5.4 primary dendrites and 45.7 branches of all orders. The average total dendritic length of the basal dendrite system was 2748 um. Thus, the basal dendrites of pyramidal neurons possess 89% of the total dendritic length of neighboring spine free nonpyramidal neurons. 59% of the total dendritic length of the basal dendrite system is contained in the 3rd and 4th order branches. The spatial properties of the basal dendrites were analyzed with stick histograms which reveal the orientation of dendrites in the frontal, horizontal,and tangential planes. The analyses revealed a highly significant ($p<.001$) orientation of basal dendrites toward the white matter in both the frontal and horizontal plane. This descending vertical orientation is quite similar to the dendrite orientation of spine free nonpyramidal neurons in the same layers. Dendritic stick histograms also revealed a significant orientation of dendrites in the tangential plane along a dorsal-ventral axis, also identical to that of the nonpyramidal neurons. We conclude that the two major cell types of the rabbits' primary auditory cortex, pyramidal neurons and spine free nonpyramidal cells, have similar, highly significant dendrite orientations. It is hypothesized that this common orientation is related to the oriented arborization of specific afferents arising from the medial geniculate. (Supported by NIH grant NS17861 to NTM).

72.14 CELLS ACCUMULATING [^{3}H]GABA IN LAYERS I-III OF CAT PRIMARY AUDITORY CORTEX (AI). Jeffery A. Winer. Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

The types of cells taking up cortically injected [^{3}H]gamma-aminobutyric acid (GABA; 39 Ci/mmol) were studied in primary auditory cortex (AI). From 0.05-1.0 µl (1-20 µCi) in sterile saline was injected uni- or bilaterally in 4 cats. The animals were perfused 20-40 minutes later with 0.12 M phosphate buffered saline, then with a mixture of 2% paraformaldehyde and 2% glutaraldehyde in buffer. The cortex was frozen sectioned or Vibratomed at 30 µm, or embedded for later, electron microscopic study. After exposure for 2-12 weeks at 4°C, the sections were developed and counterstained for Nissl substance. The major findings were: (a) some cells in every layer of AI took up silver grains in numbers greatly above background; (b) the range of somatic sizes of the labeled cells in the various layers, compared to Nissl stained, unlabeled cells, suggests that different types of neurons accumulate GABA; (c) none of the GABA labeled cells, except in the immediate vicinity of the injection site, had triangular somata or a thick apical dendrite; (d) the GABA labeled somata in layers I, II, and III had significantly smaller somata than unlabeled cells (see table); (e) some of the labeled in layers II and III were bipolar or small multipolar neurons; (f) certain large extraverted multipolar cells in layer II--not previously described in cat auditory cortex [4], were labeled; (g) only a rather small proportion of the cells in any layer accumulated GABA. The somatic size and shape of many of the GABA labeled cells are similar to those reported in the primary visual and somatic sensory cortex. This research was supported by USPHS Grant ROI NS16832.

Table
SOMATIC AREAS OF NISSL OR GABA LABELED CELLS IN LAYERS I-III*

	Nissl		GABA	
	mean±s.d.	range	mean±s.d.	range
layer I	173.1±53.1	105-320	153.2±43.0	85-250
layer II	170.1±41.3	72-254	135.0±43.2	54-259
layer III	200.4±54.9	110-392	189.6±41.6	105-273

*N=100 cells/layer (50 Nissl/50 GABA); all areas in μm^2.

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72.15 CORTICO-CORTICAL PROJECTIONS OF LAYERS II-III IN CAT PRIMARY AUDITORY CORTEX (AI). Sandra D. Winguth and Jeffery A. Winer. Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

The types of pyramidal and non-pyramidal cells (1) in cortical layers II and III contributing to the ipsilateral cortico-cortical system connecting AI to AII were studied. 18-36 hours after 0.1-4 µl of horseradish peroxidase (30%) was injected in AII, the ipsilateral cortex was cut into serial 60 µm thick sections and processed with DAB or TMB. The control for localization of the injection in AII was the pattern of retrograde labeling in the ipsilateral nuclei of the medial geniculate body. In AI, retrogradely labeled somata were found to form patches. The fields of origin of the AI to AII projections are larger than the injection sites, suggesting the intracortical projection may converge. Cells in all six layers were labeled, the main clusters of labeled cells forming vertical columns running from layer I-VI. However, among the most densely labeled patches of cells, unlabeled cells occurred--even when the appropriate thalamic nuclei were heavily labeled. In layer II, some 70% of the labeled cells had triangular somata and a stout apical dendrite, like pyramidal cells (2). The remaining 30% were classified as non-pyramidal (2); of these, at least half were bipolar while the remaining cells could not be classified. In contrast, in layer III over 90% of the labeled cells were pyramidal and less than 10% were classified as non-pyramidal. At least 3 kinds of pyramidal (3) and 2 types of non-pyramidal cells (4) were labeled. These results implicate both pyramidal and non-pyramidal cells of layers II and III in auditory cortico-cortical connections and reinforce the major connectional differences between these layers. Our findings show that the pyramidal cells of layer II are only intracortical or cortico-cortical, while layer III cells can be intracortical, cortico-cortical, or commissural (5). Since some non-pyramidal cells of layer III are also commissurally labeled and layer II cells are not, this accentuates the distinction between these layers. Thus these connectional differences imply quite a different function for the pyramidal cells of layers II and III of AI. This research was supported by USPHS Grant ROI NS16832.

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72.16 PROJECTION PATTERNS OF RAT AUDITORY CORTEX. J. Coleman, R. Rainer* and W.J. Clerici* Department of Psychology, University of South Carolina, Columbia, SC 29208.

The relationship of auditory cortex to midbrain and forebrain auditory structures was examined in Sprague-Dawley rats. We explored the anatomical distribution and specificity of output of auditory cortex. The horseradish peroxidase (HRP) method of Mesulam was used to investigate patterns of retrograde and anterograde transport. Microiontophoretic pipette injections or larger syringe injections were made into inferior colliculus or auditory cortex. After 18-24 hr survival tissue was cut at 40 um in coronal plane and processed for histochemistry. Cortical tissue contralateral to the cortical injection in other animals was flattened and cut from surface to depth.

Focal HRP injections into the inferior colliculus show that auditory cortex primarily innervates the dorsal cortex region and a medial sector. An injection centered in external cortex of inferior colliculus results in fewer cortical cells labelled. On the other hand, a small injection in the dorsal cortex broadly labels tiny clusters of cells in auditory cortex. The labelled neurons are identified as medium and large pyramidal cells of lamina V in which reaction product often appears in apical dendrites ascending toward the cortical surface. The pyramidal cells are distributed throughout cortical area 41, as well as in cortical areas 20 and 36. These results suggest a convergence of cortical information onto the inferior colliculus. HRP injection into auditory cortex results in terminal labelling in dorsal cortex of inferior colliculus and is prominent in ventral nucleus of the medial geniculate body. Cortex contralateral to injection shows anterograde label in lamina I which extends more heavily into laminae II and III. Heavy areas of terminal label are interrupted by lighter regions. Label is also observed in laminae V and VI. Retrograde label appears primarily in cells of laminae III and VI, although labelled neurons are observed in other layers as well.

73.1 PRENATAL BRAIN DAMAGE BY HYPOXIA. L. G. Cockerham and J. D. Hampton*. Department of Zoology and Entomology, Colorado State University, Fort Collins, CO 80523 and Physiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814.

To demonstrate the effects of prenatal hypoxia on the developing fetal brain, pregnant mice were subjected to reduced ambient oxygen pressure (52.8 mm Hg) for 5 hours per day during the last third of their gestation period. On the 21st day after delivery, the pups of the test dams were compared with the same age pups from control dams. Total body weight, brain weight and brain weight expressed as percent of body weight were used in the comparison. Weight differences were noted between the two groups, especially in brain weights, with the brain weight-body weight ratio significantly higher in the control animals. Histopathological comparisons were also made of the motor area of the cerebral cortex from animals of each group. Differences included shrunken and elongated neurons with decreased Vogt's Nissl staining noted in the test group. A correlation was noted between the reduced brain weight in the test animals and the degree of cellular damage. This study suggests that a severely reduced ambient oxygen pressure for the pregnant female during the last trimester of pregnancy will result in brain damage to the developing fetus.

73.2 ETHYLENE DIBROMIDE: EFFECTS OF PATERNAL EXPOSURE ON THE NEUROTRANSMITTER ENZYMES IN THE DEVELOPING BRAIN OF F_1 PROGENY. L.L. Hsu and P.M. Adams. Dept. of Psychiatry and Behavioral Sciences, Univ. of Texas Med. Branch, Galveston, TX 77550

Ethylene dibromide (EDB) is mutagenic and affects spermatogenesis in animals. We have now examined the effects of paternal exposure to EDB on several neurotransmitter enzymes in various regions of the developing brain of F_1 progeny, including acetyltransferase (ChAT), acetylcholinesterase (AChE) and glutamic acid decarboxylase (GAD). Young adult Fisher male 344 rats were used for this study. Male rats were injected with EDB, 1 mg/kg/day for 5 days, 7-14 days prior to breeding with untreated normal female animals. The F_1 progeny at 7, 14, 21 and 90 days of age were sacrificed by decapitation. Brains were rapidly removed, selected regions, including cerebellum (CB), corpus striatum (CS), frontal cortex (FC), hippocampus (HIPP) and hypothalamus (HY) were dissected out on ice and stored at - 60°C until assayed. Frozen tissues from mouse brain regions were homogenized in 5 volumes of ice-cold 0.05M potassium phosphate buffer. pH 7.2, and aliquots of the homogenates were used for ChAT, AChE, GAD and protein assays. Results indicated that in F_1 progeny of treated males at 21 days old, specific ChAT activity was significantly increased in CB by 25%, in CS by 29%, in HIPP by 45% and in HY by 28% but not changed in FC. No changes in ChAT activity were observed in brain areas of F_1 progeny at 7, 14 or 90 days after birth. In F_1 progeny of EDB treated males, at 7 days old the AChE activity was increased in CS by 37% and in HIPP by 29% but not affected in CB, FC or HY. At 14 days old, the AChE activity was significantly decreased in CB by 14%, in CS by 16%, and in HIPP by 18% but not changed in other brain areas. At 21 days old, the specific AChE activity was decreased in CB by 43%, increased in HIPP by 30% and in HY by 34% and was not affected in CS or FC. At 90 days old, AChE activity was not altered in any brain regions examined. Lastly, the specific GAD activities in various brain regions of F_1 progeny from EDB treated males were altered at 21 and 90 days but not at 7 and 14 days after birth. At 21 days old, the GAD activity was significantly increased in CS by 79%, whereas it was significantly decreased in FC by 10% but was not affected in other brain areas. Such neurochemical changes in the developing brain of F_1 progeny of EDB treated males at low doses may be associated with behavioral abnormalities observed early in their development.

73.3 LACK OF ALTERATIONS IN THE COMMISSURAL LAMINATION PATTERN TO THE DENTATE GYRUS OF ADULT RATS. S.L. Dewey and J.R. West. Department of Anatomy, University of Iowa, College of Medicine, Iowa City, IA 52242.

Recently, we demonstrated that prenatal ethanol exposure alters commissural reorganization following unilateral removal of the entorhinal cortex (West et al., Dev. Brain Res. 12:83-95, 1984; Dewey et al., Alcohol 1:81-88, 1984). Exposure to ethanol in utero has also been shown to alter normal afferent organization in the central nervous system (the hippocampal mossy fiber system, West et al., Science 211:957, 1981). Together, these studies suggest that the normal afferent organization of the commissural system may be altered by in utero ethanol exposure. The anterograde transport of Horseradish Peroxidase (HRP) was used to selectively label the commissural projection to the dentate gyrus. Ten normal, ten pair-fed and ten adult rats exposed to a liquid diet containing 35% ethanol derived calories during days 1-21 of gestation received unilateral hilar injections of a 30% (w/v) HRP (Sigma, Type VI) in 2% dimethyl sulfoxide solution. An Eye Com II/PDP-11/34 image processing system was used to quantify the overall area occupied by the commissural HRP terminal labelling at a dorsal hippocampal level. No statistically significant differences between groups were found ($F=.582$) (2,75) ($p>0.1$). The commissural projection occupied 23.8%, 24.2%, and 24.5% of the total molecular layer in normal, pair-fed and ethanol exposed animals, respectively. These results indicate that unlike certain intrinsic afferent systems in the hippocampus, not all afferent organization is affected equally by in utero ethanol exposure. Supported by Grant AA05523 from NIAAA to J.R.W.

73.4 ETHANOL EXPOSURE ALTERS HIPPOCAMPAL DEVELOPMENT DURING THE BRAIN GROWTH SPURT IN RATS. D.R. Pierce, K.M. Hamre* and J.R. West. Dept. of Anatomy, Univ. of Iowa, College of Medicine, Iowa City, IA 52242.

Using a rat model to study fetal alcohol effects (FAE) during a period of rapid brain development equivalent to the human third trimester requires exposing rats to alcohol postnatally. An artificial rearing procedure was implemented where chronic gastric cannulas were surgically implanted in rat pups on postnatal day 4. Pups were fed a variation of the Messer diet in eight 15 minute fractions daily. Suckle/Control (S/C), Gastrostomized/Alcohol Exposed (G/AE) (4% EtOH added to the diet), and Gastrostomized/Controls (G/C) were analyzed on postnatal day 10. The alcohol dose of 9.5 g/kg body weight/day resulted in blood alcohol levels (taken on postnatal day 6) in the range of 250 to 300 mg/dl. There were no significant differences in body weights between any of the groups.

Brain weight to body weight ratios revealed microcephaly with a 32% reduction of the G/AE group compared to the S/C group, a 27% reduction of the G/AE group compared to the G/C group and an 8% difference between the G/C and the S/C group. The EtOH group was significantly smaller ($p<0.01$) than the other two groups. Results for both brain volume and brain weight were similar.

Areas of the Hippocampus and the Dentate Gyrus, were measured using a Bioquant II system. Measurements for the Hippocampus Proper were significantly smaller ($p<0.05$) for the G/AE group than the G/C or S/C groups. However, the Dentate Gyrus and its subdivisions showed no differences between any of the groups. St. Oriens of the Hippocampus Proper had an area significantly smaller ($p<0.01$) for the G/AE group than both the G/C and G/S groups. Results of the measurements of St. Lacunosum-Moleculare (St. L-M) were similar ($p<0.05$). Combining the areal measurements of St. Lucidum, St. Radiatum, and St. L-M subfields indicated that the apical dendritic field in the G/AE group was significantly smaller ($p<0.05$) than that of the G/S group. These reductions in hippocampal subfields indicate a probable failure of the pyramidal cell dendrites to develop fully and suggest that the EtOH exposure might have altered the neurons' ability to establish a normal complement of synaptic connections. This study was supported by NIAAA grant AA05523 to J.R.W.

73.5 L-GLUTAMATE BINDING TO SYNAPTIC MEMBRANES: INFLUENCE OF MATERNAL ETHANOL CONSUMPTION. M.J. Druse-Manteuffel, G.M. Kelly*, D.A. Tonetti* and B.G. Oden*. Department of Biochemistry & Biophysics, Loyola University Stritch School of Medicine, Maywood, IL. 60153.

Female Sprague-Dawley rats were pair-fed control or 6.6% (v/v) (50g/L) ethanol liquid diets, containing 21% protein (Noronha, A.B.and Druse, M.J. J. Neuroscience Res., 8:83, 1982), on a chronic basis prior to parturition. Synaptic plasma membranes (SPM) were prepared from either selected brain regions or whole brain specimens (Cotman, C.W. and Matthews, D.A. Biochim. Biophys. Acta, 249: 380, 1971) from the developing offspring of control and ethanol-treated rats. Purity of the membranes was established by assaying marker enzymes. The Na^+-independent binding of [3H]-L-glutamate to synaptic plasma membranes was determined using a modification of the microfuge assay of Michaelis et al. (Mol. Cell. Biochem., 38: 163, 1981).

Blood ethanol levels in treated dams were approximately 110 mg% 2 hours after the administration of a fresh ration of liquid diet. In agreement with previous studies from this laboratory, the 14- to 26-day-old offspring of control and ethanol-treated rats had comparable brain and body weights. Relative to whole brain homogenates, SPM had a 4-fold enrichment in the specific activity of a plasma membrane marker(Na^+-K^+-ATPase), and no enrichment in the activity of markers for mitochondria (cytochrome c oxidase), myelin (2',3'-cyclic nucleotide 3'-phosphodiesterase) and lysosomes (acid phosphatase).

In the offspring of control rats, specific binding peaked between 17 and 20 days of age in agreement with Sanderson et al. (Dev. Brain Res., 2: 329, 1982) and deBarry et al. (Febs. Lett., 109: 175, 1980). In comparison to age-matched control offspring, there were no apparent differences in the Kd or Bmax of [3H]-L-glutamate binding to synaptic membranes prepared from the brains of 14- to 26-day-old offspring of ethanol-treated rats. A development related trend in specific binding (pmol/mg protein) was observed. *In utero* exposure to ethanol did not appear to affect the binding characteristics (Kd or Bmax) of synaptic membranes from cortex or cerebellum of 20-day-old animals

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73.6 THE LOCUS COERULEUS AND SENSITIVE PERIODS FOR "FAILURE TO THRIVE" FOLLOWING ACUTE PRENATAL ETHANOL EXPOSURE IN RATS. R.E. Ruth and S.K. Goldsmith. Inst. Study Dev. Disabil., Univ. Illinois, Chicago, IL 60608.

Ethanol-sensitive periods for postnatal growth failure in the rat were studied by injecting timed-pregnant rats intraperitoneally either two or three times over a given 24h period between gestation day (gd) 11 and 19; saline-injected and untreated dams were simultaneously processed. At birth (PO) all pups were individually marked and thereafter weighed daily.

Pharmacokinetic studies (to be presented) determined that over 80% of the fetal ethanol absorption occurred from the maternal bloodstream, i.e., the route was largely physiologic for the fetus. Peak fetal levels in excess of 350 mg% were required to produce postnatal growth failure. Sensitive periods were uncovered at gd 13 and gd 17; up to 35% of the pups exposed at these times showed normal growth initially but subsequently manifested severe growth retardation. Fetal exposure at other gestational ages had little effect on postnatal survival.

The number of neurons in the locus coeruleus (lc) was estimated by standard morphometry. In untreated pups the apparent mean number of lc neurons (per side) increased from approximately 1600 at P1 to 1800 at P24 and P40; at each age there was a considerable range of 500--600 counts (Ruth and Goldsmith, 1983). Thus far pups which failed to thrive after ethanol exposure on gd 13 or gd 17 had lc counts which were uniformly below or above the entire range of normal values. Their viable littermates (exposed and reared identically) had counts within normal limits, as did the few remaining cases where growth retardation was observed (from either untreated/saline-injected dams or dams injected with ethanol outside the sensitive periods).

The results indicate that gd 13 and gd 17 are ethanol-sensitive periods which influence postnatal viability in rat offspring. Gross abnormality in a central noradrenergic system is also produced at these times but is observed only in those offspring which subsequently fail to thrive. Supported in part by HD 16061 from NIH.

73.7 EARLY BEHAVIORAL ALTERATIONS IN RATS PRENATALLY EXPOSED TO RESERPINE. J. Buelke-Sam and G.L. Kimmel*, Division of Teratogenesis Research, National Center for Toxicological Research (FDA), Jefferson, AR 72079.

Reserpine has been used clinically in the management of hypertension during pregnancy, and is thought to reduce blood pressure via depletion of biogenic amines. Reserpine does cross the placenta, making it possible that such depletion also occurs in the exposed fetus. Alterations in the preweaning developmental pattern of several central and peripheral endpoints of catecholaminergic function have been reported following prenatal reserpine exposure; however, all reported behavioral testing in treated offspring has been carried out in adult animals. In this study, 3 behavioral endpoints were evaluated in preweanling rats following prenatal reserpine treatment: the negative geotaxic response on postnatal day 8 (PND 8); the developmental activity profile on PNDs 12, 16 and 20; 50-trial auditory startle habituation on either PND 19 or 20. Pregnant CD rats were injected s.c. with either 0, 0.375 or 0.75 mg/kg/day reserpine on days 12-15 of gestation. Treatment resulted in dose-related decreases in maternal weight gain over gestation and mean pup weight at birth. While there were no differences across treatment groups in the proportion of rats successfully completing the negative geotaxis test, offspring in the high-dose group turned 30% slower than controls. A normal developmental activity pattern, with peak levels on PND 16, was seen in controls. Changes in this pattern were both sex and dose-dependent in treated rats. Females from both dose groups were twice as active as controls on PND 12, then equally active on both later test days. Males from the low-dose group showed little or no change in activity compared to controls; however, the profile of high-dose males was suggestive of an overall developmental delay, reflected in lower PND 12 and 16 counts, and higher PND 20 counts than controls. In auditory startle habituation, rats from the low-dose group showed a slight but consistent reduction in startle amplitude compared to controls. In high-dose rats, both response amplitude and rate of habituation were decreased. Thus, there appear to be dose-related effects of reserpine on several preweaning behaviors thought to be mediated in part by central catecholaminergic systems.

73.8 EFFECTS OF PERINATAL PHENOBARBITAL ON LOCOMOTOR ACTIVITY AND MONOAMINE LEVELS. L. Erinoff, P.A. Bradshaw, and S.R. Snodgrass. Neurology Research, Childrens Hospital of Los Angeles, Los Angeles, CA 90054

Pregnant Sprague-Dawley rats were implanted with osmotic minipumps (2 ml total volume) to provide constant drug administration (0.5 ul/hr) for 14 days. The daily dose of phenobarbital (PB) was approximately 65 mg/kg. Pumps were implanted on day 16 of gestation and were not removed until 8 days following birth of the pups. Pups were not cross-fostered so they received drug from the lactating dams. Thus, pups were exposed to PB for 6 days prenatally and 8 days postnatally. Two litters were PB-treated and one served as vehicle control. Blood levels of PB in the two dams, determined on the final day of drug treatment, were 34 and 40 ug/ml.

PB-treated pups did not differ from control pups in body weight throughout development. Monitoring of locomotor activity for one hour in photocell cages was begun at 12 days of age on 5 pups from each litter. PB-treated pups were more active than controls through 6 weeks of age. At 6 weeks of age, the dose response function for apomorphine (0.1, 0.3, and 0.6 mg/kg) effects on locomotor activity was determined. PB-treated and control rats had similar decreases in activity at the lowest dose of apomorphine, while only control rats showed increased locomotion at the two higher doses. Both control and PB-treated rats exhibited greatly increased activity following 1.0 mg/kg amphetamine.

Rats were killed at 10 weeks of age, and their brains were dissected into the following regions: brainstem-diencephalon, cortex, hippocampus, cerebellum, striatum, and nucleus accumbens. Dopamine (DA), norepinephrine (NE), serotonin (5HT), dihydroxyphenylacetic acid (DOPAC), and 5-hydroxyindoleacetic acid (5HIAA) levels were determined by HPLC with electrochemical detection. PB-treated rats had significant increases in cerebellar NE concentration (21%) and hippocampal 5HIAA levels (36%) without concomitant changes in wet weight and protein content. The cerebellum and the hippocampus are both regions reported to show cell loss following perinatal PB (Bergman et al., Acta. Anat., 114: 185, 1982).

Our data indicate that perinatal PB exposure produced by implanting minipumps in pregnant dams leads to lasting behavioral and neurochemical changes.

73.9 RELATIONSHIP OF TESTOSTERONE TO LACTATIONAL EFFECT OF ETHANOL. F.E. Lancaster and O.A. Gutierrez*. Dept. of Biology, Texas Woman's Univ., Houston,TX 77030.

Pregnant Long-Evans rats were received on day 15 of gestation and fed liquid control diet (1226-PR-C, Bio Serv) until day 2 of lactation. On days 2-21 of lactation, dams were assigned to the following dietary groups: (1) Ethanol (ET) received 27% calories as ethanol in liquid diet (1226-PR-A) (2) Pairfed (PF) received control liquid diet isocaloric to the amount consumed by ET on the previous day, and (3) Control (CT) received control diet ad libitum. Litters of dams in each dietary group were assigned to subgroups as follows: (1) Gonadectomized plus testosterone (G^+), in which females were castrated on day 3 and given daily injections of testosterone and males were hemigonadectomized on day 1, and (2) Sham (S), in which males and females were sham operated. In the sham group, ET males were affected only in brain weight; females were affected in all organs. In the G^+ group, males and females were affected in all organs. In conclusion, female offspring were affected more severely by ethanol in the milk during lactation than were the males. Testosterone injection did not protect the female against the effects of ethanol. Hemigonadectomy in the males resulted in more severe effects than in sham operated males.

73.10 IN UTERO EXPOSURE TO ALCOHOL INDUCES CHANGES IN FETAL BEHAVIOR. W. P. Smotherman, K. S. Woodruff*, S. R. Robinson*, C. del Real*, Lab. Psycobiol. Res., Dept. Psychol., Oregon State Univ., Corvallis, OR 97331, and S. Barron and E. Riley, Dept. Psychol., SUNY, Albany, NY 12222.

Pregnant female Sprague-Dawley rats were intubated with a solution of ethyl alcohol in isotonic saline on Day 19 of gestation, yielding four groups (n = 6 rats/group) exposed to 0, 2, 4 and 8 g alcohol / kg body weight. Four hours after intubation, females were surgically prepared by chemomyelotomy, which produces permanent spinal anesthesia without drug administration. Each prepared female was placed in a temperature-controlled bath of isotonic saline, the uterus exteriorized, and fetuses delivered into the bath, preserving the attachment of the placenta to the uterus. Two subject fetuses were each observed over a 10-min observation period, recording individual movements of head, body and appendages, and totalling these frequency scores to derive measures of overall activity. Following observation, samples of maternal blood, amniotic fluid, and fetal tissue were collected for assay of alcohol content. Blood alcohol content of female rats increased from negligle concentrations in the 0 and 2 g/kg groups to intermediate ($\bar{x}$ = .089% w/v) and high levels ($\bar{x}$ = .168 % w/v) in the 4 and 8 g/kg groups, respectively. This pattern of difference was paralleled in fetal tissue (4 g/kg: .079% w/v; 8 g/kg = .160% w/v) and amniotic fluid samples (4 g/kg: .082% w/v; 8 g/kg: .210% w/v). Curiously, the alcohol content of amniotic fluid was significantly higher than fetal tissue in the 8 g/kg exposure, but no corresponding difference was apparent in the 4 g/kg exposure. Total activity of fetuses decreased with low and intermediate alcohol exposure (mean movements/10-min period: 0 g/kg = 55; 2 g/kg = 45; 4 g/kg = 27), but rebounded to control levels with high alcohol exposure (8 g/kg = 58 movements/10-min). Several specific patterns of movement showed a similar trend across the four exposure conditions; movements of Forelegs and Trunk (torsions, flexions or extensions of the body) decreased in 2 g/kg and 4g/kg groups, relative to 0 g/kg controls, and returned to control levels in the 8 g/kg condition. The lack of diminished activity in the 8g/kg group is unexpected, and may be related to the anomalously high levels of alcohol in the amniotic fluid of 8g/kg fetuses.

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73.11 TASTE PREFERENCE FOR SODIUM CHLORIDE IN DAHL SALT-SENSITIVE AND SALT-RESISTANT RATS FED HIGH OR LOW LEVELS OF SODIUM CHLORIDE IN WEANING DIETS. A. J. Lanou*, F. Ferrell* and S. D. Gray* (SPON: I. J. Miller Jr.). Depts. of Nutrition and Human Physiology, Univ. of California, Davis, CA 95616.

Dahl salt-sensitive (S) rats develop high blood pressure when fed a high-sodium diet, whereas their salt-resistant (R) counterparts remain normotensive. Thus, these two strains provide a model for studying the interaction of genotype and diet in the development of hypertension. When fed standard diets (0.5-0.75% NaCl) the S strain exhibits a significantly lower preference than R for isotonic and hypertonic NaCl solutions (Wolf et al., Proc. Soc. Exp. Biol. Med. 120:301-305, 1965). We compared effects in S and R of high and low NaCl diets on taste preference for NaCl solutions presented over a wide range of concentrations. We fed weanling S and R rats an 8.0% (Hi Salt) or 0.4% (Lo Salt) diet for four weeks. Then, while feeding all animals the Lo Salt diet, we measured their taste preferences for NaCl at 0.1 mM - 0.56M, using three 24-hr two-bottle preference tests of each NaCl concentration vs. distilled water. S rats had higher preferences than R for hypotonic NaCl, regardless of dietary NaCl level. S and R Hi Salt animals had similar preferences for isotonic NaCl, but S-Lo Salt rats had higher preferences than R-Lo for isotonic NaCl. Compared to S rats, R preferred hypertonic 0.32M NaCl more, regardless of rearing diet, and all four groups rejected 0.56 M NaCl equally strongly. During "pre-preference test" dietary NaCl loading of the Hi Salt groups, S-Hi consumed significantly more water per 100 g body wt than did R-Hi. S-Hi and R-Hi drank approximately four times, and three times, the amount of water consumed by S-Lo and R-Lo, respectively. Water consumption did not differ between the Lo Salt groups. R animals weighed more than S regardless of diet, and within each strain, Lo Salt animals weighed more than Hi Salt animals. Various differences between our findings and those of Wolf et al in 1965 for isotonic and hypertonic saline preference might be attributed to genetic drift in the animals, as they are not completely inbred (review by Rapp, Hypertension: 4: 753-763, 1982). Our findings indicate that sodium susceptible genotype and dietary NaCl level interact to influence subsequent saline taste preference.

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73.12 INTRAUTERINE DIAZEPAM EXPOSURE: EFFECTS ON PHYSICAL AND NEUROBEHAVIORAL DEVELOPMENT IN THE RAT. C.L. Ryan* and B.A. Pappas (SPON: T. Tombaugh). Unit for Behav. Med. and Pharmacol., Carleton Univ., Ottawa, Ont., K1S 5B6.

The present study investigated the effect of prenatal diazepam on general somatic growth and some aspects of CNS maturation and function.

Primiparous timed-pregnant dams (Woodlyn Wistar) were daily administered a single s.c. injection of 1.0 mg/kg (D1) or 5.0 mg/kg (D2) diazepam or vehicle (C), over gestation days 13-20. At the time of parturition, litters were counted, sexed, culled to 8 pups, and fostered to non-injected dams for further developmental testing. Maternal weight gain over the treatment period was not affected by the diazepam drug treatment. The offspring exhibited a significant dose dependent decrease in pup viability. Additionally, body weight was depressed and hair growth delayed in the D1 group. No differences were detected among the groups on either the day of emergence or criterion performance for the parameters of incisor eruption, pinna uncurling, eye opening, righting, geotaxis, acoustic startle, swimming, or forward locomotion. Rotarod performance, assessed at approximately 40 days of age, was, however, significantly affected, as animals from both D1 and D2 failed to reach the performance level of the offspring from C. Differences in body weight was again evidenced at 60 days of age with males from D1 weighing significantly less than those of D2 or C.

Long-term alterations in seizure thresholds of the adult offspring were also investigated. A dose-response study involving an acute injection of the chemical convulsant metrazol (40, 50, 60 or 70 mg/kg), did not uncover significant differences in seizure response among the groups. A subsequent kindling study, involving repeated injections of metrazol (50 mg/kg) over a number of days, revealed an increased susceptibility to stage "3" (tonic-clonic) seizures in the animals from the D1 and D2 groups. The number of injection trials required to produce a stage "3" seizure in the animals from D2 was significantly less than that for C animals. Therefore, altered metrazol seizure thresholds, due to prenatal diazepam, were revealed by repeated subconvulsant dosing, but not by an acute exposure procedure.

In summary, in the rat, prenatal exposure to clinically relevant doses of diazepam significantly altered specific parameters, producing both fetal toxicity and long-term neurobehavioral alterations.

73.13 COMPARISON OF PLASMA LEVELS OF INDOLE AMINES IN PREGNANT AND NON-PREGNANT RATS. M. Sakuma, I. Mefford, R. Grady and (Spon; R. Pourcho). Dept. of Psychiatry, Wayne State University, Detroit, MI 48207 and Dept. of Chem., Boston Col. Boston, MA 02167.

We have previously shown that prenatal tryptophan diets induced an increase or decrease of plasma tryptophan and 5-HT levels in offspring (Neuro. Sc. Meeting, 1983). The capability of prenatal diets to induce blood level changes in serotonergic amine levels raised the question of whether the blood levels of those amines in dams differ from levels obtained from controls. If blood levels of those amines in pregnant dams differ from control levels, the obtained offspring levels are necessary for interpretation, based on pregnant dams' metabolism. There is a hypothesis that serotonergic amine levels in plasma do not differ between pregnant and non-pregnant animals. This study investigated the plasma levels of tryptophan, 5-HT and 5-HIAA in pregnant rats compared with same-aged non-pregnant female rats. 12 pregnant rats at 15 days gestation showed higher serotonin levels than 14 non-pregnant female rats ($p < 0.001$). However, tryptophan and 5-HIAA levels failed to show significant differences with non-pregnant levels. The unexpected high serotonin level in pregnant rats lead us to the next experiment. Four pregnant rats (15 days) and 4 non-pregnant female rats were fed independently by 3% tryptophan diet during third trimester in the former and for a week in the latter. In pregnant rats, the prediet value of tryptophan was reduced by the delivery-end following decreased serotonin level ($p < 0.01$). While non-pregnant rats fed a high tryptophan diet failed to show a change in tryptophan levels only 5-HT levels decreased from prediet values ($p < 0.001$). These results indicate that high tryptophan diet did not affect the tryptophan level in non-pregnant rats; however, the diet brought the tryptophan level down in pregnant rats. The latter results indicate a possible effect of prediet value of high serotonin during pregnancy which is capable of regulating precursor, tryptophan levels.

73.14 METABOLISM OF INTRACRANIALLY INJECTED LINOLEIC ACID IN THE DEVELOPING BRAINS OF RATS FED CIS AND TRANS OCTADECENOIC ACIDS. N. K. Menon and J. F. Mead*. Lab. of Biomedical and Environmental Sciences, Univ. of Calif., Los Angeles, CA 90024.

On the basis of the earlier observation by Mead et al. which showed that orally-administered [^{14}C]18:2 was metabolized at a significantly greater rate by fat-deficient mice than by normals, we studied the metabolism of 18:2 by developing brain in marginal essential fatty acid (EFA) deficiency. Female Wistar rats were started on a fat-free diet containing 5% W/W of either oleic acid [c18:1 = 90-95%; 18:2 = 2%] or hydrogenated corn oil [c18:1 = 16.5%; t18:1 = 52.2% and 18:2 = 7.9%] two weeks before mating. The diets were continued through pregnancy and lactation. At postnatal day 12, six pups from each group were injected intracranially with 5 μCi of [1-^{14}C]18:2 as albumin complex. The breath CO_2 was measured at 1, 4 and 8 hours following injection. The animals were sacrificed at 8 hrs. The weights of body, brain and brain total lipids of the pups on the cis diet were reduced by 26%, 22% and 33% respectively more than those on the trans diet. There was a significant increase in the expired CO_2 ($P < 0.001$) at 1 and 4 hrs and a 38% increase in the incorporation of label into fatty acids of the brain in the pups on cis diet when compared to those on the trans. There was no difference in the fatty acid composition of the brain total lipids. Fractionation of fatty acids through SiO_2-$AgNO_3$ column chromatography showed that 16:0, 18:0, 18:2 (9,12) 18:3 (6,9,12), 20:3 (8,11,14) and 20:4 (5,8,11,14) were labelled in the brains of pups on both the diets. These results indicate that EFA deficiency produced prenatally causes an increase in the overall rate of metabolism of 18:2 in the developing progeny similar to that reported for adult animals but does not alter the capacity of the brain to elongate and desaturate 18:2.

LEARNING AND MEMORY: PHARMACOLOGY

74.1 BOTH MEMORY AND REWARD INFLUENCE THE PLACE PREFERENCE TEST. N.M. White and G.D. Carr, Dept. of Psychology, McGill University, 1205 Dr. Penfield Ave., Montreal, Quebec, Canada H3A 1B1.

The demonstration that a conditioned place preference is produced by a given treatment is generally interpreted to mean that the treatment has rewarding properties. This conclusion is based on the assumption that rewarding properties of the treatment become associated with the stimuli in the environment with which they are paired. It is obvious that animals would be unable to express a preference for these stimuli on the test day if they failed to remember this association; and the present data suggest that in fact the ability of this method to detect reward depends in part on the memory improving properties of the treatment. Our version of the place preference apparatus consists of two distinctive large boxes separated by an opaque partition, connected by a small box, or tunnel, at the rear. During training rats experience the treatment under test in one of the two large boxes and a control treatment in the other large box on alternate days. The box paired with the treatment is counterbalanced within each group. On test day the rats are placed into the small box and allowed to choose freely among all three locations for 20 min. When rats were trained with sucrose solutions (1, 4, 20, 40% W/V) in their paired boxes, conditioned place preferences of magnitude that increased with concentration were observed. However, when trained with equally preferred (by taste) solutions of saccharin (0.5, 0.8, 1.0%) no significant place preferences were observed. We interpreted this finding in terms of our previous data (Messier and White, Physiol. Beh., 32: 195-203, 1984) showing that although both sucrose and saccharin are rewarding, only sucrose improves retention. To test the hypothesis that memory improvement might be an important factor in the place preference test we injected rats with glucose (2 g/Kg) or amphetamine (2 mg/Kg) after they drank 0.5% saccharin in their paired boxes and after they were confined in their control boxes. Significant place preferences were observed in both cases. The facts that preferences were not observed with saccharin alone, but were detected when post-training injections of known memory improving agents were administered suggests that the place preference test is not a simple measure of reward; the ability of this test to detect the rewarding properties of treatments depends in part upon their memory improving properties. Food, amphetamine and morphine (White, et al., Life Sci., 23: 1967-1972, 1978) all have memory improving properties, so the place preference test can detect their rewarding properties. However, caution is indicated when interpeting the absence of place preferences when testing substances with unknown memory improving properties.

74.2 EFFECT OF GLUCOSE AND INSULIN ON MEMORY AND STEREOTYPY. C. Messier, J. Blackburn* and N.M. White, Dept. of Psychology, McGill University, 1205 Dr. Penfield Ave., Montreal, Canada, H3A 1B1.

Previous studies have shown that retention of a learned behavior is improved by post-training injections of glucose solutions (Messier & White, Physiol. Beh., 32: 195-203, 1984). In the present series of experiments, we began to study how this effect is mediated by examining the effect of different doses of glucose or insulin alone or in combination on retention. Training in the memory test consisted of two pairings of a tone and a shock. The animals were injected subcutaneously with the appropriate substance 15 min after training. The next day, the tendency of the tone to suppress drinking was tested. In the first experiment 4 doses of glucose were injected (1, 2, 3, 4 g/Kg). The effect of these doses on memory was a U-shaped function with a significant improvement only at 2g/Kg. Six doses of insulin were injected (.25, .5, .75, 1, 2, 4 I.U./Kg). None had any significant effect on retention. This lack of effect was not due to a disruptive action of insulin on memory consolidation in general since 2 I.U./Kg failed to impair retention when injected after 10 tone-shock pairings. However, when 2 I.U./Kg of insulin was combined with the 4 doses of glucose, retention was improved in the animals that received the higher doses of glucose (3, 4 g/Kg) while no changes were observed for the lower doses of glucose (1, 2 g/Kg). The fact that insulin potentiated the effect of the higher doses of glucose suggests that the two may act synergistically to improve retention. Previous research from our laboratory (Carr and White, Psychopharmacol., 82: 203-209, 1984) suggested a relationship between memory improvement and amphetamine induced stereotypy mediated by dopaminergic function in the caudate nucleus. We therefore tested the hypothesis that glucose may also act on this substrate by observing the effect of glucose injections on the stereotyped behavior produced by 2mg/Kg of amphetamine. Paradoxically, injection of 2 g/Kg of glucose suppressed stereotypy but, in parallel with its effect in the memory test, a dose of 3g/kg or higher failed to suppress stereotypy. Since amphetamine-induced stereotyped behavior is known to be mediated by dopaminergic activity in the caudate nucleus, this experiment (together with other evidence) suggests the possibility that glucose may suppress this activity. However, the facts that glucose both improves memory and suppresses stereotypy are problematic for the hypothesis that both of these actions are mediated by the same substrate.

74.3 EFFECTS OF DIAZEPAM ON SPONTANEOUS ALTERNATION AND OPERANT DISCRIMINATION LEARNING IN THE RAT. M.G. Gaston* and K.E. Gaston. California State University, Los Angeles, CA 90032 and Pitzer College, Claremont, CA 91711.

The benzodiazapines appear to induce central effects similar to those produced by anticholinergic agents. Atropinic drugs disrupt both memory and behavioral performances which require response inhibition. We investigated the effects of diazepam on memory and response inhibition in the rat, in the contexts of a delayed spontaneous alternation task and of successive operant discrimination learning and extinction.

In Experiment 1, 100-300 day-old rats were tested on 2 occasions for spontaneous alternation in a T-maze. On each occasion, a first run was either followed immediately by a second run (Zero Delay Group) or by a 1-min delay and then a second run (Delay Group). Prior to testing, the rats were water-deprived for 48 hr and given a 2-hr drinking session: Experimental rats drank a solution of injectible Valium (5 mg/kg) diluted 20:1 with water; Control rats drank water. For each delay condition, rats who dosed below (above) the median for that group were considered Low Dose (High Dose) subjects. The median High Dose was 19.23 mg/kg; the median Low Dose was 8.59 mg/kg. Amount of self-administered Valium was inversely related to age--High Dose animals were younger rats; Low Dose animals were older rats. Results showed significant Delay and Dose effects. At zero delay, Controls alternated 87% of the time, Low Dose Valium (old) rats 66%, High Dose Valium (young) rats 45%. At 1-min delay, Controls alternated 75%, Low Dose (old) animals 52%, and High Dose (young) animals 30% (less than chance). The findings suggest that diazepam both impairs memory and exaggerates prepotent response tendencies.

In Experiment 2, rats were trained to lever-press under conditions of alternating tone-on and tone-off periods. When the tone was off, a response delivered food; when the tone was on, lever-pressing was not reinforced. After asymptotic performance was attained, Experimental animals were injected i.p. with Valium (Low Dose = 2 mg/kg, High Dose = 5 mg/kg); Controls received normal saline. In a dose-dependent manner, Valium rats displayed lower than normal bar-press rates under the condition of reinforcement, but greatly increased responding under the condition of non-reward. The drugged rats made significantly more responses in the presence of the tone than in its absence. During a subsequent extinction session, the Valium rats made significantly more responses than Controls. This finding of behavioral disinhibition is similar to that found in subjects treated with anticholinergic drugs or who have received temporal lobe limbic lesions.

74.4 IMPAIRMENT OF A PASSIVE-AVOIDANCE RESPONSE BY DIAZEPAM AND ITS ACTIVE METABOLITES. Ray H. Zobrist and Harold L. Komiskey. Dept. Clin. Pharmacol., UCSF, San Francisco, CA, 94143, and Dept. Biomed. Sci., Univ. of Illinois College of Medicine at Rockford, Rockford, IL, 61107-1897.

Diazepam produces a temporary inhibitory effect on the process(es) of learning and/or memory in both animals and humans. In the present study, diazepam and its behaviorally active metabolites were individually examined for an ability to inhibit the step-down task in the Sprague-Dawley rat after intravenous (i.v.) injection. The brain levels of diazepam and its metabolites were measured by reverse isotope dilution and HPLC techniques at various times after injection of 180 µg/Kg diazepam. All three active metabolites produced a dose-dependent impairment of the step-down task. At minimum effective concentrations, the two intermediate metabolites oxydiazepam and N-desmethyldiazepam were more potent than either oxazepam or the parent drug, diazepam. However, at doses approaching human therapeutic levels, all four benzodiazepines were equipotent. The brain levels of all three metabolites after an i.v. injection of diazepam were undetectable for at least 10 min., and, when detectable, remained at very low and continuously declining levels. Diazepam levels were almost immediately detected and reached a peak level 20 to 30 times higher than metabolite peak levels. The demonstration of the above activity by all four benzodiazepines raises the question as to the role of diazepam and/or one or more of its metabolites in producing the impairment.

The role of oxazepam in the diazepam elicited impairment of the step-down task was investigated. Rats were injected i.v. with the lowest dose of ^{14}C-oxazepam (90 µg/Kg) that significantly reduced the passive-avoidance responding. Brain levels of ^{14}C-oxazepam were measured at various times after i.v. injection. The brain levels of oxazepam achieved after 180 µg/Kg diazepam failed to reach the brain levels achieved after 90 µg/Kg oxazepam. Therefore, oxazepam probably does not contribute significantly to the diazepam-induced impairment of the passive-avoidance response.

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74.5 PHENOXYBENZAMINE (PBZ) PREVENTS ANTEROGRADE AMNESIA (AA) CAUSED BY ELECTROCONVULSIVE SHOCK (ECS) IN MICE. A. Sattin. R.L. Roudebush V.A. Medical Center and Institute of Psychiatric Research, Indiana Univ. Med. Center, Indianapolis, IN 46223.

Electroconvulsive treatment (ECT) has long been recognized as the most predictable and efficacious treatment for major mental depressive disorders. The clinical popularity of ECT declined drastically following the advent of effective pharmacotherapy. Despite the measurable benefit of unilateral electrode placement, fear of transient memory and learning dysfunction (AA) following ECT is another factor that inhibits its clinical application. The demonstration by P.E. Gold and D.B. Sternberg (Science, 201: 367, 1978) that PBZ prevents retrograde amnesia (RA) induced by various convulsive and other amnesia treatments of rats and mice suggested a new approach to the prevention of AA.

Standardized hybrid mice (SEC/1ReJ X DBA/2J, F_1) were produced in our own lab. Housed in 3's in a 12-hr. normal light cycle, 6 groups of 12 mice were treated as follows: 1) Handling only, 2) Handlin + sham ECS, 3) PBZ + sham ECS, 4) PBZ + ECS, 5) Saline + ECS and 6) Saline + sham ECS. Treatment was given on 3 consecutive P.M.'s in the above order. On each treatment day ECS or sham ECS was given once and repeated 30 min. later. PBZ (1 mg/kg i.p.) or saline was given 30 min. before the first ECS or sham. ECS was transocular, 10 mA (constant current) X 1s. All ECS resulted in tonic hindlimb extension. All mice were given conditioned passive avoidance training on day 4 and retested on day 5 (see above reference for method).

All female mice were excluded from the analysis of results because of interfering estrus behavior. Two males did not survive the ECS. The residual n's in the 6 groups were 7, 7, 6, 5, 8, and 5, respectively. Every mouse exhibited maximum retest latency (300s) except for 6 of the 8 mice in the saline ECS group which entered the dark box after 14.8, 17.3, 24.0, 93.5, 207.3, and 224.0s. For this group, the difference from any other group was $p < 0.002$ (Mann-Whitney U-test, 1-tailed).

This result suggests that PBZ might be capable of preventing ECS-induced AA as well as RA. Since AA is the only significant adverse effect of clinical ECT, we plan to test this pre-treatment in humans. As the mechanism of this effect is unknown, further laboratory investigation of this paradigm will be pursued. Supported by V.A. Research Dept.

74.6 COGNITION ACTIVATING PROPERTIES OF DIHYDRO-1H-PYRROLIZINE 3,5 (2H,6H) -DIONE (CI-911) IN ANIMAL MODELS. J. G. Marriott, B. P. H. Poschel, R. E. Voigtman*, J. S. Abelson*, and D. E. Butler*. Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

Age-related cognitive impairments, such as those due to Alzheimer's disease, are a large and growing medical and social problem. Few therapies exist to treat such disorders. Those that are available produce only minimal improvement. Any new treatment which significantly reversed or retarded the progressive deterioration in cognitive function seen in elderly and demented patients would revolutionize therapy. An ongoing testing program in our laboratories to identify and develop new pharmaceutical treatments for cognitive disorders found that CI-911, dihydro-1H-pyrrolizine-3,5(2H,6H)-dione, improved memory in several animal models. Specifically, CI-911 strikingly reversed amnesia produced by electroconvulsive shock using one-trial, inhibitory avoidance training in mice. While a number of compounds have been found to have good amnesia-reversing activity, CI-911 was among the most active compounds ever tested, and this activity occurred over a very broad dose range (0.63 to 320 mg/kg, PO). Another distinguishing feature of CI-911 was its ability to improve short-term memory (STM) in animal models of normal and impaired memory. In male Long-Evans rats (4 - 30 mos of age) trained on a delayed alternation task, CI-911 significantly improved ($p < 0.01$) percent correct responding at delay intervals ranging from 15 to 120 sec. Similarly, aged rhesus monkeys performing a delayed response task showed significant improvement ($p < 0.05$) in percent correct responses at delay intervals ranging from 5 to 60 sec. The greatest improvement in memory was seen in the poorest-performing animals, those with the greatest, age-related memory impairment. The effects of CI-911 at longer delays appear to be due to direct actions upon memory and are not the result of effects upon nonspecific, performance aspects of the task, such as fatique, attention, or perception, since no changes were observed at 0-sec delay intervals. CI-911 was inactive in a general observational test for central nervous system effects and in tests of locomotor activity, intracranial self - stimulation, and self-administration in rhesus monkeys. On the basis of this pharmacology and the lack of toxicity in several animal species, CI-911 was recommended for clinical trials in patients with impaired memory function.

74.7 POTENTIATION OF THE AVERSIVE, BUT NOT REINFORCING EFFECTS OF MORPHINE USING A COMPOUND CONDITIONED STIMULUS. J. S. Miller*, D. F. McCoy*, K. S. Kelly*, and M. T. Bardo (SPON: J. F. Zolman).
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In a typical classical conditioning situation a conditioned stimulus (CS) is paired reliably with an unconditioned stimulus (US). After repeated pairings the CS alone is able to elicit a conditioned response (CR). When a compound CS is employed the individual elements are said to compete for associative strength, with the more "salient" element being more strongly conditioned. An exception to this is the case where animals are given pairings of an odor-taste compound CS followed by LiCL, an illness inducing US. The control group is given pairings of the odor alone and LiCl. When tested with the odor alone, the conditioned odor aversion is potentiated in animals trained with the compound CS. To date, studies examining potentiation in rats have utilized LiCL as the US. It is not clear how other unconditioned stimuli would function in this situation, especially one with reinforcing as well as aversive properties.

A series of studies was conducted to investigate whether the reinforcing and aversive effects of morphine could be potentiated by the use of compound cues. The first experiment demonstrated potentiation of morphine-induced odor aversion after one conditioning trial with a within-subject design. Potentiation was not obtained in one trial between groups, regardless of whether the CS was completely novel (Exp. 2) or somewhat more familiar as a result of CS preexposure (Exp. 3). However, repeated pairings did result in significant potentiation of the odor aversion (Exp. 4). Interestingly, potentiation of the reinforcing effect of morphine, as assessed in the conditioned place preference model was not obtained when external stimuli were substituted for odor stimuli (Exp. 5). These results provide a basis for comparing the effects of morphine with other primary reinforcers, as well as clarifying the situations in which potentiation of drug effects is likely to occur.

74.8 TIME-DEPENDENT EFFECT OF POST-TRAINING NALOXONE ON ONE-TRIAL APPETITIVE LEARNING. J. Zografos,* D.H. Malin, G.R. Travan* and P.K. Richardson.* (SPON: S. Burzynski).
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Post-training administration of the endorphin antagonist naloxone has been shown to improve subsequent retention of one-trial aversive learning in a time-dependent manner. The present study tested whether this effect would generalize to one-trial appetitive learning.

Subjects were 26 male rats deprived to 80% initial weight. A 36 cm square maze offered a choice between two ascending ramps, one in a highly accessible black corridor and one in a less accessible white corridor. Rats were allowed to find and eat 10 45 mg Bio Serve dustless pellets at the top of the white ramp. Their running speed was calculated as 100 times the reciprocal of their latency to reward. Immediately following reward, each rat was injected i.p. with either saline or 3 mg/kg naloxone. Each rat was retested for running speed 24 hours later. As Table 1 shows, the saline-injected rats increased their speed by 94% ($p<.005$, 1 sample t), while the naloxone-injected rats increased only 27% (NS). The running speeds on the training day did not differ significantly, but the saline rats were significantly faster than naloxone rats on the retention day ($p<.05$).

Table 1. Running speed (M±SEM) I.P. Injections Immediately Post-Training.

	Training Trial	Rentention Trial	Increase
NX 3mg/kg	2.75 ± 0.35	3.49 ± 0.57	27%
Saline	2.61 ± 0.35	5.05 ± 0.52	94%

A second experiment used similar procedures except that all injections were delayed two hours post-training. Both naloxone and saline injected groups showed excellent retention (respective increases of 85% and 93%) with no significant differences between groups.

Interference with retention of one-trial reward learning by an endorphin antagonist appears consistent with a role for endorphins in reward mechanisms as proposed by Stein and Belluzi. Naloxone's opposite effects on reward and punishment learning might reflect a tendency of endorphins as natural euphorients to attenuate effects of punishment and potentiate effects of rewards.

Supported by University of Houston-Clear Lake Organized Research Fund. Naloxone donated by DuPont Glenolden Laboratories.

74.9 EFFECTS OF OPIATE ANTAGONISTS ON SPATIAL MEMORY IN YOUNG AND AGED RATS. M. Gallagher, E. Bostock and R. A. King, Dept. of Psychology and Neurobiology Program, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514.

Recent studies reporting that aged rats exhibit impaired performance on spatial tasks have begun to explore the neural basis of these deficits. Two investigations have reported that opiate antagonists improve retention of spatial information in young rats. This investigation examined whether opiate antagonists would alter deficits exhibited by aged rats in spatial tasks using the 8-arm radial maze.

Young (4-8 mo) and aged (24-28 mo) male Long Evans rats were trained to visit each arm of an 8-arm maze only once during a session when a food pellet was placed at the end of each arm. During further training a 5 hr delay was inserted between the 4th and 5th arm choices within a session and training continued until rats achieved a criterion performance of no more than 2 errors on 3 consecutive days. Following this training phase, the experiments consisted of testing the rats on the maze in novel spatial environments, i.e., new rooms. A within-subject design was used for drug treatment. In one new room an opiate antagonist was administered; in the other room the same animals received vehicle injection. The opiate antagonist used for one group of young rats and a group of aged rats was naltrexone (1 mg/kg). For two other groups of young and aged rats the opiate antagonist was naloxone (2 mg/kg). All injections were administered IP on each day of testing at the beginning of the 5 hr delay until criterion performance was achieved.

The data from rats that received naltrexone revealed that aged rats required more trials than young rats to achieve criterion. In addition, naltrexone produced comparable improvements in both age groups ($p<.01$). The results for animals that received naloxone exhibited trends in the same direction, i.e., impaired performance in aged rats and improved performance for both age groups in the drug condition. The effect of naloxone, however, only approached significance ($p<.06$).

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74.10 EFFECTS OF NALOXONE ON RECOGNITION MEMORY IN MONKEYS. S. Martin*, T. Aigner*, R. Brown and M. Mishkin (SPON: S. Mitchell). Laboratory of Neuropsychology, NIMH, Bethesda, MD 20205 and Division of Preclinical Research, NIDA, Rockville, MD, 20852.

Administration of the narcotic antagonist naloxone has been reported to produce improvements in memory and cognitive functioning in some patients with Alzheimer's disease (Reisberg et al, New Engl. J. Med. 308: 721, 1983). Recent evidence suggests that naloxone can also facilitate performance in a spatial memory task in rats (Gallagher, Science 221: 975, 1983). To determine if opioid systems are involved in memory processes in monkeys, we administered naloxone to 5 macaques trained in delayed nonmatching-to-sample with trial-unique objects. This procedure has previously been shown to provide a sensitive measure of the effects of the cholinergic agents scopolamine and physostigmine in monkeys (Aigner et al, Soc. Neurosci. Abstr. 9: 826, 1983). In each daily session, the animals were shown a sample object every 15 sec until 40 objects had been presented. Each of these objects was then paired with a new object. Since the samples were presented in the same sequence on both occasions, approximately 10 min elapsed between the first presentation of the sample and its pairing with a novel object. The animal was rewarded for choosing the novel object in each pair. After performance had stabilized, doses of naloxone (0.1, 0.3, 1.0, 3.2 or 10.0 mg/kg) or saline were administered 20 min prior to the sessions. With the exception of the highest dose, each dose of naloxone was tested twice in an unsystematic order. At least one noninjection control session preceded each drug or saline session. Upper and lower 95% confidence intervals (CI) were determined for the mean of these control sessions for each animal (overall $\bar{X}$ = 73.1% correct). In each of the 5 animals, the percentage of correct choices was increased above the upper CI following the 0.3 mg/kg dose of naloxone (overall $\bar{X}$ = 80.8%). The effects of higher doses were more variable, either producing no effect or decreasing the number of correct choices. The results suggest that selective doses of naloxone can produce reliable, though modest, improvements in recognition memory in monkeys.

74.11 LSD: EFFECTS ON CLASSICAL CONDITIONING. C.W. Schindler, I. Gormezano and J.A. Harvey. Departments of Psychology and Pharmacology, The University of Iowa, Iowa City, IA 52242.

The effects of d-lysergic acid diethylamide (LSD) were studied on the acquisition, maintenance, extinction and differentiation of the classically conditioned rabbit nictitating membrane response. As previous research has shown that 12.9 µg/kg LSD is maximally effective in enhancing acquisition (Gimpl et al., J. Pharmacol. Exp. Ther. 208:330, 1979), this dose was used throughout the present experiments.

In the first experiment different groups of rabbits were trained with different CS-UCS (conditioned stimulus and unconditioned stimulus) intervals. Although LSD enhanced acquisition for all intervals, it was least effective at the CS-UCS interval which produced the most rapid acquisition for controls. In a second experiment, LSD was again shown to enhance acquisition. Subsequently, when LSD was given for the first time following acquisition (maintenance), no immediate change in percent conditioned responding (CRs) was observed. However, when LSD was discontinued during maintenance, there was an immediate decrease in percent responding. A similar effect was observed for extinction. During extinction, LSD given for the first time had no effect on the observed reduction in responding; however, if LSD was discontinued in extinction, there was an immediate and almost complete loss of CRs. Finally, in a fourth experiment, three different differentiation procedures were employed. For two procedures, CRs were first established for two stimuli, followed by either tone-light or tone-tone (1000 Hz-5000 Hz) differentiation. For the third procedure, tone-tone differentiation was in effect throughout training. LSD was tested only during differentiation, and failed to have any consistent effect for any of the three experiments.

The results of the experiments again indicate that 12.9 µg/kg LSD will enhance acquisition, but does not appear to affect the performance of CRs as indicated by the lack of an effect of LSD when given for the first time in either maintenance or extinction. However, the results also indicate that LSD produces asymmetrical state-dependent learning as indicated by the dramatic reduction in either extinction or maintenance responding when LSD was discontinued. Finally, despite the fact that LSD enhances CS processing (Gormezano & Harvey, J. Comp. Physiol. Psychol. 94:641, 1980), LSD had no effect on the differentiation of stimuli.

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74.12 EPINEPHRINE FACILITATES INHIBITORY AVOIDANCE RETENTION OF ADRENAL DENERVATED RATS TREATED WITH A HIGH DOSE OF DSP4. C. Bennett*, S. Kaleta*, M. Arnold*, and J.L. McGaugh. Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717

Brain norepinephrine (NE) levels within 30 minutes after training have been correlated with retention performance (Beh. Biol. 24, 168). This result suggests that the release of brain NE modulates memory formation. Further, epiniphrine (EPI) given peripherally in doses that affect retention, can potentiate the decrease in CNS NE after training (Beh. Biol. 23, 509). Therefore systemic EPI may influence memory formation by triggering the release of central NE. In the present experiment we tested the possibility that the memory modulatory effects of peripherally administered EPI, is blocked by central NE depletion.

Male Sprague-Dawley rats were adrenal denervated to deplete adrenal catecholamines (Br. Res. 201, 236). Two weeks after the adrenal surgery, they were given 0.9% saline (Control) or N-(2-chloroethyl) N-ethyl 2-bromobenzylamine (DSP4) (100 mg/kg, ip) in a divided dose to deplete central and peripheral NE (Br. J. Pharm. 58, 521). The DSP4 was synthesized by two of us (Arnold & Bennett) after the method of Krueger & Cook (Arch. Int. Pharm. 218, 96). Two days after the second dose, while sympathetic as well as central NE stores were depleted, the rats were trained on a one-trial step-through inhibitory avoidance task (8). Any animal which did not step through within 90 seconds was eliminated. Immediately after training, each rat received saline (Sal) or EPI (0.1 mg/kg, sc). Retention, measured as the latency to step through, was tested 24 hours later.

Significantly more DSP4 (8/31) than Control (1/28) rats were eliminated for failing to step through within 90 seconds (Chi Sq. = 4.04, $p < 0.05$), however, the entrance latencies of the DSP4 and Control rats which were trained did not differ significantly. The retention scores of the DSP4/Sal and the Control/Sal groups did not differ significantly, therefore, DSP4 does not appear to affect acquisition or retention of this task. However, the difference in the entrance latencies suggests that DSP4 treatment may impair spontaneous activity or response initiation. EPI significantly enhanced the retention of both Control and DSP4 groups. These data suggest that the memory modulatory effects of systemic EPI are not likely to be explained by an EPI-stimulated release of central NE.

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74.13 ROLE OF ALPHA- AND BETA-ADRENERGIC RECEPTORS IN LEARNING AND MEMORY IN MICE. G.D. Novack, D.B. Sternberg and J.L. McGaugh. Center for the Neurobiology of Learning and Memory and Department of Psychobiology, University of California, Irvine, CA 92717

Adrenergic agonists, both endogenously released and exogenously administered, have been found to enhance learning and memory. Previous research in this laboratory and others has shown both a peripheral and central role for this modulation. In an effort to classify pharmacologically the receptors involved, we evaluated the effect of various adrenergic antagonists on performance in a one-trial learning inhibitory-avoidance task in mice. Male CFW adult mice, 22-33g, were acclimated to the housing conditions for 1 week or more. Mice were weighed, marked, and trained in a two-chambered apparatus. After moving from a lighted to a dark area, mice received a 750 µA shock for 2 sec. Antagonists were administered i.p. immediately following the training trial in doses which elicit a systemic blockade. They were returned to home cages, and 24 hr later, tested in the same apparatus. A 300 sec cutoff was used on the testing day. All antagonists used block both peripherally and centrally. Preliminary results follow:

Antagonist	Receptors	Dose (mg/kg)	Effect on Performance	Dose-related
Prazosin	a-1	0.1- 1.0	Attenuation	+
Yohimbine	a-2	0.3- 3.0	Mild Attenuation	-
Phentolamine	a-1/a-2	1.0-10.0	Mild Facilitation	±
Propranolol	b-1/b-2	1.0-10.0	Attenuation	+

The attenuation of retention by adrenergic antagonists suggests a role for adrenergic agonists in learning and memory. The clear-cut dose-relationship of the effects of propranolol and prazosin suggests an involvement of a-1 and b-1/b-2 receptors. As the effects of the a-2, and mixed alpha-antagonists were mild and not clearly dose-related, the degree of involvement of these receptors is less clear.

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74.14 AMYGDALA NORADRENERGIC SYSTEM & MEMORY MODULATION: INVOLVEMENT IN THE ENHANCING EFFECT OF PERIPHERAL EPINEPHRINE. K.C. Liang, R.G. Juler* & J.L. McGaugh. Center for the Neurobio. of Learning & Memory, Univ. of Calif., Irvine, CA 92717 & Dept. of Psychol., Nat'l Taiwan Univ., Taipei, Taiwan, ROC

Evidence indicates that post-trial systemic injections of epinephrine (E) enhance retention of learned responses and alter the forebrain norepinephrine (NE) levels. In view of our recent findings that the amygdala (Amyg) is involved in the memory modulatory effect of peripheral E, we investigated the interaction between the Amyg NE system and peripheral E in modulating memory processes.

Male Sprague-Dawley rats with cannulae implanted bilaterally into the Amyg were trained on a step-through inhibitory avoidance task (0.7 mA/ 1 s footshock unless otherwise noted). Retention was tested 24 hrs later. In Exp.1, rats received bilateral intra-Amyg injections of NE or vehicle (Veh) (1 µl per side). Posttraining intra-Amyg injections of 0.1 or 0.3 g NE enhanced retention ($p<0.02$, 0.05*; respectively), while higher doses of NE had no effect. Intra-Amyg injections of 0.2 µg NE enhanced retention ($p<0.02$) if given immediately after training, but had no effect if given 3 hrs later. Further, propranolol (Prop) injected concurrently with NE into the Amyg blocked the NE enhancing effect (0.2 µg NE vs 0.2 µg NE + 0.2 or 1.0 µg Prop $p<0.02$,0.05; respectively). In Exp.2, adrenal sham operate (Sham) and demedullated (ADMX) rats were trained on 1.0 mA/1.0 s footshock and injected with 0.2 µg NE or Veh into the Amyg immediately after training. As found previously, adrenal demedullation impaired retention in the implanted rats given Veh (ADMX/ Veh vs Sham/Veh $p<0.05$). However, intra-Amyg injections of 0.2 µg NE attenuated this retention deficit (ADMX/Veh vs ADMX/NE $p<0.05$). In Exp.3, immdiately after training, rats received first an intra-Amyg injection of 0.2 µg Prop or Veh and then a s.c. injection of 0.1 mg/kg E or saline (Sal). While E enhanced retention in rats given intra-Amyg Veh (E/Veh vs Sal/Veh $p<0.02$), it had no effect on rats given intra-Amyg Prop. Thus, intra-Amyg Prop blocked the memory enhancement induced by peripheral E.

These findings, taken together, are consistent with a hypothesis that the Amyg NE system may be involved in the memory modulatory effect of peripherally administered E. *Statistics are based on two-tailed Mann-Whitney U-tests.

The present study is supported by USPHS Research Grants MH12526 and AG00538 (to JLMcG).

74.15 FLUOXETINE, A SEROTONIN UPTAKE BLOCKER, ENHANCES MEMORY PROCESSING IN MICE. A. Cherkin and J. F. Flood. GRECC, VA Medical Center, Sepulveda, CA 91343.

Meyer et al. [J Am Geriatr Soc. 25, 289-298 (1977)] administered amino acid precursors of serotonin (5-HT) and of dopamine (DA) to 10 patients with severe dementia, and observed memory improvement in 2. Few pharmacological agents are available to probe the effects of serotonin on memory in controlled laboratory experiments. 5-Hydroxytryptophan (5-HTP), the immediate precursor of serotonin, has anti-amnestic effects but only at large doses which increase plasma corticosterone substantially. These effects are non-specific because exogenous corticosterone is anti-amnestic. We now report the effects on memory retention in mice of fluoxetine hydrochloride which blocks serotonin uptake but with only limited effects on DA and norepinephrine. A high dose of fluoxetine hydrochloride (30 mg/kg) impaired acquisition when injected subcutaneously 1 hr before training in a T-maze active avoidance paradigm using the procedure of Flood et al. [Neurobiol Aging 4, 37-43 (1983)]. The ED50 was 25 mg/kg; 5, 10, and 15 mg/kg were without significant effect compared to controls. Retention measured 1 wk after injecting fluoxetine 1 hr before training was optimally improved by doses of 0.5 to 5 mg/kg. Fluoxetine also improved 1-wk memory retention when administered immediately post-training, either intracerebroventricularly (8 or 12 ug/mouse) or subcutaneously (10 or 15 mg/kg). Fluoxetine (15 mg/kg) blocked the amnesia induced by scopolamine (1 mg/kg). The results suggest that an increase in synaptic 5-HT enhances memory processing since the immediate effect of blocking uptake would be to increase the level of 5-HT in the synapse.

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74.16 FACILITATION OF RETRIEVAL FOLLOWING PRE-TEST ADMINISTRATION OF PIRENPERONE IN MICE. H.J. Normile* and H.J. Altman. SPON: H. Goldman). Lafayette Clinic, 951 E. Lafayette, Detroit, MI 48207.

There is evidence indicating that serotonin (5-HT) plays a significant role in the processing of information by the brain. Although the behavioral effects are often dependent on a number of factors, it generally appears that acute stimulation of 5-HTergic neurotransmission interferes, while disruption facilitates performance on a variety of learning and memory tasks. However, the results are not entirely consistent and often susceptable to a number of methological criticisms. Moreover, attention has generally focused on events on/or about the time of training. The following series of experiments were designed, therefore, to determine what effects a 5-HT antagonist might have on memory retrieval in Swiss-Webster mice. The 5-HT antagonist used was pirenperone (PIREN) a highly selective 5-HT type-2 receptor antagonist with little to no agonist activity. The behavioral task used was a modification of the standard thirst motivated one-trial inhibitory avoidance task (Quartermain and Altman, 1982). All injections were made prior to the retention test. In Experiments 1 and 2, dose- and time-dependent relationships were established. In Experiment 3, an attempt was made to block the PIREN induced facilitation of retrieval using 5-HT agonists and a variety of antagonists of other neurotransmitter systems. Peripheral administration of PIREN (1.0 mg/kg) 30 min. prior to the retention test results in a significant enhancement of memory. The facilitation was both time - and dose - dependent and could not be attributed to non-specific effects of the drug on behavior in general since the latencies of an independent group of non-contigently shocked animals were not significantly different from saline injected controls. The facilitation induced by PIREN could not be antagonized by any of the agonists or antagonists examined except the alpha-adrenergic antagonist phenoxybenzamine. While failing to reach statistical significance there was, however, a clear trend towards an antagonism of PIREN following haloperidol, scopolamine and bicuculline. The results confirm and extend earlier observations indicating an involvement of 5-HT in memory and suggest that interactions with other neurotransmitter systems may underlie its effects on such behavior.

74.17 NIGRAL MEDIATION OF SUBSTANCE P-INDUCED MEMORY ENHANCEMENT. M. Pelleymounter*, K. Schlesinger* and J. Stewart (SPON: L.Crnic). Dept. of Psych. and the Inst. for Behavioral Genetics, U. of Colo., Boulder, CO 80309 and the U. of Colo. Sch. of Med., Denver, CO 80262.

Peripheral injection of Substance P (SP) results in enhanced retention of passive avoidance (PA). This increased retention is accompanied by monoamine level changes in specific brain regions 24 h after treatment. This study examined some of the characteristics of these changes in relation to the behavioral effects of SP. Male HS mice were treated with infusions of the neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT) or of ascorbic acid/saline vehicle into the striatum (STR) or substantia nigra (SN). Some mice also received 6-hydroxydopamine (6-OHDA) lesions of the SN. After a two-week recovery period, mice were trained for a PA task. On the training day, latency to enter the dark chamber of the apparatus was measured prior to footshock (0.4 mA, 5 sec). Mice were then injected with SP (1ng/g, sc) or acidified saline. Latency to re-enter the dark chamber was measured 24 h later. Retention was defined as the difference in latencies. Serotonin (5HT) and dopamine (DA) depletion was verified by high pressure liquid chromatography with electrochemical detection (HPLC-ECD) analysis of frontal cortex (FC), STR and SN homogenates. Mice injected with SP following training showed better retention of the PA task than saline controls in all lesion groups except for those with SN 5HT depletion. This group showed a reversal of the SP effect. 5,7-DHT lesions resulted in substantial 5HT reduction in the FC, STR or SN, depending on the lesion site. 6-OHDA SN lesions also resulted in large reductions in STR DA. Further examination of the SN role in SP memory enhancement involved measurement of DA and 5HT release 24h after training and SP injection into intact mice. Release, defined as the ratio of amine to metabolite levels, was measured in STR, SN and FC and analyzed by HPLC-ECD. Significant changes in release were found in SN 5HT and only in groups treated with shock and/or SP. This suggests that SP-induced retention of PA may be mediated via a nigral 5HT pathway. (Supported in part by NIH grant NS 18531).

74.18 ON THE NEUROCHEMICAL NATURE OF RAT SPATIAL MEMORY FORMATION. S. J. Y. Mizumori, M. R. Rosenzweig, E. L. Bennett, V. Channon* & M. R. Pease*. Dept. of Psychology and Melvin Calvin Lab., University of California, Berkeley, CA 94720.

The neurochemical basis of the formation of spatial memories was examined using different pharmacological agents. Male Long-Evans rats were trained on a 12-arm radial maze. After extensive training with 4 hr delays interposed between choices 6 and 7, cannulae were implanted bilaterally into ventral hippocampus. Following post-surgery training to criterion, the rats were injected on different days with the protein synthesis inhibitor anisomycin (ANI) or saline either 30 min before choice 1 or 5-10 min after choice 6. ANI injected before training impaired subsequent retention over a 4 hr delay while ANI given during the delay had no effect on retention.

Analysis of the temporal course of development of the ANI-induced amnesia revealed that memory was good when either no delay or a 2 min delay was interposed between choices 6 and 7. In contrast, when rats were required to retain information over delays of 15 min or longer, ANI-treated rats performed poorly at test. These data suggest that protein synthesis is important for even temporary storage of information, provided the retention interval is long enough. Here, protein synthesis was important for normal function of a long-term component of working memory.

Based on the model of memory formation proposed by Gibbs and Ng (1977), a different class of drugs was used to investigate the neurochemical bases of a short-term component of working memory. Additional rats were trained in the radial maze as described above. When injected 30 min before choice 1, lanthanum chloride, a calcium uptake inhibitor, and glutamate significantly impaired retention over a 4 hr delay interval. Preliminary results indicate that, unlike ANI-induced amnesia, glutamate-induced amnesia is evident after a 2 min delay. Rats injected with lanthanum or glutamate 5-10 min after choice 6 showed good retention 4 hr later. Further comparisons of the temporal characteristics of the development of amnesia caused by different agents will be reported.

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74.19 PHARMACOLOGICAL DISSOCIATION OF MEMORY: ANISOMYCIN, A PROTEIN SYNTHESIS INHIBITOR, AND LEUPEPTIN, A PROTEASE INHIBITOR, CAUSE OPPOSITE EFFECTS ON MEMORY. U. Staubli*, R. Faraday* and G. Lynch (SPON:D. Needels). Center for the Neurobiology of Learning & Memory, Univ. Calif., Irvine CA 92717.

Recently we reported that intraventricular infusions of the calcium proteinase inhibitor leupeptin produced an impairment of tasks requiring spatial memory (Staubli et al., Behav. & Neural Biol. 1984,40:58-69) ; this treatment did not influence spontaneous activity, habituation to novel environment, escape conditioning or learning of inhibitory avoidance responses. We suggested that these effects were due to the inhibition of a membrane associated calcium protease thought to regulate glutamate binding sites in the telencephalon. An alternative interpretation is that spatial memory in a complex maze is more easily disrupted than avoidance conditioning. The present experiments approached this problem in two ways: 1) we tested leupeptin on a simple memory problem that is dependent on forebrain areas and 2) the effects of a treatment known to impair avoidance learning were analyzed on a leupeptin-sensitive task. The rats were trained on a series of two odor discriminations until they acquired the novel,correct odor in less than 5 trials. Leupeptin was then infused into the ventricles and the animals tested on a new series of discriminations. The drug produced a profound, reversible impairment in this test. Lesions of the hippocampus block the memory needed for this problem.(Staubli et al P.N.A.S., in press). In a second experiment dosages of the protein synthesis inhibitor anisomycin were established that produce a severe impairment of both active and passive conditioning.The same treatments were tested in the olfactory memory task using the same number of trials as in the avoidance problems and were found not to disrupt either acquisition or retention 24 hours later. Thus leupeptin blocks olfactory memory but not avoidance conditioning while anisomycin produces the opposite pattern of results--in essence a pharmacological double dissociation. This finding strongly suggests that olfactory and spatial memories are not easily disrupted and therefore that the impairments produced by leupeptin are due to selective biochemical actions of the drug, presumably on proteases. They also provide evidence that very different cellular mechanisms are involved in different forms of memory.(supported by NIMH grant 19793 and NSF grant 17370).

LEARNING AND MEMORY: CHOLINERGIC PHARMACOLOGY

75.1 SCOPOLAMINE AND BODY-ROTATION INDUCED CONDITIONED TASTE AVERSIONS IN RATS: INTERACTIVE EFFECTS. R. L. Ladowsky and K.-P. Ossenkopp. Dept. Psychology, University of Western Ontario, London, Ontario, Canada, N6A 5C2.

Both body-rotation and scopolamine hydrobromide (SHB), a centrally acting drug often used for prevention of motion sickness in humans, have been shown to produce conditioned taste aversions (CTA) in rats when administered after the presentation of a novel flavour. Whereas lesions of the area postrema, a circumventricular structure in the fourth ventricle, abolish scopolamine induced CTAs, these types of lesions enhance taste aversions formed in response to body-rotation (Ossenkopp, Beh. Neur. Biol., 1983, 38, 82). In the present experiment we examined the combined effects of scopolamine injections and body-rotation on induction of CTA in rats.

Eight groups of adult male hooded rats were kept on a $23\frac{1}{2}$ hr/day water deprivation schedule. These animals were given 4 pairings of a 0.1% sodium saccharin solution taste CS with an unconditioned stimulus (US) which differed for each group. Four groups of rats received injections of either saline (vehicle), SHB (1 mg/kg), SHB (0.1 mg/kg), or scopolamine methyl nitrate (SMN) (0.1 mg/kg), immediately after access to the saccharin, and following the drug injection these animals were subjected to 30 min of body-rotation (70 rpm, on a 15 sec on - 5 sec off schedule). Another 4 groups received the same 4 drug treatments respectively, but these groups were given sham rotation after the injection. The conditioning days were seperated by days with $\frac{1}{2}$ hr access to water only.

There were no significant group differences in water consumption on water only days. In both 1 bottle and 2 bottle choice tests, rats that received the combined US of body-rotation plus a 0.1 mg/kg dose of either SHB or SMN exhibited significantly stronger CTAs by the fourth conditioning day than the groups which received either the equivalent drug injections plus sham rotation or an equivalent amount of body-rotation plus saline. There was no significant difference between the group that received 1 mg/kg SHB plus the body-rotation and the group that received 1 mg/kg SHB plus sham rotation, possibly due to a floor effect. Thus, at a dose of 0.1 mg/kg both SHB and SMN combine with body-rotation in an additive manner to produce stronger CTAs than those produced by the drugs alone or those produced by the body-rotation alone. (Supported by Natural Sciences and Engineering Research Council grant U0151 to K.-P. Ossenkopp).

75.2 THE ROLE OF HIPPOCAMPAL CHOLINERGIC ACTIVITY IN TASTE-POTENTIATED ODOR AVERSION LEARNING. F. Bermudez-Rattoni, and J. Garcia.* Mental Retardation Research Center, UCLA, Los Angeles, California 90024, and CIFC, Universidad Nacional Autonoma de Mexico.

Flavor is primarily composed of odor (O) and taste (T) which are functionally distinct in conditioning and memory for flavor aversions. T is an excellent conditioned stimulus (CS) for a delayed emetic unconditioned stimulus (US), but O is not. However when O and T are combined into a compound OT-CS and followed by a delayed emetic US in a single acquisition trial, O proves to be even more aversive than T when each is tested alone in extinction. O appears to be "potentiated" by T, as if a sensory "and-gate" controlled by T shunts simultaneous Os into food-related memory mechanisms.

We have demonstrated that the limbic system is apparently involved in gating O; novocaine applied to the amygdala specifically disrupts O-toxin associations. Agonists and antagonists of cholinergic activity affect O-toxin more than T-toxin associations when applied to the amygdala. We are now reporting on the effects of similar treatments applied to the hippocampus and the parietal cortex.

Rats were implanted with bilateral cannulae aimed at the dorsal hippocampus or the parietal cortex. Different groups were infused with physostigmine (9 μg), Scopolamine (30 μg) or Saline for three minutes. Thirty minutes later, an OT compound CS was followed by a delayed toxic lithium US in a single conditioning trial. Beginning three days later, the rats were given a series of tests with O and T presented separately in counterbalanced order on different days.

Physostigmine applied to the dorsal hippocampus produced a reliable disruption of O-toxin aversions. Scopolamine produced the opposite effect, a reliable facilitation of O aversions. Neither drug affected the T aversions when applied to the hippocampus. When drugs were applied to the parietal cortex, no effects were observed. There appears to be an inverse relationship between hippocampal cholinergic activity and the formation of a potentiated odor aversion during acquisition of a conditioned flavor-toxin aversion.

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75.3 CHOLINERGIC BLOCKADE OF THE CAUDATE NUCLEUS AND PASSIVE AVOIDANCE. PROTECTION AGAINST RETENTION DEFICITS BY INCREASING THE VALUE OF THE NEGATIVE REINFORCER. Magda Giordano* and Roberto A. Prado-Alcalá. Psychophysiol. Lab., Sch. of Psychol., Anahuac Univ., México and Dept. of Physiol., Sch. of Med., Natl. Univ. of México, P.O. Box 70250, México, D.F., 04510.

Injections of acetylcholine receptor blockers into the caudate nucleus (CN) induce an amnestic state in animals trained in operant tasks mediated by positive reinforcers. When these treatments are given to overtrained animals, however, no retention deficits are seen. To test the generality of these findings, rats (Ss) were trained in a one-trial passive avoidance task using a two-compartment box. Ten sec after being put into the safe compartment the Ss were allowed to step into the gridded compartment where they received a five-sec footshock. The latency to step into the gridded compartment was measured, again, 1 and 7 days later. Independent groups, given a 0.25 mA footshock during training, were injected bilaterally with atropine (0, 30, 45, 60, or 90 ug/3 uL)into the anterior CN two min after training. Other groups were trained using a 0.5 or a 1.0 mA footshock and injected into the CN, also two min after training, with 60 or 90 ug/3 uL. A dose-dependent retention deficit was found in those groups that received the 0.25 mA footshock. No deficits in memory were seen in any of the other groups. These data give further support to the hypotheses that: a) cholinergic activity of the CN plays an important role in memory processes associated with the acquisition and early maintenance stages of operant behaviors, mediated by "low" values of the reinforcers, and: b) the striatal cholinergic system is not involved in memory in conditions of overtraining or when high values of a negative reinforcer are given.

75.4 CHOLINERGIC LESIONS OF THE HIPPOCAMPUS PRODUCE LONG-TERM ALTERATIONS OF REACTIVITY AND COGNITIVE FUNCTION. T. J. Walsh*, D. L. DeHaven, H. A. Tilson, R. B. Mailman, A. Fisher* and I. Hanin. (SPON: C. L. Mitchell), Lab. Behav. Neurol. Tox., NIEHS, RTP, NC; Univ. North Carolina Sch. Med. and Univ. Pittsburgh Sch. Med.

AF64A is a specific cholinotoxin which reduces presynaptic cholinergic markers including choline acetyltransferase (ChAT) activity, high affinity Ch uptake and ACh concentrations (Mantione et al., Science 213, 1981). This cholinergic deficit is persisten and occurs without concomitant alterations in other transmitters. AF64A (15 or 30 nmol) also impair retention of a passive avoidance (PA) response and performance in a radial-arm maze (RAM) following i.c.v. administration (Walsh et al., Brain Res., in press). These deficits are associated with a 50% decrease of ACh in both the hippocampus (HPC) (15 and 30 nmol) and frontal cortex (30 nmol). The content of DA, DOPAC, HVA, NE, 5-HT, 5-HIAA and Ch is not affected.

To examine the anatomical specificity of the behavioral effects the present study examined the behavioral properties of HPC cholinergic mechanisms. AF64A (4 nmol/side) or art. CSF were directly infused into the dorsal HPC of adult male Fischer rats. Motor activity, hot-plate latencies and the acoustic startle response were measured 7 days prior to surgery and again at 2, 7, 14, 21 and 28 days after surgery. Cognitive behavior was assessed 35 days after dosing with a step-through PA task and 60-99 days after dosing in the RAM.

Activity and responsiveness to acoustic stimuli were enhanced 2-3 fold in the AF64A group. This group also reacted 48-84% faster than the CSF controls on the hot-plate. This behavioral hyperreactivity persisted throughout the 28 day test period. During the PA retention test the AF64A group exhibited shorter step-through latencies and more partial entries than the controls. In the RAM the AF64A group made fewer correct responses and required more choices to complete the task. Animals were sacrificed 120 days after dosing and neurochemical assessment revealed that ChAT activity was reduced (40%) only in the HPC of the AF64A group. The regional concentrations of DA, NE, 5-HT and their metabolites were not altered; except for DOPAC which was elevated 50% in the striatum.

These studies suggest 1) that cholinergic processes in the HPC modulate behavioral reactivity and cognitive function and 2) that AF64A can be used to elucidate the cholinergic substrates of behavior.

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75.5 PERFORMANCE FACTORS IN REGARD TO IMPAIRED MEMORY AND TOLERANCE INDUCED BY ATROPINE SULFATE. G.J. Thomas, J.W. Kasckow and R.M. Herndon. Ctr. Brain Res., Univ. Rochester Sch. Med. & Dent., Rochester, NY 14642.

It has been shown that "representational" memory is impaired and tolerance to the drug scopolamine can occur after systemic injection (ip) or after intracerebral injection into hippocampus (Messer, W.S., Jr. et al., Soc. Neurosci. Abstr., 9:826, 1983). In the above study, the memory concept was derived from a paired-run procedure which presents the animal with a delayed nonmatching to sample task in a T-maze. Rats first received an information run (I, 1 door); immediately afterwards they were placed in the start box for the choice run (C, 2 door), and when the start door opened they had to choose the opposite arm. Representational memory is indicated by their accuracy of choice which depends on the memory of which T-maze arm they entered last.

The present work studied the effects of atropine sulfate, another anticholinergic drug, with the same paired-trial procedure. Intraperitoneally administered atropine sulfate impaired representational memory in a dose-related manner. Doses of 5 mg/kg produced no change in the rat's accuracy of choice. Doses of 10 mg/kg caused performance to fall to 93% ($p < .05$). Doses of 20 mg/kg caused the median percentage correct to fall to 40% ($p < .025$). All comparisons were made with the same rats doing 100% correct after control injections of saline on alternate days. There was also a tolerance effect seen after repeated injections of 20 mg/kg.

A different group of rats, trained on the same T-maze and given a dose of 20 mg/kg on 3 different days interspersed with saline injections (1 injection per day) also showed tolerance. The first 2 injections of atropine reduced accuracy to levels that differed significantly from the 100% accuracy of the same rats when given saline ($ps < .01$ and $< .05$). The third injection of atropine had no significant effect.

Both studies found marked performance effects of the drug as indicated by the response-time scores. In the first study, doses of 20 mg/kg produced significant increases in response times for both I and C runs (both $ps < .025$). In the tolerance study, there was also an increase in times for the first 2 injections (both $ps < .01$). Hence, it cannot be concluded that atropine sulfate is only affecting representational memory.

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75.6 RADIAL MAZE ACQUISITION IN RATS WITH BASAL FOREBRAIN LESIONS CAN BE ACCELERATED WITH DRUG-THERAPY. D. S. Olton and G. L. Wenk, Department of Psychology, The Johns Hopkins University, Baltimore, MD 21218.

Bilateral combined lesions of both the nucleus basalis magnocellularis and medial septal area were made by injections of 25 nmoles of ibotenic acid into each area in three groups of rats. Three days later, the first group received IP injections of pentoxifylline (20 mg/kg) and piracetam (100 mg/kg), had choline chloride (8%,w/w) added to the diet and lived in an enriched environment. The second group did not receive drug therapy, but did have an enriched environment. The third group did not receive any therapy. A fourth group, controls, did not receive lesions nor therapy. After three weeks of drug therapy, each rat was tested in an 8 arm radial maze.

At the beginning of testing, all groups had low choice accuracy; at the end of testing, all groups had improved choice accuracy. Rats with therapy acquired the task faster than those without therapy, and at the same rate as controls.

Cholinergic biochemical indicators did not differ significantly among all lesioned groups of rats, treated or untreated. Choline acetyltransferase activity, an indicator of the integrity of cholinergic neuronal terminals, decrease significantly in the cortex (-53%) and hippocampus (-51%) as compared to the values in unlesioned controls. Sodium-dependent high affinity choline uptake, an in vitro indicator of cholinergic neuronal activity, decreased slightly in the cortex (-29%), but not in the hippocampus in rats with lesions as compared to control levels. (^{3}H)-quinuclidinyl benzylate binding in the cortex and hippocampus of rats in all lesioned groups did not differ significantly from the values in controls. These results suggest that the therapy can accelerate acquisition of tasks requiring working memory in rats with basal forebrain lesions. (Supported by grant DAMD 17-82-C-2225)

75.7 MEMORY ENHANCING EFFECTS OF COMBINING CHOLINE AND PIRACETAM IN MIDDLE-AGED MICE ON A CHRONIC CHOLINE DEFICIENT DIET R.L. Dean and R.T. Bartus, Med.Res. Div. of American Cyanamid Co., Lederle Labs., Pearl River, NY 10965

Considerable research indicates that central cholinergic mechanisms play an important role in the expression of learning and memory. Reports from several neuroscience disciplines suggest that dysfunctions in some of the cholinergic mechanisms may be intimately involved with the loss of memory and other cognitive functions in aged animals and humans. Recent evidence from our lab (Bartus, et al, Neurobiol.Aging, 1981) suggests that the effect of cholinergic manipulation on memory processes, via increased acetylcholine precursor availability, may be greatly enhanced by the simultaneous administration of piracetam, a nootropic drug purported to enhance oxidative metabolism. In that series of studies, the combined administration of choline/piracetam improved performance of aged F344 rats on a passive avoidance task (a task in which aged rats demonstrate severe impairments) to a greater extent than that obtained with either drug alone.

In an attempt to gain further insight into the generality of this pharmacological effect, retired breeder C57 mice (9 mo.) were placed on a chronic choline-deficient diet (chronic manipulation of the cholinergic system via choline deficient diet produces significant changes in behavior in mid-aged mice, similar to those which occur naturally with old age (Bartus, el al., Sci., 1981).After 6 mo. on the choline deficient diet (at 15 mo. of age), the mice received one of four supplements in their drinking water for 1 wk: 1) placebo (normal water), 2) choline (100 mg/kg/dy), 3) piracetam (100 mg/kg/dy), or 4) combination of choline/piracetam (same doses as groups 2 and 3). Following this regime, the mice were trained and tested on a single trial passive avoidance task. Thirty min. prior to training and 24 hr. retention testing, each animal was given a supplemental injection, consistent with the original treatment condition.

The results demonstrate that while piracetam failed to produce significant effects on retention in chronic choline deficient mice, supplemental choline and the combination of choline/piracetam significantly improved performance. Although the mechanism(s) of action of choline/piracetam combination remain unknown, the ability of this drug combination to reduce various types of neuronal dysfunctions, including some associated with age is intriguing. Further, the possibility should be explored that other combinations using different agents may be even more effective.

75.8 THE EFFECTS OF ANIRACETAM (RO 13-5057) AND PIRACETAM ON THE ENHANCEMENT OF MEMORY IN MICE.
G. Vincent, A. Verderese*, and E. Gamzu, Hoffmann-La Roche Inc., Nutley, NJ 07110

Nootropics or cognitive-enhancing compounds are beneficial in attenuating learning and memory deficits in both humans and laboratory animals (Cumin et al., 1982; Scott, 1979). Aniracetam (1-anisoyl-2-pyrrolidinone) and its parent compound piracetam (2-pyrrolidinone acetamide) have been shown in mice to protect against retrieval disrupted by electrobrain shock (Gamzu and Perrone, 1981; Vincent et al., 1983). Here we report that aniracetam but not piracetam improved memory consolidation in mice that exhibited spontaneous deficits in acquisition (i.e., "poor learners") in an automated platform-jump avoidance proedure.

Mice were trained to avoid shock in two 5-trial sessions, separated by 4 hours. Testing was conducted 24 hours later using a 10-trial session. "Poor learning" mice, defined as those animals that failed to successfully escape shock during the first training session, were given aniracetam (10-1000 mg/kg) or piracetam (30-1000 mg/kg) immediately after the second training session. Mice given aniracetam (30, 100, 300, and 1000 mg/kg) exhibited a significant improvement in avoidance behavior during testing compared to vehicle-treated mice; in contrast, piracetam treatment resulted in no significant improvement. Aniracetam (300 mg/kg) administered 2 hours following the second training session was ineffective in enhancing avoidance behavior. These findings demonstrate a time- and dose-related enhancement of memory consolidation that is specific for aniracetam.

75.9 ELEVATIONS IN LOCAL CEREBRAL GLUCOSE UTILIZATION PRODUCED BY THE CHOLINERGIC AGONIST ARECOLINE. T.T. Soncrant*, H. Holloway*, S. Carlson*, S.I. Rapoport (SPON: N. Cutler). Laboratory of Neurosciences, National Institute on Aging, NIH, 10/6C103, Bethesda, MD 20205.

Arecoline (AREC) is a cholinergic agonist which stimulates central and peripheral muscarinic receptors. After i.p. administration to rats, AREC gains rapid entry to the brain, and produces a centrally-mediated tremor which peaks within a few min and subsides after 20-30 min. Memory-enhancing effects of AREC and of other cholinomimetics have been described in animals and in normal human subjects.

We measured local cerebral glucose utilization (LCGU), using the quantitative [14C]-2-deoxy-D-glucose (DG) method, at 3 min after administration to 3 mo Fischer rats of AREC 0.05, 0.5, 5, 15, or 50 mg/kg or saline i.p. Animals were pretreated with methylatropine (a cholinergic antagonist which does not enter the brain) 4 mg/kg s.c. to prevent parasympathomimetic side-effects of AREC. Tremor was rated subjectively at fixed intervals on a 4-point scale.

Intensity of tremor was dose-related, peaked at 2-5 min after AREC, and abated within 30 min. LCGU (measured after DG injection during peak behavior) increased after AREC in a dose-dependent fashion in most of the 95 brain regions examined. After the lowest dose of AREC, LCGU was increased significantly in only 3 regions (mean overall increase 12%), whereas the highest dose elevated LCGU in 73 regions (mean increase 156%). No declines in LCGU were observed; however, regions of the auditory pathway, and superficial neocortical layers (I-III) were generally unaffected by AREC.

LCGU rose in all regions of the hippocampus (pyramidal layer) and cerebral cortex (layers IV and VB) which have high muscarinic receptor densities. Selective (greater than overall mean) metabolic increases were produced by all doses of AREC in extrapyramidal motor regions (caudate, globus pallidus, subthalamic n., red n., substantia nigra, reticular formation) which mediate tremor. LCGU in hippocampal areas was increased by all doses of AREC, but was selectively enhanced only by the lowest 2 doses (accounting for 2 of 3 regions significantly increased after AREC 0.05 mg/kg).

Facilitation of memory by cholinomimetics bears an "inverted U-shaped" relation to dose; above the optimal dose, these agents have a smaller or detrimental effect. While selective enhancement of metabolism in the hippocampus by low doses could result in memory facilitation by AREC, generalized stimulation produced by higher doses may be responsible for their reduced effect on memory.

76.1 EFFECTS OF BUSPIRONE ON THE ACTIVITY OF NOREPINEPHRINE-CONTAINING LOCUS COERULEUS NEURONS RECORDED FROM MOUSE BRAIN SLICES IN VITRO. L.J. Henderson* and M.E. Trulson (SPON: R. Batton). Dept. of Pharmacol., Marshall Univ. Sch. of Med., Huntington, WV 25701.

Buspirone is a non-benzodiazepine anxiolytic that has attracted a great deal of scientific interest recently due to its potential clinical usefulness. A recent study has shown that benzodiazepine anxiolytics decrease the firing rate of norepinephrine (NE)-containing locus coeruleus (LC) neurons in anesthetized rats, while buspirone increases the activity of NE neurons in the LC (Sanghera, et al., Eur. J. Pharmacol. 86, 107, 1983). It is not known, however, whether the effects of buspirone are direct effects on NE neurons, or whether the drug alters some afferent input to the LC nucleus. In the present study we examined this issue by recording the activity of NE-containing LC neurons from mouse brain slices in vitro, a preparation that would sever virtually all afferent inputs to the LC. Adult Swiss-Webster mice were decapitated and the brains sectioned into 400 micron coronal slices. The slices were incubated in standard Yamamoto's solution under an oxygen-saturated atmosphere. Electrophysiological recordings were performed with single barelled micropipettes, according to standard techniques. NE neurons displayed a spontaneous discharge rate of (1.13-4.31 spikes/sec; Mean=2.27 spikes/sec). Administration of buspirone to the incubation bath produced a dose-dependent increase in NE unit activity (1μ M, +9.8%; 10μ M, +21.8%; 100μ M, +46.9%). When the incubation medium was altered to contain high magnesium (10 mM) and low calcium (0.5mM), a procedure known to block all synaptic transmission, there were no significant changes in the response of NE neurons to buspirone administration. These data suggest that buspirone exerts its effect on NE-containing LC neurons by a direct action, rather than by altering the afferent input to the LC. Whether this effect is related to the anxiolytic properties of the drug remains to be determined.

76.2 BUSPIRONE, A NON-BENZODIAZEPINE ANXIOLYTIC DRUG, CAUSES INHIBITION OF SEROTONERGIC DORSAL RAPHE NEURONS IN THE RAT BRAIN SLICE. C. P. VanderMaelen and R. C. Wilderman*. Preclinical CNS Res., Bristol-Myers Co. Evansville, IN, 47721.

Buspirone (Buspar) is a new non-benzodiazepine anxiolytic drug which is as clinically effective as diazepam and chlorazepate (see J. Clin. Psychiat., 1982, 43, pp. 4-166). Its mechanism of action is unknown. An involvement in serotonergic systems could contribute to the anxiolytic action of buspirone, as has been suggested for the benzodiazepines (e.g., Soubrie et. al., JPET, 1983, 226, 526-532). Recent work has shown that buspirone potently inhibits the firing of serotonergic neurons in the rat dorsal raphe (DR) nucleus when administered systemically or by microiontophoresis (VanderMaelen and Wilderman, Fed. Proc., 1984, 43, p. 947). The present study examined the effects of buspirone on these neurons in a brain slice preparation.

Adult male albino Sprague-Dawley rats were anesthetized with chloral hydrate and allowed to breathe 100% O_2 for at least 5 min. 400 μm thick frontal slices were cut through the DR nucleus, and placed in a chamber containing 95% O_2, 5% CO_2. They were bathed (but not submerged) in continuously flowing artificial CSF containing, in mM: NaCl, 130; KCl, 5.0; $CaCl_2$, 1.25; $MgSO_4$, 1.25; $NaHCO_3$, 24; NaH_2PO_4, 1.25; D-glucose, 10.0. Phenylephrine (10-20 μM) or DL-homocysteic acid (0.1 mM) was present to provide increased neuronal activity (see VanderMaelen and Aghajanian, Brain Res., 1983, 289, 109-119).

Buspirone, in bath concentrations of 100-400 nM, caused complete inhibition of firing of serotonergic dorsal raphe neurons. The 100 nM concentration is equal to the calculated blood concentration of buspirone immediately following an intravenous injection which produces an approximately equivalent amount of inhibition of these neurons in vivo. Studies comparing the inhibitory effect of buspirone with serotonin in the brain slice indicate that the inhibitory effect of buspirone outlasts its presence in the artificial CSF. This suggests that buspirone may stick to a binding site on the membrane, or may work through an intracellular second messenger, or some other mechanism.

This study along with previous in vivo studies indicates that the inhibitory effect of buspirone on serotonergic DR neurons is due to a potent and direct effect of the parent drug on these cells, and further suggests a role for serotonergic systems in anxiolytic therapeutic efficacy.

76.3 PHARMACOLOGIC EFFECTS OF CHRONIC ADMINISTRATION OF THE NON-BENZODIAZEPINE ANTIDEPRESSANT-ANXIOLYTIC CANDIDATE, BMY 13805. M. S. Eison, D. P. Taylor, A. S. Eison, C. P. VanderMaelen, L. A. Riblet, and D. L. Temple, Jr. Preclinical CNS Research, Pharmaceutical Research and Development Division of the Bristol-Myers Company, Evansville, IN, 47721

Symptomatology of the debilitating affective disorders of depression and anxiety are often concurrently observed in patient populations. This may reflect an individual's position along a unitary clinical continuum, suggesting a common underlying neuropathology, or may reflect the simultaneous presence of the neurochemical imbalances characteristic of each disease state. A drug capable of alleviating both classes of psychopathology would be an exciting new tool in our attempts to understand the biological basis of these disorders, as well as a boon to psychiatric pharmacotherapy.

In addition to an anxiolytic preclinical profile, the novel non-benzodiazepine compound, BMY 13805, known chemically as 4,4-dimethyl-1-[4-[4-(2-pyrimidinyl)-1-piperazinyl] butyl]-2,6-piperidinedione hydrochloride, acutely exhibits interactions with brain serotonin systems and chronically induces behavioral and neurochemical changes suggestive of antidepressant potential. Acutely BMY 13805 is active in conflict tests, inhibits shock-elicited aggression and conditioned avoidance, induces a behavioral serotonin syndrome and potently inhibits the activity of dorsal raphe neurons. Following 28 days of three times daily dosing, BMY 13805 down-regulates cortical $5HT_2$ receptors (Bmax) without altering striatal dopamine receptor number or affinity (Table 1). These binding changes are associated with behavioral subsensitivity to serotonin agonist-induced head twitches with no concomitant alterations in sensitivity to amphetamine-induced stereotypy. Additional pharmacological properties of this drug will be discussed in the context of potential antidepressant-anxiolytic indications.

Table 1. Binding Constants Derived From Saturation Analysis
Bmax (fmoles/mg protein ± SEM)

Dose (3X daily)	DA	$5HT_2$
Vehicle	205 ± 12	90 ± 4
13805 (25 mg/kg)	216 ± 21	69 ± 7*
13805 (50 mg/kg)	235 ± 16	76 ± 4*
Diazepam (50 mg/kg)	184 ± 18	87 ± 8
Haloperidol (3 mg/kg)	386 ± 31*	100 ± 5

*Significantly different from vehicle (t-test).

76.4 5-HT_1 RECEPTORS AS TARGET FOR THE PUTATIVE ANXIOLYTIC TVX Q 7821. T. Glaser*, W.U. Dompert*, T. Schuurman* and J. Traber* (SPON: F.-K. Pierau). Neurobiology Dept., Troponwerke, Neurather Ring 1, 5000 Cologne 80, West Germany.

TVX Q 7821 (2-(4-(4-(2-pyrimidinyl)-1-piperazinyl)butyl)-1,2-benzoisothiazol-3(2H)one-1,1 dioxide hydrochloride) was compared to diazepam in two behavioral tests for aggression and anxiety as well as with in vitro binding techniques in order to evaluate the mechanism of action. In a naturalistic model of aggression, TVX Q 7821 abolishes the aggressive behavior of residential rats towards intruders without suppressing other active behavioral elements. Diazepam reduced all active behaviors. In a test for anxiety, TVX Q 7821 but not diazepam reduced fear-related behavioral elements. Tritiated TVX Q 7821 bound with high affinity (K_D=1-2 nM) in a specific and saturable manner to calf and rat hippocampal membranes. No competition for these binding sites was seen with acetylcholine, noradrenaline, dopamine, histamine, GABA or diazepam. Only 5-HT interacted with TVX Q 7821 in binding studies, since 5-HT competed with ^{3}H-TVX Q 7821 and TVX Q 7821 competed with ^{3}H-5-HT for binding to hippocampal membranes. Using a number of 5-HT agonists and antagonists, a good correlation was found between binding to the TVX Q 7821 binding site and to 5-HT_1 receptors. Almost no binding was detected to 5-HT_2 receptors.

The results indicate that TVX Q 7821 binds with high affinity to 5-HT_1 receptors and that the mechanism of its anxiolytic actions is very likely serotonergic in nature.

76.5 CGS 8216 FAILED TO ANTAGONIZE MEPROBAMATE (MPB)-INDUCED OR PHENOBARBITAL (PHB)-INDUCED MOTOR IMPAIRMENT IN MICE.
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CGS 8216 is a phenylprazoloquinoline known to antagonize 3H-benzodiazepine binding [1], the anticonflict [2,3], antipentylenetetrazol, and motor deficit [2,4] activity of diazepam (DZP), and beta-CCE-induced seizures [5]. Unlike the benzodiazepine antagonist Ro 15-1788, CGS 8216 is also claimed to antagonize PHB-induced and MPB-induced anticonflict effects [2], although this observation was not confirmed by others [3].

We determined the effect of CGS 8216 on a traction wire behavioral task in male CFl mice whose motor performance was impaired by oral doses (mg/kg) of DZP (20), PHB (100), or MPB(200); such treatment produced a traction wire deficit in all mice within 15 minutes (the agonist pretreatment time). A traction wire deficit in mice is the inability to grasp with their forepaws, or to raise a hind paw to, a horizontal wire. Both Ro 15-1788 and CGS 8216 (10 and 100 mg/kg) antagonized the DZP-induced, but not the PHB-induced or MPB-induced,traction wire deficit. A dose of 300 mg/kg of CGS 8216 po or 10 mg/kg ip also failed to antagonize the PHB-induced traction wire deficit. Our work corroborates the preliminary finding of others [2], confirming the ability of CGS 8216 to selectively antagonize DZP-induced motor impairment. Ro 15-1788 at a dose of 100 mg/kg po tended to enhance the traction wire deficit induced by either PHB or MPB; these data may indicate a weak agonist activity of Ro 15-1788 in mice unmasked under these experimental conditions. The controversy over the presence or the absence of antagonism by CGS 8216 of drug-specific anxiolytic effects in animals [see 2,3] needs to be elucidated.

1. Czernik et al. Life Sci 30: 363, 1982.
2. Bernard et al. Pharmacologist 23: 201, 1981.
3. Patel et al. Eur. J. Pharm. 86: 295, 1983.
4. Keim and Furman. Fed. Proc. 43: 930, 1983.
5. Skolnick et al. Life Sci. 32: 2439, 1983.

76.6 COMPARISON OF THE EFFECTS OF BENZODIAZEPINES AND THREE SEROTONIN ANTAGONISTS ON A CONSUMMATORY CONFLICT PARADIGM.
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The consummatory behavior of rats shifted from a 32% to a 4% sucrose solution declines to a level substantially below that of unshifted controls that have only experienced the 4% solution (a negative contrast effect-NCE). Recovery from this suppression in performance is facilitated by anxiolytic agents such as the benzodiazepine (BDZ), chlordiazepoxide, ethanol, and the barbiturate amobarbital (Becker & Flaherty, Psychopharmacol. 80:35, 1983; Flaherty et al., Physiol. Psychol. 10:122, 1982). This consummatory conflict paradigm appears to be selectively sensitive to this class of drugs since neuroleptics (haloperidol, chlorpromazine) and the antidepressant imipramine do not produce similar effects. The anxiolytic action of BDZ has been suggested to be mediated by antagonism of the serotonergic (5HT) system (Stein et al., Am. J. Psychiat. 34:665, 1977). The effects of another BDZ, midazolam (MDZ) were compared with three 5HT antagonists in this consummatory conflict paradigm.

Rats were divided into shifted and unshifted groups. Shifted rats received 5 min access to 32% sucrose for 10 days and then 4% sucrose for four postshift days. Unshifted controls were maintained on the 4% solution for all 14 days. Prior to the start of the second postshift day, animals received ip. injections of either MDZ (.25, .5, 1, 1.25, 2 mg/kg), methysergide (3, 6, 12 mg/kg), cinanserin (5, 10, 15, 20 mg/kg), cyproheptadine (3, 6, 12 mg/kg), or vehicle.

NCE was reliable for all groups on the first postshift day (when no injection was given). This effect, however, was slightly reduced on the second postshift day by .25 and .5 mg/kg MDZ doses and eliminated by the 1, 1.25, and 2 mg/kg MDZ doses. There was no effect of the drug on unshifted control performance. Methysergide did not influence NCE at all doses tested while limited doses of the other 5HT antagonists reduced NCE (10 and 15 mg/kg cinanserin and 3 mg/kg cyproheptadine). Other doses were ineffective or produced general depressant effects (decreased unshifted control performance levels).

These results further substantiate the effectiveness of BDZ (chlordiazepoxide and midazolam) in reducing NCE; however, these results do not provide strong support for serotonergic mediation of these drug effects.

76.7 BEHAVIORAL EFFECTS OF DIAZEPAM IN RATS WITH MIDBRAIN RAPHE LESIONS.
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It has been proposed that certain of the behavioral actions of the benzodiazepines (Benzo's) are due to an interaction with serotonergic (5-HT) mechanisms. Were this the case, it might be expected that 5-HT depleting lesions would modify responsiveness to these drugs; however, few studies have examined this possibility. In the current study, we investigated various behavioral effects of diazepam (D) in rats with lesions of the median and/or dorsal nucleus of the raphe (MR & DR, respectively).

Adult, male rats were given an electrolytic lesion of either the MR or DR by passing a lma current through an electrode to a rectal cathode for 8 sec. Approximately 2 weeks after surgery non-deprived rats were tested for D-induced (0, 2, 4 mg/kg) eating of wet mash. Statistical analysis indicated that both the drug and the DR lesions produced increases in mash intake, but that no interaction between these two conditions was present. A second food-intake test using D (0, 2mg/kg) and a less palatable food (rat chow) indicated only a significant D effect. When animals were later tested in tilt cages for the effects of D (0, .5, 2 mg/kg) on locomotor activity, MR lesioned rats appeared to be more sensitive to the initial suppressant actions of the highest dose. Striatal and hippocampal 5-HT concentrations were reduced approximately 50-60% by the DR and MR lesions, respectively.

Other groups of rats with combined MR+DR lesions were tested 5 days after surgery on a lick-tube passive avoidance task following D (0, 3 mg/kg). Preliminary results indicate that both D and MR+DR lesions reduce avoidance behavior, although the topography of the response differs between the groups. Furthermore, there was no interaction between the two conditions. Additional studies will focus on more precisely identifying the anatomical and neurochemical substrates of the lesion effect.

The results of these studies suggest that the effects of D on food intake in non-deprived rats and on lick-tube passive avoidance do not entirely depend on intact 5-HT mechanisms originating in the midbrain raphe nuclei.

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76.8 THE EFFECTS OF NON-CONTINGENT SHOCK SUPERIMPOSED UPON A DIAZEPAM DRUG DISCRIMINATION.
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Drug discrimination procedures have been used extensively to investigate the effects of potential anxiolytic substances. It is unclear, however, whether the discriminative stimuli produced by classical benzodiazepines (BZs) are selectively mediated by the anxiolytic component of the drug. Other actions of BZs, including sedation and muscle relaxation, may contribute to BZ-induced discriminative stimuli. In an attempt to increase the anxioselectivity of the BZ discriminative cue, a modified diazepam (DZ) drug discrimination procedure was evaluated in which non-contingent shock was superimposed upon a standard DZ drug discrimination. It was hypothesized that DZ would produce a more anxioselective cue under this condition.

Two groups of male Long Evans rats were each trained to discriminate DZ (3 mg/kg i.p.) from cornstarch vehicle in 2-lever operant chambers on an FR-10 schedule of food reinforcement. During the 10 min session, non-contingent shock (1 - 1.5 mA) was delivered to one group (the shock group) of rats on a VI-20 sec schedule. The shock and no-shock groups learned to discriminate DZ from cornstarch vehicle in an equivalent number of sessions (approximately 40). The DZ stimulus was dose-dependent in each model, with an ED_{50} value of 1.79 (1.45 - 2.32) mg/kg in the shock group and an ED_{50} value of 0.43 (0.22 - 1.53) mg/kg in the no-shock group. Chlordiazepoxide, meprobamate and pentobarbital showed comparable dose-related generalization to the DZ cue in both models. The non-BZ anxiolytic, CL 218,872 also produced dose-dependent generalization in both the shock and no-shock groups as did the serotonin antagonist, cyproheptadine. Of particular interest was the finding that the muscle relaxant methocarbamol generalized to DZ in the no-shock group (ED_{50} = 168 mg/kg), yet failed to generalize in the shock group. In antagonism studies, CGS 8216 and Ro 15-1788 each blocked the DZ cue in both groups of discriminating rats. From these studies, it appears that both models identified compounds with anxiolytic activity. The ability of methocarbamol to generalize to DZ in the no-shock group, but not in the shock group, suggests that the no-shock DZ cue may have a muscle relaxant component.

76.9 THE BENZODIAZEPINE ANTAGONISTS, Ro 15-1788 AND CGS 8216 DIFFER IN THEIR INTEROCEPTIVE DISCRIMINATIVE STIMULI. D.A. Bennett, C.L. Corradi* and D.E. Wilson*. Neuroscience Res., Res. Dept., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

The pyrazoloquinoline, CGS 8216 and the imidazodiazepine, Ro 15-1788 have been reported to antagonize many of the effects produced by diazepam. Some differences, however, have been noted between these two substances. In particular, CGS 8216 has been reported to exhibit an "inverse agonist" effect (Braestrup et al., Neuropharmacology 22, 1982). The present studies investigated the intrinsic properties of these compounds using drug discrimination techniques in which CGS 8216 (30 mg/kg i.p.) or Ro 15-1788 (10 mg/kg i.p.) served as the training drug.

Male Sprague Dawley (CGS 8216) or Long Evans (Ro 15-1788) rats were trained in 2-lever operant chambers according to standard drug discrimination methods, using a food-reinforced FR-10 schedule. In the Ro 15-1788 discrimination, all 16 rats learned to discriminate Ro 15-1788 from vehicle in 56 ± 5 sessions. This cue remained stable throughout the course of the investigation. The discriminative cue was dose-dependent and the training dose of Ro 15-1788 produced a significant increase in responding. CGS 8216 showed dose-dependent generalization (ED_{50} = 0.46 mg/kg) to the Ro 15-1788 cue. Surprisingly, diazepam (1 - 3 mg/kg) also produced some generalization, as did pentobarbital. Caffeine (50 mg/kg) did not generalize to the Ro 15-1788 cue.

Thirteen of the sixteen rats that underwent training met the criterion for discriminating CGS 8216 from vehicle in 53 ± 4 sessions. However, unlike the rats in the Ro 15-1788 discrimination, many of the CGS 8216 rats showed instability and data could be collected only from a limited number of animals. The CGS 8216 discriminative cue was also dose-dependent; however, the training dose of CGS 8216 produced a significant decrease in responding. Ro 15-1788 did not generalize to CGS 8216 indicating an assymetrical cross generalization between CGS 8216 and Ro 15-1788 in the two discrimination experiments. Caffeine showed complete generalization at 50 mg/kg, while a subconvulsant dose of pentylenetetrazol (17 mg/kg) produced 86% generalization. Pretreatment with diazepam (1 - 10 mg/kg) failed to antagonize the CGS 8216 discrimination.

These results suggest that the cues produced by Ro 15-1788 and CGS 8216 are qualitatively different. These differences may be explained in part by the inverse benzodiazepine agonist properties of CGS 8216 and the mixed agonist-antagonist properties of Ro 15-1788.

76.10 TIME COURSE OF THE ELECTROCORTICAL (ECoG) AND BEHAVIORAL EFFECTS OF INTRAVENOUS DIAZEPAM OR LORAZEPAM IN SQUIRREL MONKEYS AND OF CEEG EFFECTS IN HUMANS. K.L. Keim, T. Smart*, M. Bergamo*, and °T.M. Itil, Dept. Pharmacology, Hoffmann-La Roche Inc., Nutley, N.J. 07110 and °N.Y. Med. College, Tarrytown, N.Y. 10591.

Based upon an ex vivo benzodiazepine (BZD) binding study comparing diazepam (DZP) and lorazepam (LOR), it was suggested that brain receptor binding may be more closely correlated with the clinical effects of these drugs than half-life of elimination [1]. This study estimated the time course of ECoG and behavioral effects in monkey, and of the computer analyzed EEG (CEEG) in human volunteers after intravenous (iv) administration of these BZDs.

Drugs were given iv 15 minutes prior to testing food-restricted monkeys on a 90-minute duration VI 60 second schedule of food reinforcement. Telemetered ECoG from the anterior cortex was quantified and the 0-32 Hz range analyzed by a computer and divided into eight frequency bands. Monkeys were given vehicle on Day 1 and either drug on Day 2, and the differences between frequency distributions and VI response rates were compared.

DZP's (0.5 mg/kg) peak ECoG effect occurred within the first analyzed epoch (15 to 42 min) following administration, predominantly enhancing 24-32 Hz ECoG activity. In contrast, LOR (0.1 mg/kg) primarily decreased 2-12 Hz activity and this effect peaked during the third ECoG epoch (68-96 min) post drug. The VI lever rate response rate for both drugs increased with time (up to 96 min): a finding characteristic of anxiolytic substances in monkeys.

Furthermore, similar to the time-related differences evoked by iv DZP and LOR in the ECoG of monkeys, we recently demonstrated a similar time course in human volunteers. That is, DZP (5 mg iv) rapidly enhanced 20-26 Hz activity prior to an enhanced 1.3-3.5 Hz activity increase, while LOR (1 mg iv) enhanced 1.3-3.5 Hz CEEG prior to the increase in 16-20 Hz activity which did not occur until 75+ minutes.

The ECoG changes may represent the "end organ" response to the tested BZDs and suggest a temporal relationship with the ex vivo binding characteristics of these drugs.

1. Spirt, N., G. Bautz, M. Zanko, W. D. Horst and R. O'Brien, Soc. Neurosci. Abst. 7: 865, 1981.

76.11 IN VITRO ANTIDEPRESSANT PROFILES OF THE NOVEL BICYCLIC COMPOUNDS, Wy-45,030 and Wy-45,881. E.A. Muth, J.T. Haskins, J.A. Moyer, S.T. Nielsen*, and E.B. Sigg*. Dept. of Experimental Therapeutics, Wyeth Laboratories, Inc., P.O. Box 8299, Philadelphia, PA 19101.

In an ongoing search for novel compounds with antidepressant activity, the bicyclic compounds Wy-45,030 (1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexanol, HCl) and Wy-45,881 (1-[1-(3,4-dichlorophenyl)-2-(dimethylamino)ethyl]cyclohexanol, HCl) were observed to inhibit the binding of ^{3}H-imipramine to the serotonin uptake site in rat brain (Wy-45,030 Ki=90 nM; Wy-45,881 Ki=37 nM). Further testing revealed that the compounds were indeed monoamine uptake inhibitors in rat brain synaptosomal preparations. Wy-45,030 exhibited uptake IC_{50}'s of 0.64 µM vs. norepinephrine (NE), 0.21 µM vs. serotonin (5-HT), and 2.8 µM vs. dopamine (DA). Wy-45,881 was somewhat more potent, with IC_{50}'s of 0.07 µM vs. NE, 0.08 µM vs. 5-HT, and 0.16 µM vs. DA. Neither Wy-45,030 nor Wy-45,881 exhibited any inhibition of rat brain monoamine oxidase in vitro.

The above results demonstrate that Wy-45,030 and Wy-45,881 resemble the tricyclic antidepressants in their acute biochemical actions. However, further in vitro testing suggests a much reduced side-effect liability for these compounds relative to the representative tricyclic, desipramine. Unlike desipramine, neither Wy-45,030 nor Wy-45,881 exhibited appreciable inhibition of muscarinic cholinergic (QNB) or histamine-1 receptor binding in rat brain homogenates, nor did they shift the dose-response curves to carbachol or histamine of guinea-pig ileal contraction. Also unlike desipramine, neither compound inhibited the binding of WB-4101 to the rat brain alpha-1 noradrenergic receptor. Neither Wy-45,030, Wy-45,881 nor desipramine showed any in vitro interaction with alpha-2 or beta noradrenergic receptors.

These results suggest that Wy-45,030 and Wy-45,881 may possess antidepressant activity devoid of antimuscarinic, antihistaminic, or antiadrenergic side effects in man.

76.12 IN VIVO ANTIDEPRESSANT PROFILES OF THE NOVEL BICYCLIC COMPOUNDS Wy-45,030 and Wy-45,881. J.A. Moyer, E.A. Muth, J.T. Haskins, R.W. Lappe° and E.B. Sigg*, Dept. of Experimental Therapeutics, Wyeth Laboratories, Inc., P.O. Box 8299, Philadelphia, PA 19101.

The novel bicyclic compounds Wy-45,030 (1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexanol, HCl) and Wy-45,881 (1-[1-(3,4-dichlorophenyl)-2-(dimethylamino)ethyl]cyclohexanol, HCl) have been shown to inhibit amine uptake in vitro (Muth et al., Neuroscience Abstracts, 1984). As this profile suggests possible antidepressant activity, these compounds were examined in a series of tests to determine their in vivo antidepressant activity.

Both Wy-45,030 and Wy-45,881 were found to antagonize reserpine-induced hypothermia in mice with the minimum effective dose being 10.0 and 3.0 mg/kg i.p. respectively. In comparison, desipramine antagonized reserpine-induced hypothermia at a minimum effective dose of 0.4 mg/kg i.p. Both compounds also prolonged the pressor action of spinal electrical stimulation, but not phenylephrine, in the pithed rat at 1.0 mg/kg i.v. These results suggest a blockade of norepinephrine uptake at sympathetic nerve terminals in vivo.

Since histamine-induced ACTH release in rats is attenuated by classical antidepressants, Wy-45,030 and Wy-45,881 were tested in this procedure. Both compounds (10.0 mg/kg i.p.) suppressed histamine-induced ACTH release by 26% and 56% respectively, while the standard desipramine (10.0 mg/kg i.p.) diminished this release by 52%.

Wy-45,030 and Wy-45,881 were also examined for the induction of noradrenergic subsensitivity in the rat pineal gland. Cyclic AMP response to isoproterenol (2 µmol/kg i.p.) was measured following both acute (single injection of 10.0 mg/kg i.p.) and repeated (9 injections 10.0 mg/kg i.p., b.i.d.) drug treatment. Both Wy-45,030 and Wy-45,881 reduced cAMP responsiveness in this paradigm by 47% and 74% respectively following repeated administration (desipramine = 81% reduction). However, unlike desipramine, Wy-45,030 and Wy-45,881 reduced cAMP responsiveness following acute administration (41% and 51% respectively).

These studies indicate that Wy-45,030 and Wy-45,881 show activity similar to the tricyclic antidepressant desipramine. However, unlike desipramine (and other antidepressants), these compounds produce noradrenergic downregulation following both acute and repeated treatment. Although the clinical relevance of this later feature is unknown, it may suggest a rapid onset of action.

76.13 INHIBITION OF NORADRENERGIC NEURONAL ACTIVITY BY THE NOVEL BICYCLIC COMPOUNDS, Wy-45,030 AND Wy-45,881. J.T. Haskins, J.A. Moyer, E.A. Muth and E.B. Sigg*. Dept. of Experimental Therapeutics, Wyeth Laboratories, Inc., Philadelphia, PA 19101.

The novel bicyclic compounds Wy-45,030 (1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexanol, HCl) and Wy-45,881 (1-[1-(3,4-dichlorophenyl)-2-(dimethylamino)ethyl] cyclohexanol, HCl) inhibit monoamine uptake in rat brain synaptosomal preparations and resemble tricyclic antidepressants (TCA) in their acute biochemical actions (Muth et al. and Moyer et al., this volume). These compounds were therefore tested for effects on neuronal activity in the noradrenergic nucleus locus coeruleus (LC) and compared to the standard TCA, desmethylimipramine (DMI). LC neurons were recorded with both single and seven-barrel micropipettes using standard electrophysiological techniques in chloral hydrate anesthetized rats and identified by (1) action potential waveform (2) spontaneous activity and (3) response to noxious stimuli. Compounds were administered either intravenously (via the lateral tail vein) or microiontophoretically. Intravenous administration of DMI reduced the firing rate of LC neurons in a dose dependent manner. The ED_{50} for inhibition of LC neuronal firing rate was 0.20 mg/kg as calculated using simple linear regression with inverse prediction. At intravenous doses greater than 0.05 mg/kg, Wy-45,030, like DMI, inhibited LC neuronal activity. Unlike DMI, however, an increase in activity was observed at low doses resulting in a biphasic dose-response curve. ED_{50} values for Wy-45,030 could not be calculated from these biphasic dose-response curves. Wy-45,881 closely resembled DMI in its effects on LC firing rate. A biphasic dose-response relationship was not observed with either DMI or Wy-45,881. The ED_{50} for Wy-45,881 was 0.37 mg/kg.

As expected, microiontophoretic application of DMI and Wy-45,030 inhibited LC activity. The C_{50} values were 52.5 and 92.7 respectively.

The mixed α-antagonist, piperoxane, antagonized the observed inhibitions produced by both intravenous and microiontophoretic administration of these compounds.

These studies indicate that Wy-45,030 and Wy-45,881 are similar to the tricyclic antidepressant, DMI, in their inhibition of noradrenergic neuronal activity in the locus coeruleus.

76.14 EFFECT OF BUPROPION (WELLBUTRIN®) ON THE FIRING RATES OF A9 AND A10 DOPAMINE NEURONS. V. K. Shea* and C. M. Wang* (SPON: G.T. Pollard). Dept. of Pharm., Wellcome Research Laboratories, Research Triangle Park, NC 27709.

Bupropion is a novel antidepressant that is structurally and neurochemically different from tricyclic antidepressants and MAO inhibitors. Bupropion is active in animal antidepressant tests (ED_{50}≈10 mg/kg i.p.) and stimulates locomotor activity at higher doses (20-50 mg/kg i.p.). At these higher doses, bupropion inhibits uptake of dopamine (DA) in vivo (Canning et al., Br. J. Pharmacol. 66:104-105, 1979; Cooper et al., JPET 215:127-134, 1980). The possibility exists that DA may be involved in bupropion's CNS actions. We, therefore, examined the effects of bupropion and standard antidepressant drugs administered by the i.p. route on firing rates of single DA neurons to explore the possibility that changes in firing rates might correlate with CNS activities in animal tests. Extracellular activity of A9 and A10 DA neurons was recorded using conventional techniques in chloral hydrate anesthetized rats. Data reported here were obtained from DA neurons with baseline activity exceeding 3 impulses/second.

The firing rates of DA neurons were suppressed following i.p. injections of bupropion is a dose-dependent manner with an ID_{50}≈45 mg/kg. A9 and A10 DA neurons responded to bupropion in a quantitatively similar manner. Non-DA neurons on the substantia nigra zona reticulata failed to respond to bupropion in a consistent way.

The ability of bupropion to suppress the firing rates of DA neurons was not shared by typical antidepressants tested. Neither imipramine (16 mg/kg i.p.) nor phenelzine (16 mg/kg i.p.) inhibited the firing of either A9 or A10 neurons. While bupropion suppressed DA firing rates, its ID_{50} of 45 mg/kg i.p. was more than 4 times its ED_{50} of 10 mg/kg i.p. in behavioral tests predictive of antidepressant activity. These observations suggest that bupropion's effects on DA neuronal firing rates may be unrelated to its antidepressant activity. More likely, suppression of DA neuronal firing rates by bupropion is secondary to inhibition of DA uptake in vivo (ID_{50}≈50 mg/kg i.p.), and parallels bupropion's stimulation of locomotor activity in rodents at high doses.

76.15 SUBSENSITIVITY TO NOREPINEPHRINE (NE) IN RAT BRAIN AFTER REPEATED STRESS: CHARACTERIZATION AND COMPARISON WITH EFFECTS OF STRESS HORMONES AND ANTIDEPRESSANTS. E.A. STONE, A.V. SLUCKY*, J.E. PLATT and R. TRULLAS*. Dept. Psychiatry, New York Univ. Sch. of Med., New York, NY 10016.

Previous studies have shown that repeated stress in the form of footshock or restraint reduces the function of postjunctional noradrenergic receptors in the rat brain as evidenced by a decrease in the cAMP response to NE in brain slices. The present studies were undertaken to characterize further this phenomenon and to compare it with the subsensitivity caused by antidepressants and stress hormones.

Restraint stress was administered to rats either once or repeatedly for varying amounts of time at a frequency of once or twice daily. The antidepressant drug, desmethylimipramine (DMI) was given at 10 mg/kg, ip, bid for 10 days. Corticotrophin (ACTH), NE or epinephrine (EPI) were chronically infused subcutaneously with minipumps for 10 days. Animals were killed 0 or 24 hrs after treatment.

Repeated but not acute restraint was found to reduce significantly the cAMP response to NE in slices of the hypothalamus and cerebral cortex. This reduction was directly related to the duration of stress and persisted for at least 24 hrs after the last stress session. Analysis of NE-cAMP dose response curves showed that stress reduced the maximum cAMP response but did not change the EC_{50} value of NE. A greater degree of subsensitivity was found in response to NE than to isoproterenol (ISO). Stress did not have a persistent effect on the density or affinity of beta adrenergic receptors (BARs) as measured by specific [^{3}H]dihydroalprenolol binding. DMI produced a persistent reduction in the cAMP response to both NE and ISO of equal size and also persistently reduced the density of BARs. The effects of ACTH, NE and EPI are currently under investigation.

The results indicate that restraint stress produces a persistent and dose related decrease of the function of postjunctional non-beta noradrenergic receptors in widely separated brain regions. Antidepressants, in comparison, produce persistent reductions in both beta as well as non-beta receptor function in these same brain areas. These results support the hypothesis that adaptation to stress and antidepressant treatment have in common the ability to down regulate non-beta adenylate cyclase-linked noradrenergic receptors in the brain, a phenomenon possibly related to the therapeutic effects of these treatments. (Supported by grants MH22768, MH08618 and CIRIT predoct. award to R.T.).

76.16 EFFECTS OF CHLORIMIPRAMINE ON HOARDING BEHAVIOR IN THE RAT. J.K. Nishita, G.G. Dougherty *, E.H. Ellinwood, Jr. and W.J.K. Rockwell*. Dept. of Psychiatry, Duke University Medical Center, Durham, NC 27710.

Hoarding behavior in the laboratory rat is reliably enhanced by food deprivation and by lowering body temperature. In a thermoneutral environment, male rats hoard food only when they are nutritionally depleted (Morgan, Stellar & Johnson, 1943), whereas, female rats will hoard without being food-deprived (Herberg, Pye, & Blundell, 1972). Various compensatory mechanisms related to body weight regulation and feeding have been suggested to underlie these sex differences in hoarding (Colling & Herberg, 1982; Fantino & Cabanac, 1980).

Since hoarding has been described as a "self-perpetuating habit" (Bindra, 1959) and its persistence following nutritional recovery might be analogous to the occurrence of shoplifting in patients recovering from anorexia nervosa (Herberg & Blundell, 1970), we became interested in the efficacy of chlorimipramine (CHL: a tricyclic antidepressant used in the treatment of obsessive-compulsive neurosis). We report our findings on the effects of CHL and imipramine (IMI) on the hoarding behavior of food-deprived male rats and non-deprived female rats.

Adult male and female Sprague-Dawley rats were screened for spontaneous hoarding behavior using the method described by Herberg and Blundell (1970). Rats were separated into three groups: CHL (15 mg/kg), IMI (15 mg/kg) and saline controls and tested for baseline hoarding behavior for 10 days followed by 10 days of drug treatment. Only those rats which hoarded 5 or more pellets were used. Both CHL and IMI produced significant reductions in hoarding behavior in male rats compared to saline controls ($p<.06$ and $p<.01$; respectively). IMI produced greater reductions in hoarding than CHL ($p<.10$). Similar drug effects were observed in female rats but statistical analyses were confounded by the low levels of spontaneous hoarding and the estrous cycle.

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76.17 MAO INHIBITOR DOSE-RESPONSE IN VIVO. E.L. Giller, Jr., H. Hall* and J. Lieb* West Haven VA Neuropsychopharmacology Lab, Yale Univ. Sch. of Med., New Haven, CT 06511

Inhibitors of monoamine oxidase (MAO, monoamine: O_2 oxidoreductase, EC 1.2.3.4) are clearly effective in the treatment of some types of depression, panic and/or phobia. A sufficient daily dose for a drug trial is essential but it is not clear what constitutes such a dose for many of the currently used MAO inhibitors (MAOi) except for phenelzine, for which the best clinical response occurs with doses of phenelzine sufficient to produce at least 80%-90% inhibition of platelet MAO. Animal studies show that this level of inihibition is necessary to affect neurotransmitter metabolism and that measures of peripheral MAO inhibition at drug steady-state reflect central MAO inhibition. We measured platelet MAO inhibition (tyramine substrate) in a sample of 103 patients on varying steady-state doses of isocarboxazid, phenelzine or tranylcypromine to extend pilot work in establishing an in vivo dose-response curve. Mean (±SD) pretreatment platelet MAO activity was 43.4±16.4 nanomoles tyramine deaminated/mg. protein/hr. Inhibition of platelet MAO by 90% or more was achieved by isocarboxazid 30 mg, phenelzine 60 mg or tranylcypromine 5 mg. These results agree with previous pilot work. We conclude that adequate drug trials require at least these daily dosage levels and that the hydrazine MAOi's isocarboxazid and phenelzine inhibit MAO activity 90% at clinically used doses while similar inhibition of MAO occurs at doses much lower than those used clinically with tranylcypromine.

76.18 SERUM MELATONIN IN DEPRESSED PATIENTS BEFORE AND AFTER TREATMENT WITH DESMETHYLIMIPRAMINE (DMI). J. Gottlieb*, J. Amsterdam*, S. Caroff*, A. Winokur and A. Frazer, VA Medical Center and Departments of Psychiatry and Pharmacology, Univ. of Pennsylvania, Philadelphia, PA 19104.

Previously, we have reported that the nocturnal rise of melatonin in serum was reduced significantly if rats were treated repeatedly with DMI (J. Pharmacol. Exp. Therap. 222: 534, 1982). We wanted to determine whether a similar result would occur in depressed patients treated with DMI. Seven male depressed patients (36-60 years) entered the study. On the day before treatment with DMI was initiated and again after 4 weeks of treatment, blood was taken from an indwelling venous catheter every 1.5 hours between 1630 and 0730 hrs. Lights were turned off at 2200 hr and turned on at 0600 hr. During the dark phase, the room was completely darkened and blood was drawn with the aid of a dim red light. Melatonin in serum was measured by radioimmunoassay (Endocrinol. 98: 482, 1976).

The results of the study were analyzed by a two-way ANOVA for repeated measures. Both before and during treatment with DMI, serum melatonin rose significantly during the dark phase ($F=7.4$; $P<0.001$). Prior to treatment, the average value of serum melatonin measured before the lights were turned off was 20±6.5pg/ml ($\bar{X}$±SEM) and this rose to a peak value during the dark phase of 62±11pg/ml. Treatment of the patients with DMI had no significant effect on concentrations of melatonin in serum ($F=0.39$, NS); during treatment with DMI, the average concentration of melatonin in serum before the lights were turned off was 21±3pg/ml and rose to a peak value of 62±8.8pg/ml.

Thus, the results obtained in depressed patients treated with DMI are different from that found in rats given the tricyclic antidepressant. The most likely explanation for the difference in results between rats and humans is the lower concentration of DMI in the plasma of the patients (70±9ng/ml, measured 7-12 hours after the last dose) as compared to that in the rats (347±75ng/ml, measured 24 hrs after the final dose). Under usual clinical use, plasma concentrations of DMI may not be sufficiently high to cause the degree of down-regulation of pineal beta-adrenergic receptors necessary to produce a reduction in the darkness-induced rise of melatonin. (Supported by Research Funds from the Veterans Administration.)

76.19 EFFECTS OF AMITRIPTYLINE AND IMIPRAMINE ON URINARY AND CSF AMINES AND METABOLITES IN DEPRESSED PATIENTS. C.L.Bowden, S.H.Koslow, A.Frazer, J.W.Maas, I.Hanin, J.M.Davis. Dept. of Psychiatry, The University of Texas Health Science Center, San Antonio, TX 78284

Biogenic amine hypotheses of affective disorders have been partly supported by preclinical pharmacological studies of the actions of antidepressants on amine systems. The NIMH Collaborative Study of the Psychobiology of Depression allowed study of several key questions about antidepressant drug effects in a large group of carefully studied depressed patients. The effects of amitriptyline (AMI) and imipramine (IMI) were studied in 95 unipolar and bipolar depressed patients. Urinary measurements at baseline and after three weeks treatment were obtained for MHPG, VMA, metanephrine (MET), NORMET, norepinephrine (NE) and epinephrine (E). Comparably timed CSF measurements of MHPG, 5-HIAA and HVA were obtained from 66 of the patients.

Substantial reduction in all urinary metabolites and in CSF MHPG and 5-HIAA occurred in both AMI and IMI treated patients, and in both unipolar and bipolar patients. NE, E and HVA did not change with treatment. These effects did not differ between the two drugs. When clinical outcome was considered, some differences between the two drugs were present. Greater reduction in urinary MHPG and MET occurred in patients who had a positive clinical response to AMI than those who did not. This pattern was not present in IMI treated patients. There were no differences between the two drugs on change in CSF metabolites between those who had a positive clinical response and those who did not.

Diagnostically, unipolar and bipolar patients differed on several of the measures studied. Unipolar patients who responded to drug treatment had greater reductions in MET than those who did not. Bipolar responders had lesser reductions in NORMET than non-responders, and an actual increase in NE, versus a decrease among non-responders. The pattern of change in urinary and CSF MHPG also differed, with unipolar responders having greater decrements than bipolar responders. Analyses of these outcome categories within diagnostic groups were performed with the two drug treatment groups combined.

These data suggest that study of a battery of catecholamines and their metabolites provides additional information about the effects of antidepressant drugs in depressed patients. Furthermore, such data may help to clarify biogenic amine hypotheses of depression and our understanding of the pharmacological mechanisms of tricyclic antidepressants.

77.1 DIFFERENTIAL EFFECTS OF TWO NEUROLEPTIC DRUGS ON INTRACRANIAL SELF-STIMULATION (ICSS) IN THE PRE-FRONTAL CORTEX OF THE RAT. R. Halperin, S. Mindell* and M. Jacobs*. S.U.N.Y., Purchase, Purchase, NY 10577.

According to the dopamine hypothesis of schizophrenia, neuroleptic drugs exert their therapeutic effects by inhibiting brain dopamine activity. It has been suggested that the medial pre-frontal cortex (PFC), a projection area of the mesocortical dopamine system, mediates the antipsychotic effects of these drugs. Other evidence, not necessarily linked to dopamine, suggests a role for the PFC 1) in the mediation of pathology associated with schizophrenia (Buchsbaum et al., Arch. Gen. Psychiatry, 39, 1982), and 2) as a site where neuroleptic drugs alter neural activity (Bacopoulos, J. Pharmacol. Exp. Ther., 219, 1981). To date, none of the behavioral models used in animals to screen novel compounds for their potential as antipsychotics assesses activity of the PFC. Further, all models use behavioral measures that reflect primarily dopamine activity.

The PFC is a site that supports ICSS in the rat. Dopamine appears to be involved in (Mora and Meyers, Science, 197, 1977), but does not exert exclusive control (Simon et al., Behav. Neurol. Biol., 27, 1979) over ICSS at this site. This experiment was conducted in the hope of developing a more relevant animal model. We have investigated the effect on ICSS in the PFC of chronic administration of two neuroleptics, only one of which is potent in ameliorating psychotic symptoms.

Each rat was stereotaxically implanted with an electrode aimed at the PFC. After stable ICSS rates were obtained at a range of current intensities, rats were given haloperidol (HAL), metoclopramide (MET) or sucrose alone in sweetened drinking water for 14 or 21 consecutive days. ICSS rates were measured daily for seven days prior to drug, 14-21 days during drug, and 21 days after the termination of drug.

Rats treated with HAL exhibited a marked suppression of ICSS during drug treatment, an immediate and short-lived increase in sensitivity to lower current intensities after drug treatment, and a rapid return to baseline behavior. Rats treated with MET showed a small suppression of ICSS during drug treatment, and a marked and longlasting increase in ICSS upon termination of drug treatment.

Further studies must be conducted to determine whether this paradigm is distinguishing the structural or therapeutic properties of these drugs.

77.2 COMPARATIVE EFFECTS OF MJ-13859, A POTENTIAL ANTIPSYCHOTIC DRUG, AND CLASSICAL NEUROLEPTICS ON RAT BRAIN DOPAMINERGIC NEUROTRANSMISSION. Brian A. McMillen and Helen L. Williams*
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The potential antipsychotic drug, MJ-13859 or 8-[4-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl]butyl]-8-azaspiro (4,5)decane-7,9-dione, has been described as having an atypical profile for ratio of ED50's in conditioned avoidance responding and induction of catalepsy (1). We have observed that MJ-13859 has an IC50 = 6.4 nM against D2 receptor binding of 0.1 nM ^{3}H-spiperone, which is similar to trifluoperazine (TPZ). However, MJ-13859 was less potent than TPZ both for induction of catalepsy and inhibition of amfonelic acid (AFA)-induced hyperactivity. This drug potently increased striatal and frontal cortex dopamine metabolism (increased DOPAC concentrations) with a maximum effect at a dose of 3.0 mg/kg s.c. in female rats. A rapid first pass effect was indicated by diminished levels of DOPAC in response to i.p. injection of drug. Also, female rats showed a larger and longer response than male rats, which is in harmony with the known greater drug metabolizing capacity of male rats. At 3.0 mg/kg MJ-13859, AFA-stimulated behavior was completely inhibited and a synergism on DOPAC levels occurred. These results indicated that at this dose level a strong in vivo blockade of striatal D2 receptors had developed.

For sub-chronic dosage of MJ-13859, 2 week osmotic minipumps were implanted in the scapular region delivering 1.0 or 3.0 mg/kg/day. After 2 weeks the rats were challenged with 0.1 mg/kg haloperidol (HALO), but exhibited near normal increases of DOPAC concentrations in striatum and frontal cortex. When rats received 2x8.0 mg/kg s.c. fluphenazine decanoate 1 week apart, there occurred a marked tolerance to HALO challenge. Additional rats received 2 weeks of either 1.0 mg/kg/day HALO or 3.0 mg/kg/day MJ-13859 and allowed a 4 day drug washout before performing Scatchard analysis of striatal ^{3}H-spiperone binding. Only the HALO treated group exhibited a significant increase (5%) of D2 receptors. Thus, although MJ-13859 acutely elevated DOPAC levels, caused catalepsy and potently bound the D2 receptor, neither decreased sensitivity to acute neuroleptic challenge nor D2 receptor supersensitivity, common to classical antipsychotic drugs, occurred after sub-chronic dosage with this drug. These data suggest that MJ-13859 may have fewer extrapyramidal side-effects than do classical antipsychotic drugs.

(Supported by a contract from the Bristol-Myers Co.)

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77.3 ANTIPSYCHOTIC DRUG EFFECTS IN THE AMYGDALA: FAILURE TO SUPPORT A CATECHOLAMINERGIC OR CHOLINERGIC MECHANISM OF ACTION. G. D. Anderson and G. V. Rebec. Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

Our previous work has demonstrated that, unlike the nucleus accumbens and the neostriatum, the amygdaloid complex is differentially responsive to classical and atypical antipsychotic drugs. Haloperidol, for example, fails to alter the activity of amygdaloid neurons even at doses that produce catalepsy, whereas clozapine routinely increases firing rates. In addition, clozapine, but not haloperidol, blocks the depression of amygdaloid activity produced by amphetamine. With long-term treatment, amygdaloid neurons become even more responsive to clozapine, whereas haloperidol fails to alter amygdaloid activity even when pretreatment is continued for 13 consecutive days (Anderson, G.D. & Rebec, G.V., Soc. Neurosci. Abstr., 9:425, 1983).

If clozapine is acting via a catecholaminergic mechanism, then multiple injections of this drug should alter the neuronal response to amphetamine. We also examined haloperidol in this paradigm because withdrawal from this drug produces an increase in the sensitivity of catecholaminergic receptors. Rats received 2 daily injections of saline, 1.0 mg/kg haloperidol, or 10.0 mg/kg clozapine for 6 consecutive days. Following a 4-day withdrawal, the animals were prepared for single-unit recording and challenged with repeated iv injections of 0.2 mg/kg d-amphetamine. Surprisingly, in all groups, the amphetamine response was comparable. Thus, this drug produced a 50% inhibition of activity between the 6th and 9th injection regardless of pretreatment. Spontaneous firing rates were also similar in each group. These results argue against an effect of either clozapine or haloperidol on catecholaminergic mechanisms in the amygdaloid complex.

Clozapine, unlike haloperidol, appears to act, at least in part, as a muscarinic antagonist. If this is the case in the amygdala, then scopolamine, a well-known muscarinic blocker, should mimic the effect of clozapine in this site. To test this hypothesis, .25 mg/kg scopolamine (iv) was administered to a separate group of rats at 2-min intervals. Cumulative doses as high as 2.0 mg/kg failed to alter amygdaloid activity. Taken together, these results suggest neither catecholaminergic nor cholinergic mechanisms can completely explain the differential effects of haloperidol and clozapine in the amygdaloid complex.

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77.4 ASCORBIC ACID POTENTIATES THE BEHAVIORAL RESPONSE TO HALOPERIDOL: IMPLICATIONS FOR THE MECHANISM OF ACTION OF ANTIPSYCHOTIC DRUGS. G. V. Rebec and J. M. Centore*.
Dept. Psychology, Indiana University, Bloomington, IN 47405.

We have previously shown that a systemic injection of ascorbic acid (AA) accelerates the activity of neurons in the neostriatum--an effect that is mimicked by haloperidol, a widely prescribed antipsychotic drug (Ewing, A.G. et al., Brain Res., 261:101, 1983). In fact, AA potentiates haloperidol-induced catalepsy in rats suggesting that AA enhances the ability of this drug to block neostriatal dopamine (DA) receptors (Rebec, G.V. et al. Soc. Neurosci. Abstr., 9:124, 1983). This hypothesis is supported by in vitro evidence that haloperidol binding in the neostriatum requires the presence of AA (Hadjiconstantinou, M. and Neff, N.H., Neuropharmacology, 22:939, 1983). Because many of the behavioral effects of haloperidol are due, at least in part, to its high affinity for DA receptors, it is conceivable that AA modulates the antipsychotic potency of this drug.

To begin to test this hypothesis, we examined the ability of AA or AA combined with haloperidol to block the stereotyped behavior produced by amphetamine in rats, a popular animal model of antipsychotic efficacy. The animals were housed individually in sound-attenuating chambers for at least 48 hr prior to the onset of the experiment. Ten minutes before receiving a subcutaneous injection of 1.0 mg/kg d-amphetamine, the rats received an intraperitoneal injection of saline, 1000 mg/kg AA, 0.1 mg/kg haloperidol, or 1000 mg/kg AA mixed with 0.1 mg/kg haloperidol (in each case, AA was pH adjusted to 7.0 and maintained under nitrogen until administration). Individual items of open-field behavior were monitored at regular intervals throughout the amphetamine response. AA alone failed to exert a significant effect although several items of behavior were slightly reduced compared to saline pretreated controls. When combined with haloperidol, however, AA completely blocked the amphetamine response. In fact, this combination was significantly more effective in reducing amphetamine-induced locomotion, rearing, sniffing, and head bobbing than haloperidol alone. These results provide further evidence that an AA-DA interaction may play an important role in mediating the behavioral effects of haloperidol and related antipsychotic drugs.

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77.5 PHARMACOLOGIC ACTIVITY OF DIFFERENT FORMS OF FLUPHENAZINE AND METABOLITES. J.I. Javaid, B. Duslak* and J.M. Davis.* Illinois State Psychiatric Institute, Chicago, IL 60612

There is a large body of persuasive evidence implicating the dopamine system in the mechanism of action of neuroleptic drugs. All of the clinically useful neuroleptics, even though structurally different, have been shown to interfere with dopaminergic mechanisms and this common feature, among a variety of other pharmacologic actions, provides the best correlation with their efficacy.

The ability of neuroleptics to induce catalepsy has been used as an in vivo test for neuroleptic activity. It has been suggested that the major mechanism of action of neuroleptic induced catalepsy involves blockade of postsynaptic dopamine receptors.

It has been shown by in vitro studies that radiolabelled butyrophenones, such as ^{3}H-haloperidol and ^{3}H-spiroperidol, bind selectively and with high affinity to dopamine receptors in mammalian brain. The in vitro potencies of different neuroleptics in blocking dopamine receptors are linearly correlated to their daily doses required for therapeutic treatment in patients.

In the present studies the DA receptor blocking activities of different forms of fluphenazine and its metabolites and butaperazine and its metabolites were determined by their abilities to inhibit ^{3}H-spiroperidol binding to rat caudate in vitro. The in vivo neuroleptic activity of these compounds was assessed by their ability to produce catalepsy in rats. The onset latency of catalepsy was dose-dependent. Butaperazine derivatives (sulfone and sulfoxide), which did not inhibit spiroperidol binding in vitro, also failed to produce catalepsy. On the other hand, fluphenazine sulfoxide and fluphenazine N-oxide were active in both tests. These results suggest that fluphenazine metabolites can cross the blood brain barrier and have pharmacologic activity.

77.6 COMPARABLE EFFECTS OF HALOPERIDOL AND PARTIAL REINFORCEMENT ON THE RESISTANCE TO EXTINCTION OF A FOOD-REWARDED RUNWAY TASK IN RATS. C.H.Camp* and A.Ettenberg (SPON: H.Carlisle). Dept. Psychology, Univ. California, Santa Barbara, CA 93106

It is well established that dopamine antagonist neuroleptic drugs produce dose-dependent reductions in reinforced operant behaviors. This finding is consistent with the view that neuroleptics reduce the rewarding effects of positive reinforcers. However, since dopamine neurons are involved in extrapyramidal motor function, it is difficult to dissociate between reward- and performance-attenuating explanations of neuroleptic action. One means avoiding this problem would be to test animals after the drug has cleared their systems. For example, rats trained under partial reinforcement schedules later show an increased resistance to extinction. If neuroleptics attenuate or block the rewarding properties of food, then pretreatment on some training trials should have a similar effect as no-reward on some training trials i.e. increased resistance to extinction. The present study was devised to test this hypothesis.

The experiment consisted of two phases: 1) an acquisition phase during which each animal traversed a straight runway once a day for a reward of ten 45mg food pellets, and 2) an extinction phase of 21 days during which no food reward was provided. Latencies to leave the start box and to reach the goal box were recorded on every trial. Rats were assigned to either a partial reinforcement group (PRF) or one of two continuous reinforcement (CRF) groups (n=10/gp). PRF rats were not rewarded on 10 randomly spaced trials during acquisition while both CRF groups were rewarded on every trial. On the 10 days that the PRF rats were not reinforced, the CRF rats were injected (45min pretest) with either 0.15mg/kg haloperidol (CRF/HAL) or an equal volume of its lactic acid vehicle solution (CRF/VEH).

Rats that experienced non-rewarded trials during acquisition (PRF group) continued to run during extinction long after the CRF/VEH controls had ceased doing so. Although haloperidol reliably attenuated running latencies on drug days (suggesting a motor impairment), the CRF/HAL group demonstrated a prolongation of extinction that was indistinguishable from that produced by partial reinforcement. Since there was no drug present during the extinction phase of the study these data demonstrate a neuroleptic-induced reward deficit which cannot easily be accounted for by reductions in general performance capacity.

77.7 EFFECTS OF THE PUTATIVE D-1 ANTAGONIST, SCH 23390, IN RODENT AND PRIMATE MODELS OF STRIATAL DOPAMINE ANTAGONISM. S. Gerhardt*, R. Gerber and J.M. Liebman (SPON: M. Roffman). Neuroscience Res., Res. Dept., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901

Two types of brain dopamine receptors, designated as D-1 (adenylate cyclase-linked) and D-2, respectively, have been postulated on the basis of receptor binding and pharmacological studies. Virtually all marketed antipsychotic drugs antagonize D-2 receptors, and are associated with extrapyramidal symptomatology. Although the function of brain D-1 receptors is unclear at present, the recent discovery of a putative selective D-1 antagonist, SCH 23390 (Iorio et al., J. Pharmacol. Exp. Ther. 226:462, 1983) offers an opportunity to clarify the possible role of striatal D-1 receptors in extrapyramidal dysfunction. The present experiments assess SCH 23390 in two animal behavioral models of extrapyramidal (striatal) involvement.

Drugs that induce an acute dyskinetic syndrome in monkeys also produce extrapyramidal side effects in humans (Liebman and Neale, Psychopharmacology 68:25, 1980). At doses that blocked conditioned avoidance responding in squirrel monkeys, SCH 23390 (1 and 3 mg/kg p.o.) did not produce the acute dyskinetic syndrome in any of six monkeys. Even at 10 mg/kg p.o., only one of six monkeys showed this extrapyramidal syndrome. Haloperidol (1.25 mg/kg p.o.) induced the acute dyskinetic syndrome in all six monkeys. These results are consistent with the suggestion (Iorio et al, 1983) that SCH 23390 may have low extrapyramidal potential.

SCH 23390 was further investigated for its ability to block apomorphine-induced climbing and stereotyped sniffing in mice. The induced climbing has been attributed to stimulation of dopamine neurons in nucleus accumbens (Costall et al., Eur. J. Pharmacol. 96:201, 1983), while the stereotyped sniffing appears to be striatal in origin. SCH 23390 inhibited apomorphine-induced climbing (ED_{50} = 0.78 mg/kg) but only partially blocked stereotyped sniffing at doses as high as 30 mg/kg. Other antipsychotic drugs with D-2 antagonist properties blocked both behaviors at similar doses. It is possible that the behavioral effects of D-1 antagonists may be mediated largely by mesolimbic rather than striatal neurons.

77.8 DIFFERENTIAL EFFECTS OF PIMOZIDE AND CLOZAPINE ON FIXED INTERVAL FOOD REINFORCED RESPONDING IN RATS. G. Kaempf* and J. Porter*(SPON: M. Lynch). Dept. of Psychol., Virginia Commonwealth Univ., Richmond, VA 23284.

This study assessed the acute and chronic effects of pimozide (PMZ: 0.0, 0.1, 0.3, 1.0 mg/kg) and clozapine (CZP: 1.0, 3.0, 10.0 mg/kg) on fixed interval 60 sec (FI-60) responding and spontaneous motor activity (SMA) across 10 consecutive days of drug treatment. Daily operant sessions were 30 min in length and occured 4 hr after PMZ injection and 1 hr after CZP injection. SMA was measured during a 10 min period immediately following the operant sessions on the first 4 and eighth days of treatment. Overall response rates (RR), index of curvature (IOC: Fry,W., Kelleher,R., Cook,L., J. Exp. Anal. Beh., 3, 193, 1960), and the number of reinforcers delivered (SR) served as indices of operant responding.

PMZ produced a significant dose-related suppression of operant RR and SR that persisted across all 10 sessions; however, PMZ failed to affect IOC. In contrast, CZP significantly suppressed operant responding as indicated by all 3 measures. The rats developed tolerance to this CZP-induced suppression of operant responding in that all CZP-treated groups returned to vehicle control response levels by the seventh session. Both PMZ and CZP suppressed SMA in a dose-related manner that persisted through Day 8.

Several conclusions were drawn from these data. First, PMZ and CZP produced different profiles of effects on operant behavior. Also, simple response rates provided inadequate information to differentiate these two neuroleptics. Other measures of operant performance (eg. IOC) are required to provide adequate classification of compounds. Finally, acute dosing procedures may not provide a reliable basis for differentiating between typical and atypical neuroleptic compounds. The development of tolerance to CZP's disruptive effects provides evidence that the mechanisms underlying the suppression of operant behavior after acute treatment are qualitatively different for CZP and PMZ.

77.9 CHARACTERIZATION OF THE BUPROPION CUE IN THE RAT: LACK OF EVIDENCE FOR A DOPAMINERGIC MECHANISM. R.D.Blitzer*and R.E.Becker. Dept. Pharm., Univ of Rhode Island, Kingston, R.I. and Dept. Psychiatry, SIU School of Medicine, Springfield, IL 62708.

Using a 2-lever operant task rats were trained to discriminate, from saline, the cue produced by 40mg/kg i.p. of bupropion, a non-tricyclic antidepressant. Despite bupropion's established dopaminergic activity in vitro and in vivo, it was found that the bupropion cue was neither mimicked by the dopamine agonists L-DOPA and bromocriptine nor blocked by a variety of neuroleptics (haloperidol, thioridazine and thiothixene). In addition, bupropion was active in attenuating the behavior-suppressing effects of haloperidol, in contradistinction to amphetamine and the atypical antidepressants nomifensine and viloxazine. The bupropion cue was not mimicked or disrupted by adrenergic or serotonergic drugs, but it did generalize to some stimulants (amphetamine, cocaine and caffeine) as well as to nomifensive and viloxazine. The generalizations were blocked by neuroleptics. These data indicate that bupropion's cue properties are not based on its dopaminergic activity even though its generalization to drugs appears to be mediated through dopamine receptors. The possible involvement of phenylthylamine in the bupropion cue is also discussed.

77.10 SERUM LEVELS OF A NEW ANTIPSYCHOTIC, BMY 13859, IN CLINICAL TRIALS DETERMINED BY RADIORECEPTOR ASSAY. D. K. Hyslop, J. D. Arnold,*[1] W. J. R. Taylor,* and D. P. Taylor. CNS Research, Pharmaceutical Research and Development Division, Bristol-Myers Company, Evansville, IN, 47721, and [1]Quincy Research Center, Kansas City, MO, 64127.

The new psychotropic agent BMY 13859 has demonstrated preclinical activities which suggest future utility as an antipsychotic drug (Riblet et al., Soc. Neurosci. Abstr. 8: 470, 1982). These include inhibition of conditioned avoidance responding and blockade of apomorphine- and amphetamine-induced stereotyped behavior. Despite a lack of potent in vitro or in vivo cholinergic receptor interaction, this potent D_2-dopamine antagonist (IC_{50} = 10 nM in vitro against [^{3}H]spiperone on striatal membranes) when administered orally does not induce a neuroleptic-like catalepsy in rats. Receptor analysis following chronic administration to rats reveals no change in [^{3}H]spiperone binding to dopamine sites and suggests that BMY 13859 will not cause symptoms of tardive dyskinesia after long-term clinical use. In the dog, oral administration of BMY 13859 blocked amphetamine-induced stereotyped behavior for 6 hours, and this effect was accompanied by sustained serum levels of [^{3}H]spiperone-displacing substances derived from BMY 13859 (Eison et al., Methods and Findings, 1984, in press). Recently, we have analyzed serum samples from subjects receiving 70 mg doses of BMY 13859. In preliminary experiments with spiked samples of control serum, BMY 13859 appeared to be associated with (presumably) serum proteins since incubation (4 hours at 22°) shifted the compound's IC_{50} from 92 nM to 163 nM. Clinical samples revealed serum concentrations equivalent to 10 nM haloperidol or 160 nM chlorpromazine at one hour after oral dosing. These levels of [^{3}H]spiperone binding activity are consistent with those seen with conventional antipsychotics. Moreover, levels of [^{3}H]spiperone-displacing material were detectable 6 hours after dosing as well. Thus, BMY 13859 may represent a step forward in efficacy and safety in the treatment of psychoses.

77.11 WITHDRAWAL OF ANTICHOLINERGIC MEDICATION FROM PATIENTS TREATED WITH ANTIPSYCHOTICS D.B. Menkes, G. Caradoc-Davies,* H.O. Clarkson,* and P.E. Mullen,* Dept. Psych. Med., Univ. of Otago, NEW ZEALAND.

Extrapyramidal side-effects (EPS) occuring in patients receiving antipsychotics (APs) are effectively suppressed by the co-administration of antimuscarinic anticholinergic drugs (ACs). In order to prevent EPS, many patients on APs are given ACs prophylactically; it is of interest to establish whether such treatment is necessary or desirable for the majority of patients, particularly since ACs add cost, inconvenience and the possibility of their own side-effects to the AP treatment regimen. The present study was designed to examine the use of these drugs in a population of chronically hospitalised patients treated in a large state psychiatric institution. Of 680 inpatients 253 (37.2%) were found to be on APs. Of these, 179 (70.8%) were simultaneously treated with ACs. Due to the duration of hospital stay in many of these patients, it was not possible to document the incidence of EPS at treatment initiation in this population; nonetheless, the overwhelming majority appeared to have been treated with ACs prophylactically since the onset of AP therapy. In a pilot study, 6 patients chronically on APs (mean ± S.E.M. chlorpromazine equivalents = 1796 ± 832 mg/day) were withdrawn from ACs and evaluated for EPS using the modified Columbia rating scale. In one case (chlorpromazine equivalents = 2400 mg/day), noticeable tremor and bradykinesia developed, but the remaining patients suffered no significant EPS after AC withdrawal. In order to provide an unbiased appraisal of the consequences of AC withdrawal, a cohort was selected (from the 179 patients on both APs and ACs) by excluding children under 15 yrs, the aged (over 65 yrs), those with epilepsy or recent E.C.T., those with tardive dyskinesia or other movement disorders, those on low doses of APs (under 100mg chlorpromazine equivalent), and those unable or unwilling to cooperate. These criteria left 45 patients for a 12 week, double-blind, placebo-controlled, gradual withdrawal from a standardized AC regimen (benztropine 2mg b.d.) The results of the withdrawal in terms of EPS and severity of psychosis will be detailed and the implications discussed.

77.12 **EFFICACY AND SAFETY OF BW234U, AN ATYPICAL ANTIPSYCHOTIC, IN HOSPITALIZED SCHIZOPHRENIC PATIENTS.** **A.T. Dren, A.R. Hickey*, M.J. Dalton* and P.J. Manberg. Burroughs Wellcome Co., Research Triangle Park, NC 27709.**

BW234U, a novel carbazole derivative, exhibited pharmacologic properties in animals indicative of antipsychotic activity but with a mechanism of action different from that of the conventional neuroleptics (Ferris et al. J. Pharm. Pharmacol. 34:388, 1982).

Clinically, BW234U was initially evaluated in a multicenter, 28-day, open-label trial in 59 schizophrenic patients hospitalized with an acute exacerbation of the disorder. Forty-eight patients completed at least 2 weeks of treatment on a flexible b.i.d. dose regimen of 50-600 mg/day. Clinical improvement was evident on all psychiatric rating scales without the occurrence of significant extrapyramidal or autonomic side effects. Consequently, placebo-controlled and chlorpromazine-controlled multicenter investigations were initiated and are currently ongoing to confirm the therapeutic efficacy of BW234U in schizophrenia.

The placebo-controlled trial employs two fixed dose regimens of BW234U (100 and 300 mg/day) and placebo, administered on a b.i.d. basis. The chlorpromazine study employs flexible dose regimens of chlorpromazine (400-1600 mg/day) and BW234U (200-800 mg/day and 100-400 mg/day), also administered b.i.d. Measures of efficacy consist of the CGI and BPRS scales. Safety assessments include an Adverse Events Checklist and vital sign, EKG, EEG, ophthalmologic, and clinical laboratory measurements. Simpson-Angus and AIMS ratings are performed at weekly intervals to evaluate alterations in extrapyramidal neurological function.

Interim analyses of these studies have shown that BW234U, has efficacy superior to placebo at 300 mg/day, and, efficacy comparable to chlorpromazine in the dose range of 100-400 mg/day. Compared to chlorpromazine there has been a noticeable lack of acute extrapyramidal side effects (dystonias, akathisia, etc.) with BW234U treatment. In addition, anticholinergic and cardiovascular complaints have been rare and sedation minimal. Clinical trials continue in a variety of settings to better define the therapeutic potential of this unique antipsychotic.

77.13 THE PHARMACOLOGY OF A NEW ANTIPSYCHOTIC AGENT FREE OF DIRECT DOPAMINE BLOCKING ACTIVITY. R. Ferris, G. McKenzie, J. Howard, F. Soroko*,M. Harfenist*† and R. Maxwell*. Depts. of Pharmacology and Organic Chemistry†, The Wellcome Research Labs, Research Triangle Park, NC 27709.

BW 234U cis-9-[3-(3,5-Dimethyl-1-piperazinyl)propyl]carbazole·2 HCl is a novel antipsychotic agent, which like neuroleptics blocks apomorphine (APO)-induced aggression in rats and APO-induced climbing in mice (limbic mediated behaviors). Unlike neuroleptics, it does not antagonize APO-induced sterotyped behavior, does not produce catalepsy and does not block conditioned avoidance response (striatal mediated behaviors).

BW 234U was also an effective antagonist of aggressive behavior in several other animal models. The compound blocked muricidal (mouse killing) behavior in isolated male rats and blocked isolation-induced aggression in mice. Of great interest is the finding that the selective blockade of limbic mediated behaviors is achieved without any direct effect of the compound on dopaminergic receptors. Concentration of 10^{-4}M or greater are necessary to achieve weak, if any, effects on dopamine-stimulated adenylate cylase activity (D_1), ^{3}H-spiroperidol (D_2) or ^{3}H-dopamine binding in limbic or striatal areas of rat brain. The extremely weak potency of BW 234U as a blocker of D_2 receptors in brain is readily apparent when compared to the potencies of several other neuroleptics. For example, BW 234U was 160 times weaker than sulpiride, the neuroleptic studied with the least potency for D_2 receptors, and 10,000 times weaker than haloperidol, the neuroleptic studied with the greatest potency for D_2 receptors. Bw 234U has no effect on the enzymes involved in catecholamine synthesis and metabolism in vitro and does not effect the synthesis of ^{3}H-dopamine from ^{3}H-tyrosine in vivo. The compound has moderate potency as an inhibitor of $5HT_2$ receptors but only negligible effects on cholinergic, α_1-, α_2-, ß-adrenergic, benzodiazepine, Gaba receptors and calcium channels as judged by binding assays.

These data, taken in aggregate, suggest that BW 234U has a specific but indirect effect on dopaminergic activity in limbic areas, and thus, should be a useful antipsychotic agent in man. The absence of any direct or indirect effects on striatal areas suggest it should not produce extrapyramidal side effects in man. Preliminary results of these and other studies have been reported by Ferris et al., J. Pharm. Pharmacol., 34:388-390 (1982).

77.14 A COMPARISON OF THE BEHAVIORAL EFFECTS OF A NOVEL ANTIPSYCHOTIC, BW 234U, WITH TWO TRADITIONAL NEUROLEPTICS. James L. Howard, Gerald T. Pollard, Kenneth W. Rohrbach* and S. Teresa McBennett*. Department of Pharmacology, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

BW 234U is a novel compound which has been shown to possess antipsychotic efficacy in acute schizophrenia. The capacity of BW 234U to block apomorphine-induced aggression in rats predicted its antipsychotic activity. Its failure to block apomorphine-induced stereotypy and its lack of effect on dopamine systems in brain suggested that it would not cause extrapyramidal side effects (Ferris et al., J. Pharm. Pharmacol., 1984, 34, 388). BW 234U was compared to the classical antipsychotics haloperidol (H) and chlorpromazine (C) for effects on locomotor activity, two-way avoidance, lever-pressing for electrical stimulation of the medial forebrain bundle, and lever-pressing for food under multiple fixed-interval/fixed-ratio and differential reinforcement of low rate schedules in rat. At doses that blocked aggression, H and C reduced locomotion and responding on all tasks, but BW 234U had no effect. BW 234U did moderately reduce locomotion and responding for brain stimulation and food at doses two to four times its ED_{50} for blockade of aggression, but at no dose tested did it affect avoidance responding. The behavioral profile of BW 234U is different from that of H and C, supporting the view that it represents a new, "non-neuroleptic" class of antipsychotic drugs.

77.15 EVIDENCE THAT THE NOVEL ANTIPSYCHOTIC, BW 234U ACTS IN LIMBIC STRUCTURES OF BRAIN TO ANTAGONIZE APOMORPHINE-INDUCED AGGRESSION. Barrett R. Cooper, Gregory N. Ervin*, and Kaido Viik*. Dept. of Pharmacology, Wellcome Research Labs, Research Triangle Park, North Carolina 27709.

BW 234U is a novel compound with reported antipsychotic effects in acute schizophrenics in open studies and in double-blind placebo controlled clinical trials. BW 234U was developed on the basis that it blocked apomorphine-induced aggression like haloperidol and chlorpromazine, but unlike these two neuroleptics, BW 234U does not antagonize apomorphine-induced stereotyped behavior (Ferris et al., J. Pharm. Pharmacol. 34, 388, 1982). These effects suggested a selective antagonism of limbic system dopaminergic hyperactivity. Studies with ^{14}C- and ^{3}H-BW 234U showed that it has a differential distribution in brain with highest amounts in frontal cortex, lateral septum, amygdala, and hippocampus and lowest amounts in striatum, cerebellum and olfactory bulb. Although this distribution differs markedly from the ^{3}H-spiroperidol (^{3}H-spiro) distribution in brain both agents do accumulate in frontal cortex and lateral septum. While pretreatment with various dopamine antagonist neuroleptic drugs will prevent the selective accumulation of ^{3}H-spiro in frontal cortex, BW 234U does not effect the ability of ^{3}H-spiro to accumulate selectively anywhere in brain, nor does pretreatment with various neuroleptics effect the distribution of ^{3}H-BW 234U. These results suggest that the same sites (e.g. DA receptors) do not influence the accumulation of these two drugs in vivo and that BW 234U is not metabolized to a neuroleptic-like drug in vivo that would compete with ^{3}H-spiroperidol.

Brain lesions or direct injection of BW 234U into various brain structures has been used to determine if the areas that concentrate BW 234U participate in its antiaggressive (hence presumed antipsychotic) action. The ED_{50} for BW 234U as an antagonist of apomorphine-induced aggression was shifted from 12.5 mg/kg to 37 mg/kg i.p. by electrolytic lesions of the lateral septum or frontal cortex. Direct injections of BW 234U into frontal cortex and lateral septum antagonized APO aggression while injections into the lateral ventricle, nucleus accumbens septi or striatum did not. These findings add to current evidence that suggests BW 234U acts in the limbic system to antagonize apomorphine-induced aggression by a non-dopaminergic mechanism. This limbic action may be relevant to its antipsychotic efficacy and supports the view that BW 234U represents the first of a new "non-neuroleptic" class of antipsychotic drugs.

77.16 THE EFFECTS OF THE NOVEL ANTIPSYCHOTIC BW 234U ON MONOAMINES AND THEIR METABOLITES IN DIFFERENT REGIONS OF RAT BRAIN: COMPARISON TO HALOPERIDOL. Dana M. Vaughn, C.M. Wang* and Barrett R. Cooper. Dept. of Pharmacol., Burroughs Wellcome Co.,Research Triangle Park, North Carolina 27709.

BW 234U is a novel compound reported to have antipsychotic effects in schizophrenics in open studies and in double blind placebo controlled clinical trials. This agent was developed on the basis of blocking apomorphine-induced aggression in rats like haloperidol and other neuroleptic drugs but differs in that it does not block apomorphine-induced stereotyped behavior or bind to dopamine receptors in vitro (Ferris et al., J. Pharm. Pharmacol., 34, 388, 1982). In these experiments the effects of BW 234U and haloperidol on neurochemical measures of the activity of monoamine neural systems were studied using doses of each drug that were equated for potency in the apomorphine-induced aggression model (½X, 1X, 2X ED_{50} values by the i.p. route). The purpose of this experiment was to determine if these two antipsychotics shared a common action on one or more monoamine systems and at one or more sites in brain that could be correlated with both drug's ability to antagonize apomorphine-induced aggression. HPLC procedures were used to determine levels of NE, DA, DOPAC, HVA, 5HT and 5HIAA in 6 brain regions selected on the basis of the distribution of BW 234U in brain and of terminals of the dopamine, norepinephrine or 5HT neural systems. The only common neurochemical effect of the two drugs that has been noted thus far is a 20-30% dose related increase in 5HIAA in the frontal cortex, nucleus accumbens, and olfactory tubercular areas of brain. Dopamine levels or its metabolism to DOPAC or HVA were not effected by BW 234U in a dose dependent manner nor were changes greater than 25% while haloperidol produced the expected large dose-dependent increases in dopamine metabolites in brain areas receiving dopamine innervation (striatum, n. accumbens-olfactory tuberculum, lateral septum, amygdala and frontal cortex). Norepinephrine levels were unaffected by either drug. Results so far suggest that if any monoamine system participates in the common ability of both BW 234U or haloperidol to antagonize apomorphine-induced aggression, it is likely serotonin and further studies to evaluate this possibility are planned. Thus BW 234U is a novel antipsychotic that differs from existing neuroleptic antipsychotics in that it does not directly influence the activity of dopamine neural systems.

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NO ABSTRACT

80 SYMPOSIUM. MULTIMODAL MAPS IN THE SUPERIOR COLLICULUS. J. C. Middlebrooks, Stanford University (Chairman); B. E. Stein, Medical College of Virginia; J. T. McIlwain, Brown University; D. L. Sparks, University of Alabama in Birmingham.

The superior colliculus mediates shifts of gaze directed to the sources of visual, auditory, and somatic stimuli. The deeper layers contain coincident maps of sensory and motor space. This symposium will compare the representations of different sensory and motor modalities and will consider the translation of sensory signals into motor commands.

Auditory neurons in the superior colliculus are selective for the location of a sound source. Middlebrooks will describe the spatial pattern of neural activity in the colliculus elicited by a sound source at a given location. Most sounds activate a substantial fraction of the auditory neurons in the contralateral superior colliculus, yet the location of the source is represented by the position of a restricted population of neurons activated to near their maximum firing levels.

Many neurons in the superior colliculus respond to multiple sensory modalities. Stein will review the characteristics of the map of the body surface in the colliculus, then will discuss the nature of multimodal convergence at the single unit level. Multimodal interactions elicited by visual, auditory, and somatic stimuli can be facilitatory or inhibitory.

A punctate visual stimulus activates units throughout an area of the superior colliculus approximately 3 mm in diameter, and a saccadic eye movement elicited by electrical stimulation of the colliculus is preceded by widespread activation of collicular output cells. Nevertheless, the direction and magnitude of an electrically-evoked eye movement depends critically on the position of the stimulating electrode. McIlwain will consider the problem of transforming the activity in a large population of collicular neurons into a unique signal to the oculomotor system.

The deeper layers of the superior colliculus contain neurons which discharge before saccadic eye movements. Sparks will present data demonstrating that, at the level of the superior colliculus, auditory and visual signals have been translated into common "motor" coordinates and share neural pathways for the initiation of saccades.

INVERTEBRATE LEARNING AND BEHAVIOR I

81.1 A CELLULAR MECHANISM FOR THE TEMPORAL SPECIFICITY OF CLASSICAL CONDITIONING OF THE SIPHON-WITHDRAWAL RESPONSE IN APLYSIA. G. A. Clark* (SPON: H. Chiel). Center for Neurobiology & Behavior, Columbia University, College of Physicians & Surgeons, and New York State Psychiatric Institute, New York, NY 10032.

Like several forms of learning in mammals, classical conditioning of the siphon-withdrawal response in Aplysia exhibits a precise temporal specificity. Learning is optimal if the conditioned stimulus (siphon or mantle touch CS) precedes the unconditioned stimulus (tail shock US) by approximately 500 msec; longer intervals, backward pairings, and explicity unpaired training all result in relatively less enhancement of the siphon-withdrawal response (Hawkins et al., 1983). Previous experiments have suggested that differences between paired and unpaired training may be accounted for by an activity-dependent enhancement of presynaptic facilitation: when spike activity in the siphon sensory cells precedes a facilitatory input (activated by tail shock), it results in greater presynaptic facilitation than in unpaired controls (Hawkins et al., 1983; Walters & Byrne, 1983). In the present study, I examine the temporal specificity of this effect more closely, and find that activity-dependent facilitation may account for differences between forward and backward conditioning as well.

To compare effects of forward and backward pairing, I recorded intracellularly from two LE siphon sensory cells which projected to a common postsynaptic target (a siphon motorneuron or interneuron). Each sensory neuron was stimulated intracellularly (4 tests at 3 min intervals) to establish a baseline for the monosynaptic EPSPs elicited in the postsynaptic cell. Spike trains (7 spikes, 10 Hz) in the two sensory neurons were then paired with a pedal (tail) nerve shock US (10 Hz, 1 sec). For one sensory neuron (forward paired cell), the spike train began 500 msec before the US; for the other sensory neuron (backward paired cell), the spike train began 500 msec after the US. Training consisted of two such pairings, and was followed by two post-training tests. To control for possible intrinsic differences in facilitation in the two cells, I rested the preparation for 15 min, and then began another training series in which the pairing conditions of the two sensory cells were reversed; thus, each cell was tested under both forward and backward training conditions. In 8 preparations, I found that forward pairing produced a significantly greater facilitation of EPSPs than did backward pairing (64% $\pm$ 11% increase vs. 22% $\pm$ 11% increase, $p < .01$). These results indicate that spike activity must precede the arrival of the facilitatory input for activity-dependent facilitation to be maximally effective; thus, both the order and temporal proximity of these two events are important.

81.2 EVIDENCE THAT ACTIVITY-DEPENDENT FACILITATION UNDERLYING CLASSICAL CONDITIONING IN APLYSIA INVOLVES MODULATION OF THE SAME IONIC CURRENT AS NORMAL PRESYNAPTIC FACILITATION. R. D. Hawkins and T. W. Abrams. Center for Neurobiology & Behavior, Columbia University, and New York State Psychiatric Institute, New York, N.Y. 10032.

The siphon withdrawal reflex of Aplysia exhibits sensitization in response to shock to the tail, and classical conditioning when a weak stimulus to the siphon or mantle shelf is paired with tail shock (Carew, Walters, and Kandel, 1981; Carew, Hawkins, and Kandel, 1983). Cellular studies indicate that a mechanism of the conditioning is activity-dependent amplification of the mechanism of sensitization of the reflex: presynaptic facilitation due to broadening of action potentials in the sensory neurons (Hawkins, Abrams, Carew, and Kandel, 1983). Broadening of the action potentials could in turn be due to changes in several different ionic currents. Previous studies have shown that normal presynaptic facilitation involves a decrease in a specific ionic current, the serotonin and cAMP-sensitive K^+ current, I_S (Klein, Camardo, and Kandel, 1982; Siegelbaum, Camardo, and Kandel, 1982). In the experiments reported here we investigated whether activity-dependent facilitation involves modulation of the same ionic current.

The training procedure was identical to that used previously to demonstrate differential facilitation of EPSPs in the circuit for the withdrawal reflex (Hawkins et al., 1983). Briefly, intracellularly produced action potentials in one siphon sensory neuron were paired with shock to the tail nerve, while action potentials in another sensory neuron were specifically unpaired with the tail nerve shock. Before and after training, we voltage clamped each sensory neuron with a single electrode clamp (Dagan 8100) and measured the outward current during depolarizing voltage clamp pulses (500 msec and 20 mV, from a holding potential of -50 mV). Five minutes after 5 training trials, there was a significantly greater decrease in the net outward current (leakage subtracted) in the paired than in the unpaired sensory neurons (mean decrease = 15.3% $\pm$ 2.7% in the paired neurons and 5.0% $\pm$ 3.7% in the unpaired neurons in 12 experiments, $t=3.15$, $p < .01$). Since the parameters of the voltage clamp pulses were chosen so that most of the outward current was carried by I_S, it is likely that this differential decrease in outward current was due to a differential decrease in I_S. Moreover, the time and voltage dependences of the current which was modulated differentially in these experiments were similar to the time and voltage dependences of I_S. These results suggest that activity-dependent facilitation underlying conditioning of the withdrawal reflex involves modulation of the same ionic current (and thus perhaps the same cAMP cascade) as normal presynaptic facilitation underlying sensitization of the reflex.

81.3 POSSIBLE ROLES OF CA^{2+} and cAMP IN ACTIVITY-DEPENDENT FACILITATION, A MECHANISM FOR ASSOCIATIVE LEARNING IN APLYSIA. T.W. Abrams, L. Bernier, R.D. Hawkins, and E.R. Kandel. Center for Neurobiology & Behavior, Columbia Univ., College of P & S, and NYS Psychiat. Instit., New York, NY 10032.

In the classical conditioning of the siphon withdrawal reflex of Aplysia, the tail shock US produces presynaptic facilitation of synaptic transmission from siphon sensory neurons (SNs) of the CS pathway. The facilitation is enhanced if the SNs have fired action potentials just prior to receiving facilitatory input, as occurs during training when the CS precedes the US.

To analyze the cellular basis of this associative mechanism, we have been using a 1 sec puff of 5-HT in place of the US (the facilitatory input from the tail). Paired spike activity also enhances the facilitatory response to a single 5-HT puff, as indicated by broadening of the action potential (Abrams et al., 1983). Previous experiments in which extracellular Ca^{2+} was greatly reduced suggested that Ca^{2+} influx is critical for this activity-dependent enhancement. Since Ca^{2+} manipulations may have diverse effects, we have tried to test this Ca^{2+} hypothesis using additional approaches. Recently, we have begun to study the effects of intracellular injections of EGTA, a Ca^{2+} chelator, on activity-dependent facilitation. Our initial results are consistent with the hypothesis that it is the transient elevation of intracellular Ca^{2+} during spike activity that enhances the facilitation response.

Since activity-dependent facilitation appears to involve modulation of the same cAMP-sensitive K^+ channel as does conventional facilitation (Hawkins et al., 1984), we have asked: Does paired activity result in an increased cAMP response to facilitatory input? cAMP levels were compared in SN clusters receiving a puff either alone or immediately after a train of five action potentials. Experiments were carried out with synaptic transmission blocked to prevent the spike activity in the SN from itself producing any detectable facilitation. In preliminary experiments, clusters that fired spikes prior to the puff had four-fold higher cAMP levels than clusters receiving the puff alone (see also Ocorr et al., 1983).

These differential levels of cAMP in cells receiving puffs paired with activity could be due to either of two possible effects of spike activity and Ca^{2+} influx: 1) increased cAMP synthesis or 2) decreased cAMP degradation. To discriminate between these possibilities, we have looked at the effect of elevated Ca^{2+} levels on degradation of cAMP in Aplysia neurons. As in other species, cAMP hydrolysis was not inhibited but stimulated by Ca^{2+}, via calmodulin. While still preliminary, these results suggest that by potentiating the synthesis of cAMP, elevated intracellular Ca^{2+} levels enhance the facilitation response to the US in SNs that have recently been excited by the CS.

81.4 A CALCIUM-STIMULATED SUBPOPULATION OF ADENYLATE CYCLASE IS A PUTATIVE COMPONENT OF A MEMORY APPARATUS IN DROSOPHILA. Yadin Dudai, Department of Neurobiology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Adenylate cyclase activity in Drosophila homogenates is heterogeneous with respect to its subcellular distribution, affinity for MgATP and responsiveness to divalent cations. When subjected to discontinuous sucrose gradient centrifugation, all the activity in the head and >90% of the activity in the thorax sediment in a particulate fraction, but up to 50% of the activity in the abdomen is soluble. The affinity of the enzyme for MgATP is lower in the soluble fraction then in the particulate fraction. Kinetic studies further suggest heterogeneity in the affinity for MgATP in washed, particulate fractions. This apparent heterogeneity is not abolished by GTPγS (10μM). Low concentrations of Ca^{2+} ($<10^{-7}$M) stimulate the particulate enzyme and high concentrations ($>10^{-6}$M) inhibit it; the Ca^{2+}-stimulation is much more pronounced in the thorax and the abdomen than in the head. Free Mg^{2+} and free Mn^{2+} (at 10^{-4}–10^{-3} M) increase the Vmax of the particulate enzyme with little effect on the Km. The conditioning mutant rutabaga (rut) lacks ca. 30% of the total particulate adenylate cyclase activity. The Ca^{2+}-stimulation of the enzyme is not detected in the mutant. The defective, or missing, enzyme is a form which is also strongly activated by free Mg^{2+} in the mM range, and its activity is reduced in the presence of high concentrations of free Mn^{2+} (ca. 10^{-2}M). The enzyme in rut is still capable of being efficiently stimulated by F^-, Gpp(NH)p, GTPγS and octopamine, although none of the above agents or their combinations, in the presence of MgATP or MnATP, raise the defective activity to normal levels. Our results may be explained by assuming that the rut gene codes for a subpopulation of the catalytic subunit of adenylate cyclase, which can be stimulated by low Ca^{2+}, or for a separate polypeptide which regulates the Ca^{2+} and Mg^{2+} responsiveness of catalytic subunit(s) of the enzyme. Since rut is a memory mutant, it is tempting to assume that a Ca^{2+}-activated cyclase is a component of a molecular apparatus that is required for memory formation. It should be noted that a cyclase system which is responsive both to a neurotransmitter and to intracellular Ca^{2+} has been suggested to serve as a molecular substrate for the interaction of paired stimuli during associative conditioning in Aplysia (Hawkins et al., Science 219, 400 (1983)). Purification of the Ca^{2+}-stimulated adenylate cyclase from Drosophila is currently underway. (Supported by a grant from the Forcheimer Centre for Molecular Genetics).

81.5 STRONG CLASSICAL CONDITIONING IN NORMAL AND MUTANT DROSOPHILA REVEALS SHORT-TERM MEMORY DEFICIENCIES IN MUTANTS. T. Tully* and W.G. Quinn*. (SPON: F. Rob Jackson), Dept. of Biology, Princeton University, Princeton NJ 08544

By making shock inescapably paired with one of two odors in a differential (discriminative) conditioning procedure, we have found that 95% of trained, wild-type flies will avoid the previously shocked odor in a T-choice test trial compared to 50% avoidance of naive flies. Maximal conditioned avoidance results from a delay conditioning procedure. Trace conditioning shows the usual decline in conditioned avoidance as the CS-US trace interval is lengthened, and backward conditioning produces no conditioned effect. Furthermore, conditioned avoidance increases with increasing CS or US saliency.

Three nonassociative control procedures yield no increased avoidance. Presenting the US alone (pseudoconditioning procedure) has no effect on avoidances of either odor, while presenting CS1 and CS2 without shock (sensitization procedure) produces some habituation to each odor. An explicitly unpaired procedure also has no effect on conditioned avoidance. Single-gene mutants, originally isolated because they failed to learn in an instrumental conditioning procedure (Quinn et al., 1974, PNAS 71: p.708) all show moderate conditioned avoidance using the classical conditioning procedure. More importantly, their conditioned avoidance levels are strong enough to produce relatively long-term retention. Comparison of retention curves from the mutants, dunce, rutabaga and amnesiac, to that from wild-type flies reveals that each of these mutations cause short-term memory to decay three times faster than in wild-type flies, while long-term memory is similar in all four strains.

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81.6 HABITUATION IN STENTOR IS DEPENDENT ON BOTH REPEATED MECHANICAL STIMULATION AND THE PRODUCTION OF ACTION POTENTIALS. D.C. Wood, Psychobiology Program, Psychology Department, University of Pittsburgh, Pittsburgh, PA 15260.

The ciliate, Stentor coeruleus, contracts to an initial mechanical stimulus but habituates rapidly during repeated 1/min stimulation. Mechanical stimuli elicit graded receptor potentials capable of triggering action potentials. Action potentials elicit contractions. During the course of repeated mechanical stimulation receptor potential amplitude decreases progressively as the probability of action potential elicitation and contraction decreases. Action potential amplitudes remain unchanged. The decrease in receptor potential amplitude, i.e., habituation, is due to a shift in the curve governing the voltage dependence of the mechanoreceptor conductance.

On the other hand, mechanical stimuli which elicit only receptor potentials and not action potentials do not result in a decrease in receptor potential amplitude during repeated stimulation. Similarly repeated elicitation of action potentials with depolarizing current pulses does not produce a decrease in the amplitude of receptor potentials elicited by test mechanical stimuli. Only if both receptor potentials and action potentials are elicited on each trial does the receptor potential amplitude decrease rapidly. Action potentials elicited 1 sec before, 30 sec before and 200 msec after the mechanical stimulus are equally effective in producing the decrease in receptor potential amplitude.

The mechanism which makes action potential production necessary for habituation was studied in voltage clamped cells. Analogous to the previously observed decreases in receptor potential amplitude, inward receptor currents elicited by repeated mechanical stimuli decreased rapidly if each was followed by a 600 msec long voltage step from resting potential (-50 mV) to 0 mV (a mock action potential). Similarly, if the mechanical stimulus was followed by a voltage step to +60 mV, resulting in only outward current through the "action potential" channels, a rapid decrease in receptor current was observed. Even voltage steps to -30 mV, which produce negligible inward "action potential" currents, result in significant decreases in receptor current. Thus depolarization, not inward current, appears to be the signal by which the action potential initiates the habituation process.

81.7 SIMULATION OF NONASSOCIATIVE AND ASSOCIATIVE NEURONAL MODIFICATIONS IN APLYSIA. K.J. Gingrich and J.H. Byrne, Dept. of Physiology and Cell Biology, University of Texas Medical School, Houston, Texas 77025.

Defensive reflexes in Aplysia have proven to be attractive systems for analyzing neural mechanisms contributing to simple forms of learning such as habituation, sensitization and classical conditioning. Previous studies have shown that habituation of the gill-withdrawal reflex is associated with synaptic depression and sensitization with presynaptic facilitation of transmitter release from sensory neurons mediating the reflex. The synaptic depression, in turn, is associated with a decrease in Ca^{++} currents in the sensory neurons, while presynaptic facilitation with increased Ca^{++} currents produced indirectly by a decrease in a novel secretonin-modulated K^+-current (for review see Kandel & Schwartz, 1982). The present work represents an initial quantitative examination of the extent to which these mechanisms account for the synaptic plasticity. A lumped parameter mathematical model of the sensory neuron release process was constructed. Major components of this model include Ca^{++} channel inactivation, Ca^{++}-mediated neurotransmitter release and mobilization, and readily releasable and upstream feeding pools of neurotransmitter. The model not only simulates the data of synaptic depression and recovery from depression (Byrne, 1982) but also qualitatively predicts other features of neurotransmitter release that it was not designed to fit. These include features of synaptic depression with high and low levels of transmitter release, post-tetanic potentiation, and enhanced release produced by broadening the sensory neuron action potential. The model suggests that empirically observed somatic Ca^{++}-current kinetics cannot fully explain synaptic depression. Rather a large component of synaptic depression is due to reduction of the pools of releasable neurotransmitter (depletion). In order to simulate presynaptic facilitation an additional compartment describing cAMP regulation was added with cAMP levels used to produce spike broadening and enhance mobilization. Associative neuronal modifications experimentally produced by pairing spike activity with modulatory input (Walters & Byrne, 1983; Hawkins et al, 1983) were simulated by incorporating a Ca^{++}-mediated amplification of cAMP levels. The model is speculative but it has begun to indicate mechanisms that may underlie various aspects of synaptic plasticity in these cells.

81.8 DIFFERENTIAL CLASSICAL CONDITIONING OF TAIL AND SIPHON WITHDRAWAL IN APLYSIA. D.A. Ingram* and E.T. Walters, Dept. of Physiology & Cell Biology, Univ. of Texas Medical School, Houston, TX 77225

By pairing intracellular activation with noxious cutaneous shock and measuring changes in monosynaptic EPSPs, Walters and Byrne (1983) showed that individual mechanosensory neurons innervating the tail of Aplysia are capable of associative conditioning. If this associative cellular modifiability occurs naturally, one would predict that classical conditioning of behavioral responses could be produced using tail stimulation as a conditioned stimulus (CS). To test this prediction we implanted insulated silver electrodes bilaterally near the edge of the tail and a third electrode in the floor of the mantle cavity. The two tail electrodes were used to deliver weak discriminative stimuli (CS+ and CS-: 400 msec ac) while the mantle floor electrode delivered a strong unconditioned stimulus (US: 400 msec ac). The paired CS+ (chosen randomly) was delivered 500 msec prior to the US. The unpaired CS- was delivered either 150 sec before or 150 sec after the US. The US intensity was increased during training if the unconditioned responses showed any habituation to the US. Two blocks of 5 trials were run, with an intertrial interval of 5 min and an interblock interval of 40 min.

We used two behavioral indices of conditioning, both of which are known to involve synaptic excitation from identified tail sensory neurons: (1) tail withdrawal magnitude, judged on a 4 point scale and (2) the duration of siphon withdrawal. When tested (blind) the day following training the tail withdrawal responses to the CS+ were significantly greater than responses to the CS- ($U_{8,8} = 11.5$, $P < .02$). Similarly, CS+ siphon withdrawals were significantly longer than CS- siphon withdrawals ($t_{14} = 2.64$, $P < .01$). Selective CS+ effects also included the initiation of escape locomotion and elicitation of mantle pumping movements after training.

These results are consistent with the postulated role of activity-dependent neuromodulation in the tail sensory neurons as a cellular mechanism of associative learning (Walters and Byrne, 1983). To explore further the role of this cellular mechanism in learning we can now examine whether, during this form of behavioral conditioning, similar changes occur in tail sensory neuron properties.

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81.9 ASSOCIATIVE-TRAINING CORRELATED CHANGES IN I_{Ca-K} IN HERMISSENDA TYPE B PHOTORECEPTORS. J.Farley*, M.Sakakibara§*, and D.L.Alkon§. *Princeton Univ.,Princeton,NJ 08544 and §Lab. of Biophys., Sect. Neural Systems, NIH-NINCDS, Woods Hole,MA 02543

We report here that in Hermissenda B photoreceptors on retention days following associative training, a Ca^{++}-activated K^+ current, I_{Ca-K}, is reduced (see also Forman et al., this volume) as was previously observed for a rapidly inactivating K^+ current, I_A (Alkon et al., Science, 1982). Twenty-four or 48 hours after paired or random presentations of light and rotation (50 trials per day/3 days) retention tests were made and voltage-clamp analysis of I_{Ca-K} and a voltage-dependent inward Ca^{2+} current (I_{Ca}) were conducted on isolated medial and intermediate B cells, without knowledge of animals' conditioning histories. Steady state current measurements in ASW with 5 mM 4-AP and 100 mM TEA at both -10 and 0 mV (V_H = -60 mV) during a 1 sec depolarizing command step (750 msec) revealed significantly smaller I_{Ca-K} currents for paired [Mean ± S.E.M.: 2.33 ± 0.82 nA; 5.35 ± 0.98 nA; n=6] vs. random control animals [4.94 ± 0.71 nA; 11.50 ± 0.99 nA; n=8; t(12)'s=2.21; 3.99; $p<0.05$, 0.01]. I_{Ca} was first measured by substituting 10 mM Ba for Ca^{2+} and raising external K^+ from 10 mM to 300 mM K^+, and determining E_K to be 0 ± 2 mV (n=3). The steady state inward current at 0 mV (from V_H=-60) was significantly greater for paired [-8.29 ± 0.73 nA] vs. random animals [-5.13 ± 0.35 nA, $t(9)=3.16$, $p<0.01$]. In a second experiment, 5 mM 4-AP and 100 mM TEA were added to a 300 mM K^+/10 mM Ba^{2+} bath to further remove any residual K^+ current contribution to the I_{Ca} measurements. Significantly greater inward currents were measured for paired [-10 and 0 mV: -6.44 ± 0.37 nA, -8.04 ± 0.29 nA; n=7] vs. random control animals [-2.38 ± 0.70 nA, -4.58 ± 0.84; n=6, t(11)'s=4.92, 3.80, $p<0.01$]. Because values of I_{Ca} measured in these first two ways could be confused by the presence of residual I_{Ca-K} when E_K was not exactly 0 mV, I_{Ca} was measured with another protocol. The isolated cells, 24 hrs after training, were first repeatedly (~ 20X) washed in 0 Ca++-ASW with 5 mM 4-AP. Following penetration at -60 mV (under voltage clamp) the cells were washed (~ 10X) in ASW with 0 Ca++, 10 mM Ba++, 5 mM 4-AP and 100 mM TEA. I_{Ca} values under these conditions for Paired vs. Naive (without training) animals were: at -10 mV (-1.8 ± 0.44 nA, n=8, vs. -2.9 ± 0.33 nA, n=8, $t(14)=1.82$, N.S.), at -5 mV (-2.08 ± 0.41 nA, n=6 vs. -3.2 ± 0.28 nA, n=8, $t(12)=2.36$, $p<0.05$) and at 0 mV (-2.76 ± 0.30 nA, n=5, vs. -3.5 ± 0.22 nA, n=7, $t(10)=2.07$, N.S.).

81.10 REDUCED WITHDRAWAL FROM SHADOWS: AN EXPRESSION OF PRIMARY NEURAL CHANGES OF ASSOCIATIVE LEARNING IN HERMISSENDA. I. Lederhendler* and D.L. Alkon (SPON: G.H.Acheson). Lab. of Biophysics, NINCDS-NIH, Marine Biological Laboratory, Woods Hole, MA 02543.

When the marine mollusc, Hermissenda, encounters a rapid reduction in illumination (a shadow) while locomoting in an otherwise uniformly illuminated field, it stops and turns back into the light. This response is fast (seconds), and it is dependent on light intensity and dark adaptation. After associative training with light and rotation stimuli--a procedure known to produce a learned suppression of phototaxis--paired animals were less able to reverse their direction of locomotion at the shadow edge and spent significantly more time in the dark than the random and naive control groups (24 hr retention test: $F_{(2, 31)}=5.5$, $p<0.01$; 48 hr: $F_{(2,31)}=4.01$, $p<0.05$). However, the speed of locomotion through the field of uniform illumination remained unchanged.

After 13 min of dark adaptation the shadow response of naive animals was less apparent when the animals encountered the edge the first time than the second. This difference in responsiveness to the edge paralleled a difference in the long-lasting depolarization (LLD) of the type B photoreceptor recorded after the first light step following dark adaptation compared to the LLD following the second step ($t=4.07$, $p<0.01$). LLD magnitude then, was closely related to the magnitude of the shadow response whether affected by conditioning, light intensity, or dark adaptation.

Recently, several studies have reported that certain components of the phototactic pattern in gradients of illumination changed with conditioning including: start latency, speed, and time spent in the light. Our present finding indicating a functional relationship between LLD enhancement and the behavioral response to light-dark differences suggests a common underlying mechanism for these various components of modified phototaxis. More recently, we reported that short latency, and specific, muscular responses of the foot to the onset of light were modified by associative training (Biol. Bull., 1983, 165:529). Thus it is possible that the onset and the offset of light have different behavioral consequences which may be associated with different phases of photoreceptor activity. The close relationship between LLD magnitude and time course, and the learned behavioral changes measured in a variety of ways, is one more indication that the membrane changes which cause the LLD difference actually store the learned association for later recall.

82.1 SOMATOSTATIN- AND SUBSTANCE P-LIKE IMMUNOREACTIVITY WITHIN NEURITIC PLAQUES. D. M. Armstrong, D. Shields* and R. D. Terry*. Department of Pathology (D.M.A. & R.D.T.) and Department of Anatomy (D.S) Albert Einstein College of Medicine, The Bronx, New York, 10461.

Alzheimer's disease or senile dementia of the Alzheimer type (SDAT) is a progressive neurodegenerative disorder that is characterized pathologically by two types of microscopic lesions in the neocortex: the neurofibrillary tangle and neuritic plaque. The concentration of neuritic plaques is correlated with significant reductions in the level of the neuropeptide systems, somatostatin and substance P, in autopsied brains of patients with SDAT. In the present study we employed light microscopic immunocytochemistry to determine whether somatostatin- and substance P-containing neurons participate in the formation of neuritic plaques.

Autopsied material from selected cortical regions of 12 patients with SDAT were sectioned on a vibrating microtome, and immunolabeled for somatostatin or substance P by the peroxidase-antiperoxidase method. Subsequently, the tissue sections were counterstained with Thioflavine-S to visualize neuritic plaques.

Within the neocortex and hippocampus approximately 20% of the plaques contained somatostatin. The dystrophic somatostatin-positive processes typically appeared swollen, and often formed grape-like clusters within the peripheral (i.e. neuritic) portion of the plaque. Similarly, distended profiles containing substance P-like immunoreactivity were observed within plaques. However, the percentage of plaques associated with substance P was somewhat lower than observed with somatostatin. Together, our data suggest that somatostatin- and substance P-containing neurites participate in the formation of neuritic plaques. The dystrophic somatostatin-positive processes probably originate from local somatostatinergic neurons. In contrast, our own data suggest that the substance P-containing processes may arise from a group of neurons in the lateral dorsal tegmental nucleus, known to contain both substance P and acetylcholine. This research was supported in part by the McKnight Foundation and NIH Grants #AG-02478, AM-21860, and DS holds a Research Career Development Award #1 K04 AM-01208.

82.2 TRANSMITTER SPECIFICITY OF NEURITES IN SENILE PLAQUES OF AGED MONKEYS. C. A. Kitt, D. L. Price, R. G. Struble, L. C. Cork*, L. C. Walker, W. C. Mobley, M. W. Becher*, T. H. Joh and B. H. Wainer. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

In this study, immunocytochemical methods were used to define the transmitter specificity of some of the neurites in senile plaques of aged macaques. Neurites, enlarged neuronal processes consisting primarily of axons, are the principal neuronal elements in senile plaques. These abnormalities occur in the brains of aged monkeys and humans and, in much greater numbers, in individuals with Alzheimer's disease (AD). In normal aging, mild reductions in several neurotransmitter markers have been described and, in AD, significant decreases occur in markers for cholinergic, catecholaminergic, and somatostatinergic neurons. There is a correlation between the density of plaques and the reduction in several of these markers. It has been suggested that dysfunction and/or death of certain neuronal populations may account for these decrements. We have hypothesized that dysfunction of these neurons in aging and disease may be associated with the formation of neurites in plaques.

To test this hypothesis, we used immunocytochemical methods to visualize choline acetyltransferase (ChAT), tyrosine hydroxylase (TH), and somatostatin which are markers for cholinergic, catecholaminergic, and somatostatinergic systems, respectively. The availability of these specific antibodies allows delineation of the transmitter specificity of some of the neurites in plaques of aged monkeys. In conjunction with silver stains and thioflavin-T staining (amyloid), our investigations reveal that some neurites in the neocortices and amygdalae of aged monkeys show either ChAT-, TH-, or somatostatin-like immunoreactivities and that clusters of immunostained neurites frequently are present in proximity to amyloid. These neurites are the same size and shape of neurites seen in silver and AChE stains. Results of the present study provide the first direct evidence for the participation of cholinergic, catecholaminergic, and peptidergic systems in the formation of neurites in plaques in aged nonhuman primate brain. Similar pathological processes involving a variety of neuronal systems may occur in aged humans and in individuals with Alzheimer-type dementia.

82.3 AROMATIC MONOAMINES IN NUCLEUS BASALIS OF MEYNERT: CHANGES RELATED TO ALZHEIMER'S DISEASE AND TO NORMAL AGING. D.L. Sparks* and J.T. Slevin. Sanders Brown Research Center on Aging and the Department of Neurology, University of Kentucky, Lexington, KY 40536.

The source and quantity of monoaminergic projections to the nucleus basalis of Meynert (NbM), the stability of these afferents during normal aging and their possible alteration in Alzheimer's Disease (AD) are poorly understood. In contrast, synaptic markers of noradrenergic and serotonergic cortical afferents have been shown to be altered and cortically-bound cholinergic efferents from NbM are generally accepted as markedly diminished in AD.

We here report the levels of aromatic monoamines, precursors, and metabolites in samples of NbM using HPLC-ECD methodology. Aliquots for analysis were taken from tissue samples immediately ventral to anterior commissure of human autopsy material obtained within 24 hours of death. Serotonin and 5-HIAA levels in NbM did not change with age, in contrast to age-related decreases observed for DOPA, dopamine, DOPAC and HVA. There were profound reductions of transmitter and metabolites of both serotonergic and dopaminergic systems in NbM of AD patients (< 75%).

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82.4 CATECHOLAMINE RELEASE AND AGING. H. McIntosh and T.C. Westfall. Dept. of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

We are currently involved in a systematic study of adrenergic function during the aging process. Present experiments have been carried out utilizing Fischer 344 (F-344) rats at 2-3 mos., 11-13 mos. and 24-25 mos. as well as Long-Evans rats at 14 weeks and 14 months to assess in vitro release and in vivo turnover of catecholamines in various brain regions.

We have previously observed that the stimulation induced release of ^{3}H-norepinephrine (^{3}H-NE) from the hypothalamus or cortex or ^{3}H-dopamine (^{3}H-DA) from the striatum produced by 56 mM K^+ or 10^{-5}M amphetamine showed no age related changes (Fed. Proc. 43:625, 1984). In vitro release was accomplished by 2 minute periods of field stimulation of superfused brain tissue which had been preincubated with ^{3}H-NE (0.3 x 0.3 mm diced hypothalamus and occipital cortex) or ^{3}H-DA (0.5 mm striatal slices). All incubation and superfusion was done in Krebs-bicarbonate buffer under a constant flow of 95% O_2/5% CO_2 at 37°C. Fractional release was calculated for each stimulation peak. Field stimulation resulted in a frequency dependent increase in the fractional release of ^{3}H-NE or ^{3}H-DA from the various regions obtained from Sprague-Dawley rats which was dependent upon the presence of calcium in the superfusion buffer. No significant differences in release of ^{3}H-DA from the striatum were apparent in 3, 13 or 25 month old F-344 animals. However the evoked release of ^{3}H-NE from the hypothalamus and cortex of 24 month old animals seemed less than from corresponding 3 or 12 month old animals.

Catecholamine turnover was measured in vivo by following the decrease in catecholamine levels following inhibition by α-methyl-p-tyrosine (αMPT). Long-Evans rats (14 wks. and 14 mos.) were injected i.p. (200 mg αMPT/kg or an equivalent volume of saline) at zero time and 4 hours later. Animals were sacrificed 8 hours after the initial injection. Endogenous levels of NE and in some cases DA were measured by HPLC with electrochemical detection in 6 brain areas (cortex, hypothalamus, brainstem, striatum, cerebellum, and midbrain) and 5 peripheral tissues (heart, portal vein, spleen, vas deferens, and caudal artery). αMPT resulted in a marked decrease in catecholamine levels in the various regions. No significant age related differences in turnover were observed. (Supported by NS16215, DA02668 and NIA.)

82.5 SEX DEPENDENT DECREASES OF ChAT IN BASAL FOREBRAIN NUCLEI DURING AGING. V.N. Luine, J.C. Rhodes* and K.J. Renner* (SPON: S. Schwartz-Giblin). Lab. of Neuroendocrinology, The Rockefeller University, New York, N.Y. 10021.

In senile dementia of the Alzheimer type, the number of cholinergic cells in the basal forebrain dramatically decreases, and the activity of Choline Acetyltransferase (ChAT) decreases in projections from the basal forebrain to hippocampal and cortical regions. Thus, we measured activity of ChAT in discrete basal forebrain nuclei and projection areas of young and old male and female rats to determine whether activity of ChAT changes during normal aging.

The activity of ChAT was radiochemically measured in samples microdissected from the brains of 4 and 24 month old male and female Fisher 344 rats (National Institute on Aging). Activity was measured in the vertical nucleus of the diagonal band, horizontal nucleus of diagonal band (nhDB), medial septal nucleus, substantia inominata, ventral globus pallidus (GP), CA1 of dorsal hippocampus, frontal cortex, and frontoparietal cortex. In addition, activity of glucose-6-phosphate dehydrogenase (G6PDH) was measured in pituitaries. In females, activity of ChAT did not differ between 4 and 24 month rats in any area examined except the GP. In the GP activity of ChAT in the aged rats was 30% lower than the young rats (Aged = 189 and young = 273 nmoles acetylcholine/mg protein/hr). Activity of Acetylcholine esterase also decreased by 40% in the GP of the older female rats. In 24 month old male rats, activity of ChAT was not different from 4 month old rats in any area examined except the nhDB. Activity of ChAT in nhDB of 24 month old males decreased by 50% (Aged = 87 and young = 184 nm/mg protein/hr). In the pituitary, the pattern of enzyme activity in aging was also different between the sexes. Pituitary G6PDH activity decreased by 30% in the aged females and increased by 40% in the aged males.

These results show sex dependent neurochemical differences in aging within specific basal forebrain nuclei and pituitary. Since the ventral GP is the rodent homologue of n. basalis of Meynert in humans, decreases in ChAT in female rats raises the possibility that females maybe at greater risk for dementia. Questions of possible gonadal hormone involvement in aging of basal forebrain and contributions to the etiology and/or progression of dementias are also raised. (Support: NIA #AG04388 and Alzheimer's Disease and Related Disorders Ass'n).

82.6 AGING-RELATED INCREASES IN DOPAMINE AND NOREPINEPHRINE IN FRONTAL LOBE, AND THEIR REVERSAL BY IMIPRAMINE. R.Elul. Psychiatry Dept.,Loma Linda Univ.Sch.of Med., and Research Service,Pettis Memorial VA Hospital,Loma Linda,CA 92357

Male Fisher rat littermates (F-344) were reared in individual cages from age 4 weeks. Animals were sacrificed at different ages by decapitation, the brain dissected quickly (2 min) into different regions. Regional catecholamine levels were deteremined with HPLC, adjusting for brain weight and running catecholamine standards between tissue assays.

In the frontal lobe, both dopamine and norepinephrine are very substantially increased in old animals compared to their littermates sacrificed at young age: In the age span from 6 mo. to 2 yr. dopamine increases more than 4-fold. Lesser, but still marked increase was observed for norepinephrine (over 30%). Using Student's T-test, the difference in dopamine levels in aging was significant at p 0.0025; for norepinephrine the significance level was 0.02 .

Imipramine was administered orally at 10 mg/kg daily dose to some littermates kept in the same environment. In both young and old animals, long-term imipramine treatment decreased dopamine level: In 5-month old animals, imipramine administration for 3 weeks decreased dopamine in frontal lobe by 34%. In 2-year old rats, receiving imipramine continuously for 20 mo., dopamine decreased nearly 2-fold. Using the T-test, the decrease in the aged animals receiving the drug was significant at p 0.01. Analogous, but considerably smaller and not always statistically significant effects of imipramine were observed on norepinephrine.

These results indicate that catecholamine neurotransmitters in the rat, at least in the frontal lobe, increase rather than decrease with age. While this increase is in level, and not necessarily in metabolism or in receptor sites (and indeed may be compensatory for a possible decrease in receptors), nevertheless the magnitude of this effect is quite marked, and is likely to be biologically significant. The clear effect of imipramine, as well, suggests that dopamine and norepinephrine levels in the frontal lobe may respond to biological stimuli.

A second point of interest is the decrease in catecholamine neurotransmitter levels induced by long-term treatment with imipramine. The original catecholamine hypothesis of affective disorders postulated increase in catecholamines by antidepressant drugs, but this was difficult to demonstrate in long-term antidepressant studies. The present results suggest a more complex mechanism of action of antidepressants.

82.7 NORADRENERGIC MODULATION OF CEREBELLAR PURKINJE CELL RESPONSES TO AFFERENT INPUTS IS DIMINISHED IN AGED RATS Paula C. Bickford[1], Barry J. Hoffer[1] and Robert Freedman[1,2,3]. [1]Departments of Pharmacology and [2]Psychiatry, Univ. Colorado Health Sciences Center, and [3]Denver Veterans Administration Medical Center, Denver, CO 80262.

Norepinephrine (NE) enhances the relative responsiveness of cerebellar Purkinje neurons to afferent inhibitory and excitatory inputs. This modulatory effect can occur at levels of NE below those which cause inhibition of spontaneous action potential discharge. We have previously shown that NE is more potent in young (3-month) than in aged (18- to 20-month) rats, in terms of its ability to inhibit spontaneous activity. We now compare the effects of NE on the responses to afferent input from climbing fibers, mossy fibers, and cerebellar interneurons in young and aged rats. Complex spike excitation, simple spike excitation and inhibition of Purkinje cell discharge were elicited by electrical stimulation of the appropriate afferent pathways and quantitated by computing post-stimulus time histograms of the neuronal response, recorded extracellularly. Histograms were compared before, during and after local ejection of NE from multibarrelled micropipettes. The effect of NE in younger rats was to reduce spontaneous activity more profoundly than either simple or complex spike excitation. The inhibitory response of the Purkinje cell to activation of basket and stellate cell afferents was also potentiated by NE. As in previous studies, this effect was often seen at dosages causing only a minimal change in spontaneous activity. In old rats, the NE-induced potentiation of both excitatory and inhibitory responses was significantly diminished. The loss of noradrenergic modulation in senescent animals may relate to behavioral deficits seen in aging. (Supported by AG 04418 and the VA Medical Research Service.)

82.8 DIAZEPAM PHARMACOKINETICS IN AGED MICE. C. Rolsten*, P. Hicks, C. Harrington*, C. Davis*, T. Samorajski and J. Schoolar*. Texas Research Institute of Mental Sciences, 1300 Moursund, Houston, Texas 77030.

Increased pharmacological response to diazepam and other benzodiazepines by long-lived rodents and humans has been repeatedly demonstrated by several laboratories. The mechanism for the increased responsiveness has not been identified, but must be associated with age-associated pharmacodynamic or pharmacokinetic differences. We have measured both plasma and brain concentrations of diazepam and its biologically active metabolites, desmethyldiazepam and oxazepam, to assess the role of pharmacokinetics in the age-associated differences in pharmacological response.

Male 12-, 18-, and 28-month old C57BL/6J mice were used that had been purchased as retired breeders from Jackson Laboratories (Bar Harbour) and aged in our animal facilities. Diazepam was administered intraperitoneally at weekly intervals at a dose of 45/mg/kg. Sleeping times were assessed weekly following diazepam administration for the first six weeks. On the seventh week, mice were sacrificed at various times between 1/3 to 6 hours when blood and brain cortex were collected. Brain cortex weights were recorded. Plasma and brain cortex were frozen at -70°C until analysis. Diazepam, desmethyldiazepam and oxazepam were measured by high performance thin-layer chromatography.

Plasma and cortex show peak concentrations of diazepam, desmethyldiazepam and oxazepam at 1/3, 2 and 8 hours, respectively. Peak concentrations occured at approximately the same times for all age groups. The brain concentrations were one to three times the concentrations in plasma. The desmethyldiazepam concentrations in brain and plasma were much higher than diazepam or oxazepam concentrations. The plasma and brain concentrations were generally higher in the oldest mice for desmethyldiazepam and oxazepam, between one to six hours. No age-associated differences were present for diazepam concentrations.

The increased pharmacological response to diazepam in long-lived mice is not associated with changes in diazepam pharmacokinetics, but could be explained by the age-associated greater concentrations in brain and plasma of its biologically active metabolites, desmethyldiazepam and oxazepam. These data emphasize the importance of evaluating all biologically active metabolites when assessing the age-associated pharmacological response of an organism to a particular agent.

82.9 CEREBROSPINAL FLUID VASOPRESSIN IN AGING AND ALZHEIMER'S DISEASE. M.A. Raskind*, E.R. Peskind*, D.M. Dorsa (SPON: P. Prinz). GRECC, VA Medical Center, Seattle, WA 98108.

The neuropeptide vasopressin (VP) plays a role in learning and memory processes, both of which are affected by aging and severely impaired in Alzheimer's disease (AD). Although some controversy exists as to whether these behavioral effects of VP are centrally or peripherally mediated, the preponderance of data supports a role for central nervous system (CNS) VP in learning and memory. VP is measurable in CSF, and seems to originate from brain tissue rather than from plasma.

We measured VP concentrations in cerebrospinal fluid (CSF) and plasma in patients meeting stringent criteria for AD (n=6, age=67±3yrs), healthy older adults (n=9, age=67±2yrs), and healthy young adults (n=8, age=24±1yrs). All lumbar punctures were performed at the same time of day, and the same aliquot of CSF was used for VP determination in each subject. VP was extracted from CSF or plasma using a SepPak C18 column and eluted with acid/ethanol, dried, reconstituted, and then measured by a radioimmunoassay with a sensitivity of 0.05μU VP per ml.

CSF VP differed significantly between groups ($f=8.75$ ($df=2,20$), $p<.001$). CSF VP was significantly lower ($p<.01$) in normal elderly subjects (0.23±0.04μU/ml) than in young normal subjects (0.37±0.03μU/ml). In addition, CSF VP in AD patients (0.13±0.01μU/ml) was significantly lower than age-matched elderly controls ($p<.05$) and normal young adults ($p<.001$). There were no significant differences between plasma VP in AD (0.29±0.05μU/ml), older adults (0.72±0.26μU/ml) or in young adults (0.63±0.05μU/ml). Also, there were no significant differences in serum sodium levels measured in AD (141.5±0.4meq/L), older adults (140.2±0.5meq/L) or young adults (139.1±0.9meq/L).

These results are compatible with decreased CNS vasopressinergic activity in both aging and AD, and may have implications for the memory and learning decrements associated with aging and AD.

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82.10 NEURON AND NEURITE LOSS IN ALZHEIMER'S DISEASE. C.G. Rasool[2], J. Rogers[1], and D.A. Drachman[2*]. [1]Dept. of Neurology, U. Mass. Medical School, Worcester, MA 01605. [2]Mailman Research Center, McClean Hospital, Belmont, MA 02178.

The widespread neuropathology observed in Alzheimer's Disease (AD) makes it difficult to accept that there would not be some accompanying neuron and neurite loss. Unfortunately, such data--particularly for neurites--remain scant owing to methodologic problems.

We have employed a new, digital imaging approach (c.f., Rogers et al., this volume) to estimate neuron and neurite losses in AD. Because the method is computer assisted, it is objective, rapid, and capable of screening large areas of tissue. It is thus a favorable alternative to previous hand counting techniques. Because the method uses a monoclonal neurofilament antiserum which marks neurons only, it is unconfounded by problems discriminating neurons from glia. It is thus a favorable alternative to previous computerized approaches using conventional stains, where discrimination of glia by geometric rules or subjective light pen editing is necessary. Because the method provides separate estimates for neurite staining (as opposed to perikarya staining), it is also a favorable alternative to Golgi techniques for neurite quantification. Golgi methods suffer several shortcomings for neurite estimation, including selectivity of impregnation and extremely low yields of stained cells.

Inferior temporal and superior frontal gyrus sections from 4 AD and 4 age and sex matched nondemented elderly controls were processed for neurofilament immunocytochemistry, then visualized at a final magnification of 160X. Fields bordering the pial surface, white matter, and intermediate lamina were quantified for total, perikarya, and neurite neurofilament reactivity, as well as cross-sectional areas of the stained neural elements. Each field encompassed 20,800 um^2. Consistent perikarya and neurite losses were obtained for AD patients at all cortical depths in both gyri. The 20-30% AD deficits obtained were highly significant.

Supported by a grant from the Alzheimer's Disease and Related Disorders Association (J.R.).

82.11 INCREASES IN COLD-INSOLUBLE AXONAL TUBULIN DURING AGING. Scott T. Brady. Dept. of Developmental Genetics and Anatomy, Case Western Reserve University School of Medicine, Cleveland, OH 44106

Previous studies on the properties of axonal tubulin have indicated that more than 50% of the tubulin labeled by axonal transport is cold-insoluble (Brady et al, J. Cell Biol. 1984 in press). Much of this tubulin is in the form of cold-stable segments of microtubules (Sahenk and Brady, J. Cell Biol. 97:210a, 1983). Such stable microtubule segments would be expected to alter the dynamics of the axonal cytoskeleton by stabilizing the microtubule component Since reorganization of microtubules is thought to be essential for growth of neurites, it was proposed that reduction in the plasticity of neuronal connections in aging animals might be accompanied by an increase in the relative proportion of cold-insoluble tubulin in axonal transport. Slow Component a (SCa; the microtubule-neurofilament network) was labeled with ^{35}S-methionine in the optic nerves of 6 month and 24 month old male Fischer 344 rats. Ages were chosen to permit comparison between mature adults and old animals at the 50% survival level. Extraction of nerve homogenates with MTG buffer (100 mM MES, 1 mM GTP, 0.5 mM $MgCl_2$,and 1 mM EGTA, pH 6.7) at 4°C for 30 minutes solubilizes 42-43% of the axonal tubulin in 6 mo. animals, but only 31-32% in 24 mo. animals. Reextraction of the pellet with CMTG buffer (MTG buffer with EGTA replaced by 5 mM $CaCl_2$) solubilizes another 6-9% of the axonal tubulin in both 6 mo. and 24 mo. animals. 61-62% of the axonal tubulin in the 24 mo. animals resisted extraction by both cold and Ca^{++}, as compared to 49% in 6 mo. animals. All differences were significant at p 0.025. Experiments using 24 mo. animals displayed more variability and in some nerves as much as 80-85% of the tubulin was insoluble. These observations are consistent with the suggestion that one characteristic of the aging nervous system is increased stability of axonal cytoskeletal elements. Such increased stability may be a factor in the reduced capacity of aging neurons for regeneration and remodeling of terminal fields.

82.12 ZINC LEVELS IN THREE DIFFERENT BRAIN REGIONS OF YOUNG AND OLD FISHER 344 RATS. H. Haigler, A. Miller* and J. North*, Searle Research and Development, 4901 Searle Parkway, Skokie, IL 60077

A chronic zinc (Zn) deficiency prevents potentiation of field potentials produced by low frequency stimulation of the mossy fibers in the hippocampus of rats (Hesse, G.W., Science 205:1005, 1979). Zn is associated with the synaptic apparatus between the mossy fibers and CA_{3-4} hippocampal pyramidal cells (von Euler, C., Physiologie de l'Hippocampe, 135, 1962). Zn is located in the same synaptic terminals as enkephalin; both are localized in the mossy fiber system in the CA_3 region of the hippocampus (Stengaard-Pedersen, K., et al., Brain Res. 212:220, 1981). Neurofibrillary tangles and brain atrophy are typically correlated with mental deterioration in patients with Alzheimer's disease (Schneck, M.D., et al., 139:2, 165, 1982). These changes may reflect a change in and disruption of the dendritic environment in the hippocampus (Ibid).

A general hypothesis based on the above data is that a depletion of Zn in the hippocampus may occur as a function of aging either as a cause or an effect of hippocampal deterioration. To test this hypothesis, the levels of Zn in three different brain regions were measured in Fisher 344 rats (n=20/group) that were old (26 months) or young (6 months) using the following technique. The rats were decapitated, the brains rapidly removed and placed on a cold surface. The brains were dissected into three areas; the cortex, dorsal hippocampus and the brainstem. Zn was measured using atomic absorption spectrophotometry after digesting the brain tissues with concentrated nitric acid (HNO_3). The specific hypothesis was that Zn would be significantly lower in the brains of old rats when compared to young rats.

The results demonstrated that there was no significant difference between Zn levels in the brains of old and young rats ($P>0.05$). The values for Zn in ppm were nearly equal in the three different areas of brains in the old and young Fisher 344 rats (hippocampus: old=58; young=47) (cortex: O=54; Y=50) (brainstem: O=25; Y=22). Therefore we conclude that there is no change in the levels of Zn in the brains of Fisher 344 rats as a function of age.

83.1 PHORBOL ESTERS: POTENT INHIBITORS OF EXCITATORY TRANSMITTER ACTION IN GUINEA PIG ILEUM AND RAT UTERUS. J.M. Baraban, R.J. Gould, I.J. Reynolds and S.H. Snyder. Dept. of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Phorbol esters (PEs) are potent inflammatory and tumor-promoting agents. Specific PE receptors have been identified which co-purify with protein kinase C, a calcium and phospholipid dependent enzyme stimulated by PEs. The dense concentration of PE receptors in nervous tissue suggests that they play a key role in neurotransmission. Therefore, we examined the activity of a series of PE analogues in two well-characterized physiological preparations, the guinea pig ileum and rat uterus.

Strips of guinea pig ileum or rat uterus were suspended in modified Tyrode's solution for recording of isometric contractions. Phorbol dibutyrate (PDBu), 50-100 nM, rapidly and reversibly inhibited contractions of ileum strips produced by oxotremorine, a muscarinic agonist. The potencies of PDBu and other PE analogues in blocking oxotremorine induced contractions paralleled their reported activities in displacing specific ^{3}H-PDBu binding (PNAS, 77:567, 1980).

The existence of a class of PEs which are highly inflammatory but only weakly tumor-promoting has led to the subdivision of PE receptors into inflammatory and tumor-promoting subtypes. The finding that 12-deoxy-phorbol ester analogues of the highly inflammatory class also inhibit oxotremorine's action on ileum suggests that the inflammatory subtype of PE receptors mediate this action.

The effect of PDBu on the response to other excitatory neurotransmitters was also examined. PDBu blocked ileum stimulation produced by histamine or bradykinin. In contrast, PDBu did not alter the contraction stimulated by KCl. In addition, PDBu did not block the ability of the β-adrenergic agonist, isoproterenol, to produce relaxation of a KCl induced contraction. PDBu produced a similar pattern of effects in rat uterine muscle strips. It inhibited contractions initiated by oxotremorine, serotonin or bradykinin, but did not alter the contractile response to depolarization with KCl.

These results demonstrate that PEs can potently and selectively antagonize the action of excitatory neurotransmitters. Furthermore, these studies suggest that the inflammatory PE receptor plays an important role in the regulation of neurotransmission.

83.2 NICOTINICALLY-MEDIATED TRANSMISSION IN AUTONOMIC GANGLIA IS IRREVERSIBLY BLOCKED BY LOPHOTOXIN. R. B. Langdon[1], and R. S. Jacobs*[2]. Dept. of Anatomy, School of Medicine, Vanderbilt University, Nashville, TN 37232[1]; and Dept. of Biological Science, University of California, Santa Barbara, CA 93106[2].

Lophotoxin (LoTx) is an uncharged diterpenoid found in soft corals of the genus Lophogorgia. Its irreversible anti-nicotinic action at frog and rat neuromuscular junctions has been previously characterized. We now report studies regarding the specificity of LoTx action. Action upon autonomic nicotinic receptors was assessed in grass frog (Rana pipiens) and bull frog (Rana catesbiana) paravertebral sympathetic ganglia and in guinea pig (Cavia porcellus) and rabbit (Oryctolagus cuniculus) ileum, all in vitro. Compound action potentials (CAPs) were recorded from frog sciatic nerve after (preganglionic) electrical stimulation of the lumbar sympathetic chain rostral to ganglion IX. These post-ganglionic CAPs were gradually and irreversibly eliminated over a 30 min period beginning concurrently with and continuing after 10 to 20 min of exposure to LoTx (32uM), a pattern of onset that resembled that of LoTx-induced block of frog sartorius end-plate potentials. The time course and amplitude of directly conducted CAPs (elicited by stimulation of a sciatic nerve root) was unaffected. The action of LoTx on nicotinic and muscarinic reception in ileum was assessed by recording its effect on contractions elicited by challenges with nicotine and acetylcholine. Treatment with 16 uM LoTx for 60 min failed to produce any significant decrement in the level of response to these agonists. However, treatment with 32 uM LoTx for 80 min nearly eliminated responses to nicotine (100-125 uM) for at least 5 hours. Responses to acetylcholine (1 to 2 uM) were unaffected. As others have reported, alpha-bungarotoxin was ineffective against the actions of these agonists. These studies distinguish the specificity of LoTx from that of alpha-bungarotoxin, the action of which appears to be very limited in the vertebrate central nervous system. These findings suggest that LoTx may serve as a unique probe for nicotinic receptors in autonomic nervous systems, and that it may be found to possess more widespread central actions than the alpha-neurotoxins.

83.3 THE ACTION OF PCP AND PCP METABOLITES ON GLUTAMATE AND GABA MEDIATED SYNAPTIC TRANSMISSION. C.A. Colton*, R.C. Kammerer* K.H. Tachiki* and J.S. Colton*. (SPON: S. Eiduson). Georgetown Sch. of Med., Washington, D.C. 20007; NIMH, Rockville, MD 20857; UCLA Sch. of Med., Los Angeles, CA 90024; and VA Med. Center, Sepulveda, CA 91343.

The action of phencyclidine (PCP) and two of its metabolites, 1-phenylcyclohexylamine (PCHA) and 5-(1-phenylcyclohexylamino) valeric acid (PCHAV) was studied on synaptic transmission at the lobster neuromuscular junction. Both PCP and PCHA decreased the amplitude of the excitatory junction potential (ejp). 1×10^{-4} M PCP produced a 65% fall in ejp amplitude at 30 minutes while 1×10^{-4} M PCHA produced a 28% fall in amplitude. PCHAV, on the other hand, produced a slight rise in ejp amplitude over the same time period. In addition to its effect on glutamate release, 1×10^{-4} M PCP completely blocked inhibitory (GABA) junctional transmission within 15 minutes. This blockade could be reversed by washing. Input resistance, (R_o) of the resting postjunctional membrane was also affected by PCP and its metabolites. PCP and PCHAV produced a rise in R_o by 30 minutes (18%-vs-9%, respectively) while PCHA produced no significant change in R_o. These experiments show that the biologically-found metabolites of PCP and PCP itself, have an effect on glutamate (excitatory) and gamma aminobutyric acid (inhibitory)-mediated transmission at the lobster neuromuscular junction. The effect of PCP and PCHA is generally depressive on excitatory and inhibitory transmission while PCHAV slightly enhances excitatory transmission. Whether the effect is on the postjunctional receptors or on prejunctional receptors is currently being investigated.

Support for this research was provided by research funds from a grant from NIH (NS-16526) and from the Veterns Administration.

83.4 ADENOSINE SELECTIVELY BLOCKS PARALLEL FIBER-MEDIATED SYNAPTIC POTENTIALS IN THE RAT CEREBELLAR CORTEX. D. L. Eng* R. B. Bhisitkul*and J. D. Kocsis. (Spon: M. E. Smith) Dept. of Neurology, Stanford Univ. Sch. of Med. and Veterans Administration Medical Center, Palo Alto, CA 94304.

Adenosine receptors have been described at a variety of presynaptic and postsynaptic sites in the brain. Presynaptic adenosine receptor activation is associated with a reduction in transmitter release. In a recent autoradiographic study it was demonstrated that the molecular layer of the cerebellar cortex has an extremely high density of adenosine receptors (Goodman et al, Science 220:967, 1983) located presynaptically on the parallel fibers (Pfs). In this study we used electrophysiological techniques to compare the effects of adenosine on the convergent Pf and climbing fiber (Cf) synaptic inputs to the Purkinje cell. Wistar rats were anesthetized with xylazine and ketamine, placed on a respirator in a stereotaxic frame, and the cerebellar vermis was exposed. Oxygenated Krebs solution into which drugs could be added was continuously superfused over the cerebellum. Teflon coated bipolar stainless steel stimulating electrodes were placed on the cerebellar surface and another pair was positioned in the medulla to activate Cfs. Field potentials were recorded with glass microelectrodes filled with 3M NaCl. Stimulation of the cerebellar surface leads to field potential responses which correspond to the action potential activity of the Pfs (N1) and to synaptic activation of molecular layer dendritic elements (N2). Application of adenosine or 2-chloroadenosine to the superfusion pool led to a reversible reduction in the N2 synaptic component of the field potential, but latency and amplitude of N1 did not change. The block of the N2 component was dose-dependent and antagonized by caffeine and theophylline. 4-aminopyridine also reversed the adenosine blockade of N2. However, adenosine did not reduce the Cf mediated response. The relative refractory and supernormal periods of the Pfs did not change after adenosine application, indicating that Pf excitability was not affected. The effects of adenosine on convergent inputs to the same neuronal population have not been previously studied. These results indicate that adenosine is selective in its presynaptic blocking action on parallel fiber synaptic transmission and that it does not have a generalized blocking effect on synaptic transmission, nor does it alter excitability properties of the presynaptic axons. Supported in part by the NIH and the Medical Research Service of the Veterans Administration.

83.5 EFFECT OF AGING ON PHOSPHOLIPID SENSITIVE-Ca^{++} DEPENDENT PROTEIN KINASE IN THE RAT BRAIN. G. Calderini, F. Bellini*, A.C. Bonetti*, E. Galbiati*, S. Teolato* and G. Toffano. Department of Biochemistry, Fidia Research Laboratories, 35031 Abano Terme, Italy.

One of the major goals in biomedical research is to understand the mechanism by which extracellular messages produce biological responses in specific target cells. Many experimental evidences suggest that the mechanism of transmembrane signaling involves the phosphorylation of specific substrate protein. In 1979 Takai, Y. et al. (J. Biol. Chem., 254:3692) reported the existence in the brain of a specific phospholipid sensitive Ca^{++}-dependent protein kinase. Since unsaturated diacylglycerol is able to activate this enzymatic pathway, a link has been proposed between the stimulus-induced activation of phosphatidylinositol turnover and the phospholipid-Ca^{++} dependent protein kinase activity. We now report that the aging process affects the activity of this system. Protein kinase activity has been characterized as a function of age both in the cytosolic and particulate fraction at different Ca^{++} and phosphatidylserine (PS) concentrations. The effect of the simultaneous presence of unsaturated diacylglycerol was also investigated as well as the effect of a long-term treatment with low doses of bovine brain phosphatidylserine (BC-PS) (Toffano, G. and Bruni, A., Pharmacol. Res. Commun., 12: 829, 1980). The data obtained so far indicate that in the rat brain the cellular responses to biological signals are reduced by the aging process and possibly restored pharmacologically.

83.6 CISAPRIDE BLOCKS THE ACTION OF SEROTONIN ON MYENTERIC NEURONS. C. A. Ort, P. R. Nemeth*, D. M. Zafirov* and J. D. Wood. Dept. of Physiology, Sch. of Med., Univ. Nevada, Reno, NV 89557.

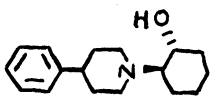
Serotonin (5-HT) has three actions on myenteric neurons in guinea-pig small intestine, when it is applied in short pulses from fine-tipped microejection pipettes. One action is a slowly-rising depolarization associated with increased input resistance and discharge of spikes that lasts for time periods three to four orders of magnitude greater than the duration of the 5-HT application. The second action is a transient depolarization associated with decreased input resistance and brief discharge of spikes. This response desensitizes quickly and can be evoked only at intervals of 2 to 3min. The third action of 5-HT is presynaptic inhibition of acetylcholine release at nicotinic synapses. Methysergide blocks the first response, does not affect the second and mimics the third. The purpose of the study was to investigate the effects of cisapride on the various actions of 5-HT. Cisapride (R51 619) is a recently developed compound (Janssen Pharmaceutica, Beerse, Belgium) that acts at serotonergic receptors. Conventional intracellar methods with 3M KCl-filled microelectrodes were used to record and inject electrical current into myenteric neurons of guinea-pig small intestine **in vitro**. Synaptic activity was evoked by electrical stimulation of interganglionic connectives. Cisapride, applied in the superfusion solution (Krebs solution), reduced or abolished both the slow and fast responses to 5-HT. The threshold concentration for reduction of the responses was 0.1uM and the responses were abolished at 1.0 to 10uM. Cisapride (1uM) abolished stimulus-evoked slow EPSPs in the same cells for which cisapride blocked the slow responses to 5-HT. There were no effects of cisapride on resting electrical behavior. Dose-response curves showed that further addition of 5-HT did not completely overcome the blocking action and suggested that cisapride acted by noncompetitive antagonism. Cisapride, like methysergide, reduced the amplitude of fast cholinergic EPSPs, suggesting that it behaved as an agonist at the presynaptic serotonergic receptors.

83.7 ACIDIC AMINO ACID ANTAGONISTS BLOCK SYNAPTIC TRANSMISSION IN THE VESTIBULAR NUCLEAR COMPLEX OF THE FROG. S.L. Cochran, P. Kasik*, and W. Precht*. Brain Research Institute, Univ. of Zürich, August-Forel-Str. 1, CH-8029 Zürich, Switzerland.

The vestibular nuclear complex of the frog (Rana temporaria) has been investigated electrophysiologically in the isolated, intact medulla to determine the nature of the excitatory transmitters afferent to these neurons. Electrical stimulation of the ipsilateral VIIIth cranial nerve evokes graded field potentials consisting of presynaptic and postsynaptic components. Bath application of glutamic acid diethyl ester (10 mM; GDEE) has no effect upon the evoked fields. D-α-aminoadipic acid (αAA), 2-amino-4-phosphonobutyric acid (APB), 2-amino-5-phosphonovaleric acid (APV), γ-D-glutamylglycine (γDGG), and kynurenic acid (KENYA) reversibly reduced the amplitude of the postsynaptic component without affecting the presynaptic volley. KENYA, γDGG, APV, and APB (1-5 mM) are more effective in abolishing the postsynaptic response than is αAA. Curare (0.1-0.5 mM) does not reduce the amplitude of these fields. Evoked, unitary spike potentials are also reversibly blocked by these antagonists. Ipsilateral VIIIth nerve stimulation evokes graded mono- and polysynaptic EPSP's in these cells. 5 mM KENYA reversibly abolishes the EPSP's with the exception of very short latency depolarizations (<1 msec onset latency), that are resistant to high frequency stimulation (50-100 Hz). These short latency EPSP's are most likely mediated through electrically-coupled presynaptic elements. Ipsilateral cerebellar and contralateral brainstem stimulation evokes mono- and polysynaptic EPSP's in these cells which are also blocked by KENYA. In no case did curare (0.1 mM) block evoked EPSP's. Bath application of 0.1 mM 4-aminopyridine (4AP) or 10 μM bicuculline methiodide (BIC) results in periodic oscillations of the cells' membrane potentials, with depolarizations accompanied by an increase in spontaneous synaptic potential, dendritic spike, and action potential frequency. Bath application of KENYA reversibly abolishes this oscillatory behavior. These findings suggest that acidic amino acids, such as glutamate, are the principal transmitters from afferents to these neurons. The ability of KENYA to block the 4AP- and BIC-induced seizure-like activity of these neurons indicates that this activity involves presynaptic elements.

83.8 A STRUCTURE - ACTIVITY STUDY OF AH5183, A NEW ANTICHOLINERGIC WHICH BLOCKS SYNAPTIC VESICLES. S.M. Parsons, D.C. Anderson†, B.A. Bahr†, L.M. Nilsson†, and G.A. Rogers*. Department of Chemistry, University of California, Santa Barbara, 93106.

The drug 2-(4-phenylpiperidino)cyclohexanol (AH5183) inhibits storage of newly synthesized acetylcholine (ACh) by synaptic vesicles in a wide variety of intact terminals, this leading to potent presynaptic blockade of cholinergic transmission. The drug also inhibits storage of ACh by purified Torpedo electric organ synaptic vesicles with an IC_{50} value of 40 nM. Many analogs and derivatives of AH5183 have been synthesized and screened with the Torpedo vesicles to determine a structure-activity relationship for this new class of drug. The phenyl ring contributes over 1000-fold to the drug potency, and changing its relationship to the piperidino ring or substituting it in the ortho or meta positions impairs potency. Para substitution is allowed. Integrity of the piperidino ring is critical, and methylation of the nitrogen or introduction of bulky substituents or conjugating groups at the piperidino 4-position compromises the drug. The cyclohexanol ring contributes over 1000-fold to the potency. No tested changes in its structure are tolerated well except those involving introduction of hydrophobic groups in the 4- and 5-positions which promote a trans-diaxial relationship between the hydroxyamino groups. Among over 50 drugs screened only 1 is significantly more potent than the parent, and only two synthetically readily accessible entry points into the structure have been found which will allow synthesis of potent affinity label ligands potentially useful in the identification of the drug binding site.



AH5183

83.9 RECEPTOR OCCUPANCY AND TURNOVER IN CHOLINERGIC TRANSMISSION
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The functional recovery of cholinergic transmission after cholinergic blockade with α-bungarotoxin (α-BTX), and its relationship to the recovery of acetylcholine receptors (AChR) were studied. The recovery was expected to depend on AChR turnover rate, on the number of receptors initially blocked by α-BTX, and on the fraction of receptors which must be occupied by ACh for full response. Turnover rates of junctional AChR have been previously described. However the relationship of the receptor turnover to transmission recovery and to receptor occupancy requirement is lacking. In this study, nicotinic transmission and its recovery were assayed by intracellular recording of the action potential elicited by indirect stimulation. The transmission was correlated with the number of free receptors remaining after blockade with α-BTX as measured by ^{125}I-αBTX bound radioactivity. When 80% of endplate AChR were blocked transmission was absent. Recovery of an additional 6% of blocked receptors (20 hours after exposure) to α-BTX) restored transmission. Consequently, complete functional recovery is provided by minute recovery of acetylcholine receptors. The slow turnover ($t_{1/2}$=11 days) of the junctional receptors is sufficient to provide the transmission recovery observed. Estimates of AChR occupancy indicate that about 26% of the endplate receptors are just sufficient for full response (action potential generation). Good agreement was observed between the percent of receptors and the percent of maximum quantal release of ACh required for transmission. The remaining 74% of AChR may be considered reserve, or may have function in transmission under variety of other conditions.

83.10 INOSITOL LIPID LABELING PRODUCED BY MUSCARINIC, HISTAMINE H_1 AND THROMBIN-RECEPTOR STIMULATION IN NEUROBLASTOMA CELLS.
R. M. Snider, S. A. Kyes*, E. B. Seguin* and B. W. Agranoff. Neuroscience Laboratory, University of Michigan, Ann Arbor, MI 48109.

Murine neuroblastoma clone N1E-115 cells are known to possess muscarinic, histamine H_1 and α-thrombin receptors which are coupled to cyclic GMP formation. The cyclic GMP response stimulated by each of these three receptors displays pharmacological specificity, as well as nearly identical time courses and ionic requirements (Snider et al., PNAS, in press, 1984). Since it has been observed that cyclic GMP responses parallel inositol phospholipid labeling effects in many systems, this relationship is being explored in N1E-115 cells.

Muscarinic and histamine H_1 receptor stimulation produced qualitatively similar patterns of de novo ^{32}P labeling into phosphatidylinositol 4,5-bisphosphate (PIP_2), phosphatidylinositol 4-phosphate (PIP), phosphatidylinositol (PI) and phosphatidic acid (PA) which are blocked by atropine and pyrilamine, respectively. Specifically, at 5-60 min of incubation in the presence of agonist and $^{32}P_i$ (10 μCi/3 x 10^5 cells) 2-5 fold increases in ^{32}P incorporation into PI and PA were observed. The percent of total recovered radioactivity present in each of the labeled phospholipid species reveals significant decreases in ^{32}P incorporation into PIP_2 and PIP, suggesting that muscarinic or histamine H_1 receptor stimulation decrease PIP_2 and PIP labeling or, more likely, that it increases their degradation. Stimulation of intact cells with α-thrombin results in a significant decrease in ^{32}P incorporation into PIP_2 and PIP with little change in PI and PA. For such studies on polyphosphoinositide breakdown, more direct information is gained by prelabeling cells with $^{32}P_i$ prior to ligand addition. In prelabeling experiments with neuroblastoma cells, α-thrombin elicited the release of water-soluble radioactive substances comigrating on high voltage electrophoresis with inositol bis- and trisphosphate (IP_2 and IP_3, respectively). Experiments are in progress to analyze the receptor-mediated phospholipid changes and release of IP_2 and IP_3 in intact neuroblastoma cells following addition of other ligands to which they are known to respond. (Supported by NIH grants NS 20920 and NS 15413).

83.11 VISUALIZATION OF ^{3}H-METHOXYVERAPAMIL BINDING SITES IN RAT BRAIN.
I.J. Reynolds, E. DeSouza, R.J. Gould and S.H. Snyder. Johns Hopkins University, Dept. of Neuroscience, Sch. of Med., Baltimore, MD 21205.

Numerous studies have shown that dihydropyridine calcium channel blockers, such as nitrendipine, bind to specific sites in brain, heart, smooth and skeletal muscle. While physiological studies have failed to show an action of these drugs in the brain, autoradiographic studies with ^{3}H-nitrendipine have shown marked regional variations resembling neurotransmitter receptors.

Phenylalkyamine calcium channel blockers including verapamil and methoxyverapamil interact with ^{3}H-nitrendipine in an allosteric fashion. This study examines the distribution of ^{3}H-methoxyverapamil binding sites in rat brain to clarify the interaction between phenylalkylamines and dihydropyridines.

Slide mounted 8 μM coronal and sagittal sections of rat brain were incubated in 0.32 M sucrose/HEPES buffer, pH 7.6 containing 1 nM ^{3}H-methoxyverapamil for 15 min. at 0°C. Non-specific binding was defined as that remaining in the presence of 3 μM unlabelled methoxyverapamil. Sections were washed 3 times for 30 s each in buffer at 0°C, dried, and exposed on X-ray film for 21 days.

Highest grain densities are seen in the dentate gyrus and Ammons horn, entorhinal and olfactory cortex and superficial layers of the cerebellum. Moderate levels are bound in other regions of cerebral cortex, striatum, hypothalamus superior colliculus and periaqueductal grey matter. Specific binding is absent in white matter tracts such as the corpus callosum. Interestingly, binding is higher in anterior than posterior pituitary. Densitometric quantification showed levels 1.7 x higher in the former than the latter. The reason for this contrast is not clear.

These results demonstrate a similar localization of sites labelled with ^{3}H-methoxyverapamil and ^{3}H-nitrendipine, supporting a common locus of action. Low levels of non-specific binding, and the ratio of specific to non-specific binding (approximately 3.5:1) make ^{3}H-methoxyverapamil a useful ligand for labelling these sites in rat brain.

83.12 METABOLISM OF CATECHOLAMINES IN THE DEVELOPING SPINAL CORD OF THE RAT.
John W. Commissiong, Department of Physiology, McGill University, Montreal, Quebec, Canada H3G 1Y6.

In a previous communication to the Society, it was shown that the spinal cord catecholaminergic nerves develop at a rapid rate, and that they possess an enormous reserve capacity to synthesize transmitters from injected precursors (Soc. Neurosci. Abs. vol. 9, p. 471). It has now been demonstrated that the developing cord, beginning at fetal day (FD) 16, also possesses the full range of metabolic enzymes for catecholamines. Using 100 mg/kg i.p. of L-DOPA as the test dose of precursor, the concentration of DOPAC and HVA in the zona intermedia of the thoracic cord were 54 ± 14 (N = 5) and 16 ± 5 (N = 5) nmol/g respectively at 1 hr after the administration of the drug, in the 12 hr old animal (ND 0.5). This very high metabolic capacity is already highly developed at FD 16, peaked in all five regions studied in the first half of neonatal life (before ND 15) and was substantially reduced by the end of neonatal life (ND 28) and in the young adult. In all regions studied, MAO activity precedes COMT activity. Control experiments done in the young adult, suggest that substantial parts of the synthesis (from L-DOPA) and metabolism of dopamine described above do not occur in monoaminergic nerve terminals. In contrast to DA, after L-DOPA, the synthesis and metabolism of norepinephrine (NE) are much less prolific, and occur entirely in noradrenergic nerves. The ventral horn of the lumbar region possesses the greatest noradrenergic synthetic capacity during development. In contrast to the prolific synthesis and metabolism of DA observed after L-DOPA, its normal synthesis and metabolism during development, up to ND 20, are either just detectable or not measurable (in the femtomolar range, 10^{-15} mol). These results indicate clearly, that the enzymes involved in catecholamine synthesis and metabolism develop very quickly in the spinal cord. In the case of DA at least, their development precedes by several weeks, the functional development of spinal dopaminergic nerves. Secondly, it is now quite clear that during development, the synthesis and metabolism of both DA and NE can occur at high rates under experimental conditions that are independent of catecholaminergic nerve activity.

Supported by the Medical Research Council of Canada.

83.13 FACILITATION OF NEURALLY EVOKED SECRETION OF CATECHOLAMINES FROM THE PERFUSED RAT ADRENAL GLAND BY TETRAETHYLAMMONIUM.
Arun R. Wakade, T.R.Sharma*, J.C.Prat* and Taruna D.Wakade*. Dept. of Pharmacology, SUNY, Downstate Medical Center, Brooklyn, NY 11203.

Tetraethylammonium (TEA) causes facilitation of stimulation-evoked release of transmitter substances from different types of peripheral nerve terminals by its well-known blocking action on K conductance, thereby enhancing influx of calcium. However, nothing is known about the effects of TEA on the secretion of catecholamines (CA) from the adrenal gland (AdG). One obvious reason is that TEA also is an effective antagonist of nicotinic receptors, which makes it difficult, if not impossible, to study CA secretion dependent on the activation of nicotinic receptors of the AdG. Recently we have demonstrated that nicotinic as well as muscarinic receptors are involved in the secretion of CA from the rat AdG (Wakade & Wakade, Neuroscience 10: 973, 1983). Therefore, it was decided to study the effects of TEA on CA secretion evoked by muscarine, nicotine, and stimulation of splanchnic nerves (SpN) in the isolated perfused AdG of the rat. [Under normal conditions, transmural stimulation (1 msec duration; 120 mA current strength and variable frequency) of AdG primarily activates SpN without exerting any direct effect on chromaffin cells (Wakade, J. Physiol. 313: 481, 1981)].

As expected, CA secretion evoked by nicotine (1 µg) was completely blocked, whereas that evoked by muscarine (100 µg) remained unchanged by 5 mM TEA. Increase in frequency of stimulation from 0.5, 1 to 10 Hz (300 pulses), led to an increase in secretion of CA (0.13, 0.16 and 0.57 ng/pulse, respectively). In the presence of 5 mm TEA, secretion evoked at 0.5 and 1 Hz was facilitated over 5-fold, and that evoked at 10 Hz remained unchanged. 0.3 µM tetrodotoxin or chronic splanchnectomy abolished CA secretion evoked by all the frequencies of stimulation in TEA. At first the potentiating effect of TEA on electrically evoked secretion of CA was attributed to enhanced release of acetylcholine (ACh) from SpN and its action on chromaffin cells via muscarinic receptors still active in the presence of nicotinic antagonist, TEA. However, 0.5 µM atropine failed to reduce the secretion. We suggest that activation of SpN results in not only release of principal transmitter, ACh, but also of other putative transmitter(s) capable of stimulating chromaffin cells and thereby evoking CA secretion.
(Supported by NIH Grant #HL18601 and NSF Grant #BNS7923019.)

CATECHOLAMINES: RECEPTORS I

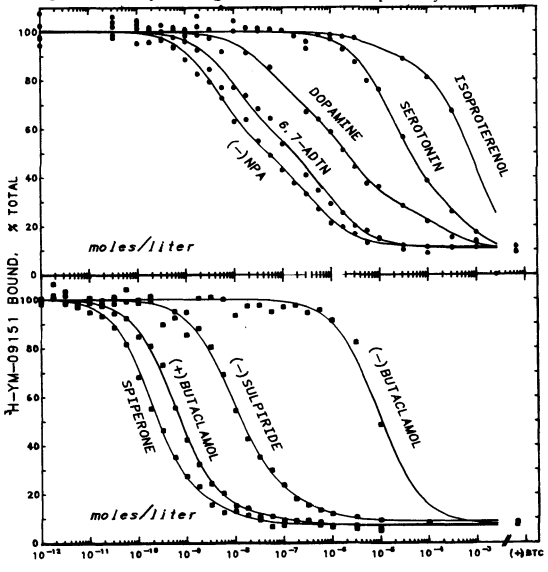
84.1 **CHARACTERIZATION OF DOPAMINE RECEPTORS IN A TUMOR OF THE RAT ANTERIOR PITUITARY GLAND.**
Christopher Lin*, Paul McGonigle*, and Perry B. Molinoff. (SPON: R.B. Murray). Dept. of Pharmacology, Univ. of Pennsylvania, Phila., PA 19104.

Dopamine receptors in the 7315a transplantable rat anterior pituitary tumor were characterized using radioligand binding assays with (^{3}H)-spiroperidol (10 pM to 1 nM) and assays of adenylate cyclase activity. Scatchard analysis of (^{3}H)-spiroperidol binding yielded linear plots and a Kd value of 150 pM. Nonspecific binding was measured in the presence of 2 µM (+)-butaclamol. Studies of the inhibition of (^{3}H)-spiroperidol binding were performed with a series of competing ligands, including the antagonists domperidone, (+)-butaclamol and sulpiride, and the agonists dopamine, bromocriptine, and N-propylnorapomorphine. The inhibition curve for dopamine was shifted to the right and the Hill coefficient increased to approximately 1.0 by the addition of 300 µM GTP. The inhibition of (^{3}H)-spiroperidol binding by the serotonin antagonist ketanserin was also studied. The low affinity of ketanserin for these sites indicated that the radioligand was not labeling 5HT-2 receptors in this tissue. The inhibition curves for the competing ligands (in the presence of GTP for the agonists) showed Hill coefficients close to 1.0, suggesting the presence of a single class of spiroperidol binding sites. Values for the equilibrium dissociation constants of the compounds were calculated using the Cheng and Prusoff equation. When compared to values obtained for the D-2B receptors found in the striatum, a correlation coefficient of 0.99 was observed, suggesting that these receptors are in fact D-2B receptors. Studies of inhibition of adenylate cyclase activity in this tissue revealed that 5 and 100 µM dopamine inhibited forskolin-stimulated synthesis of cAMP. The dopaminergic agonist N-propyl-norapomorphine was found to be a more potent mediator of this inhibition than was dopamine. The K_i value of spiroperidol for the inhibition of the dopamine-mediated effect was obtained by Schild analysis and was in good agreement with the K_i value obtained from studies of the inhibition of the binding of (^{3}H)-spiroperidol. Moreover, this value correlates well with the value calculated for the D-2B receptor found in the rat striatum. Dopamine receptor-mediated stimulation of adenylate cyclase activity was not observed, suggesting that this tissue does not contain D-1 receptors. Thus, the 7315a transplantable rat anterior pituitary tumor appears to contain a single population of D-2 receptors that appear to be linked to inhibition of adenylate cyclase activity. The properties of this receptor are similar to those of D-2B receptors found in rat striatum. The tumor may represent a useful model system with which to study dopamine receptors.
(Supported by NS 07272 and NS 18591.)

84.2 DOPAMINE D_2 RECEPTORS LABELED BY A NEW HIGH-AFFINITY LIGAND: ^{3}H-YM-09151.
H.B. Niznik, D. Grigoriadis, I. Pri-Bar*, O. Buchman* and P. Seeman. Dept. of Pharmacology, University of Toronto, Toronto, Canada M5S-1A8 and Dept. of Radiochem., Nuclear Research Center-Negev, Israel.

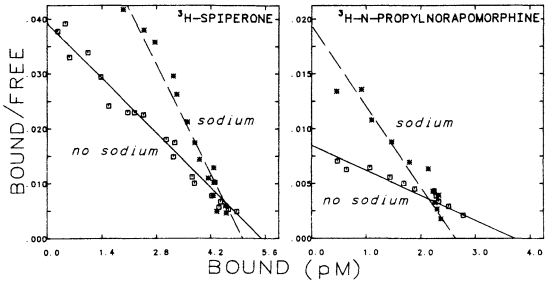
Since ^{3}H-spiperone labels both dopamine and serotonin receptors, we wanted to develop a more selective high-affinity ^{3}H-ligand for dopamine D_2 receptors. YM-09151 was tritium-labeled to 26.7 Ci/mmol.

^{3}H-YM-09151 binding to canine striatal membranes revealed a density of 36 pmol/gram of tissue and a K_D of 50 pM. In the absence of sodium the K_D of ^{3}H-YM-09151 was 500 pM without any change in receptor density. ^{3}H-YM-09151 labeled 40% more sites than ^{3}H-spiperone in the same tissue preparation. Agonists and antagonists inhibited ^{3}H-YM-09151 (80 pM) binding with a dopaminergic rank-order of potency.



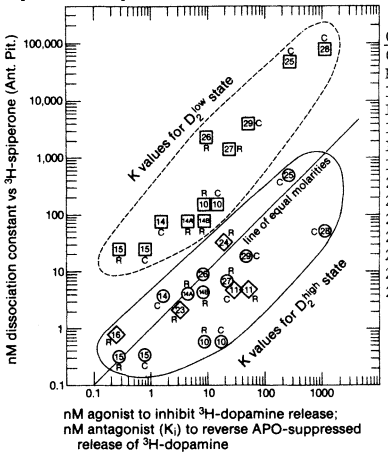
84.3 D_2 DOPAMINE RECEPTOR INTERACTIONS WITH AGONISTS AND ANTAGONISTS ARE MODULATED BY SODIUM IONS.
S.R. George, M. Watanabe* and P. Seeman. Depts. of Pharmacol. & Medicine, Univ. of Toronto, Toronto, CANADA. M5S 1A8.

Many receptor systems negatively coupled to adenylate cyclase are regulated by sodium ions. D_2 dopamine receptors are associated with inhibition of adenylate cyclase in brain and pituitary and may also be modulated in a similar manner. The addition of sodium chloride 100mM to membrane homogenates of porcine anterior pituitary resulted in 2-10 fold increases in the affinity of D_2 receptors for antagonists such as spiperone, haloperidol, molindone and the benzamides. In the presence of endogenous or exogenous dopamine, the density of sites detected by ^{3}H-spiperone was increased by sodium. Since this effect was abolished in well-washed membranes, and could be restored by subsequent readdition of dopamine, it was suggestive of displacement of dopamine by sodium from receptor sites that became available for binding by antagonist. Sodium chloride addition also reduced the proportion of D_2 receptors detected by agonists with high-affinity by conversion to a low-affinity state, as determined by agonist competition studies of ^{3}H-spiperone binding. This effect was more marked with weaker agonists and consistently apparent at higher incubation temperatures. Direct binding studies with the agonist ^{3}H-n-propylnor-apomorphine confirmed these observations as there was a reduction in the density of sites detected in the presence of sodium. Thus, sodium ions increase the total number of D_2 receptor sites available for radioligand binding and mediate partial conversion of D_2 high agonist-affinity sites to low agonist-affinity.



84.4 PRESYNAPTIC DOPAMINE RECEPTORS OPERATE IN THE HIGH-AFFINITY STATE FOR DOPAMINE. POSTSYNAPTIC ONES WORK IN LOW-AFFINITY STATE. P. Seeman, S.R. George and M. Watanabe*. Departments of Pharmacology and Medicine, Univ. of Toronto, Toronto, Canada M5S 1A8

The D_2 dopamine receptor can exist in a high-affinity state for dopamine, D_2^{high}, and a low-affinity state, D_2^{low}. We wanted to determine which of these states was functional. We obtained the dissociation constants, K, of dopaminergic drugs by computer analysis of their inhibition of ^{3}H-spiperone binding at D_2^{high} and D_2^{low} of pig anterior pituitary tissue. We found that the agonist K values for the high-affinity state were identical to the known drug concentrations which inhibited the release of either prolactin (ant.pit.) or ^{3}H-dopamine (striatum); the K values at the low-affinity state were 2 orders higher. The data suggest that the high-affinity state of the D_2 dopamine receptor is functional in the pituitary, and presynaptically in brain. The K values at D_2^{low}, however, were the same as the agonist concentrations which inhibited ^{3}H-acetylcholine release (striatum), suggesting that D_2^{low} functions at post-synaptic dopamine receptors.



Caudate slices:
C:Cat (Langer)
R:Rabbit(Starke)
10.Apomorphine
11.Bromocryptine
14.Pergolide
15.(±)-NPA
16.Haloperidol
23.Chlorpromazine
24.(-)-Sulpiride
25.(+)-Apomorphine
26.BHT-920
27.Dipropyl DA
28.(-)-NCA
29.(±)-LY 141865

84.5 NEUROCHEMICAL AND NEUROBEHAVIORAL ACTIONS OF THE D1 ANTAGONIST SCH23390: EVIDENCE FOR LONGTERM ACTIONS AT A NON-RECEPTOR SITE. D.W. Schulz, L.J. Staples*, C.D. Kilts*, T.D. Ely*, and R.B. Mailman. Dept. Psych. and Pharm., UNC Sch. Med., Chapel Hill, NC 27514 and Duke Univ. Med. Ctr., Durham, NC 27710.

SCH23390 (SCH) [R-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol] potently inhibits dopamine-stimulated adenylate cyclase (DA-AC) in vitro while displaying a very low affinity for other receptor sites. Although it has been suggested that this compound is a specific D_1 antagonist, SCH administered to rats IP or ICV potently inhibits apomorphine-induced stereotypy or amphetamine induced locomotion (Mailman et al., 1984), behaviors which are often linked to D_2 receptor activation. The present study has characterized the time course of the effects of SCH on both amphetamine-induced locomotion and DA-AC, and correlated these with preliminary pharmacokinetic analysis. Male Sprague-Dawley rats (250-450 g) were injected IP with SCH or vehicle at various times prior to treatment with 1 mg/kg amphetamine IP. Locomotion was monitered via photocell-equipped activity cages for 2 hours following amphetamine injection. Effects on DA-AC were assessed by sacrificing animals at specified times following SCH injection. Striata were immediately dissected, tissue homogenates were incubated in various concentrations of DA, and cAMP synthesis determined by the method of Schulz and Mailman (1984). Plasma levels of SCH were determined using reverse-phase HPLC with electrochemical detection. Amphetamine-induced locomotor activity was significantly attenuated 4 hours (but not 8 hours) after injection of 0.3 mg/kg SCH (10 times the ID_{50}). However, inhibition of DA-AC was still in evidence 12 hours after treatment with 0.1 mg/kg SCH. The plasma half-life of SCH was estimated to be less than 30 minutes. The present demonstration that SCH inhibited DA-AC at times when amphetamine-induced locomotor activity was unaffected is evidence that the antidopaminergic behavioral effects caused by SCH may be mediated by a biochemical locus other than the purported D_1 receptor. Moreover, the presence of biochemical and behavioral antidopaminergic effects at times when there is no longer active drug in the blood is in marked contrast to what is found with available antipsychotic drugs. These data suggest that SCH causes its antidopaminergic behavioral effects by a mechanism other than blockade of the populations of dopamine receptors usually studied. (Supported by ES-01104, HD/MH-16834 and HD-03310)

84.6 DOPAMINE INHIBITS NEUROTENSIN-RECEPTOR OPERATED CALCIUM CHANNELS IN RAT ANTERIOR PITUITARY. M. Memo, C. Missale & P.F. Spano. Inst. Pharmacol. Exp. Ther., Univ. Brescia, Italy.

Treatment of anterior lobe of rat pituitary gland with mechanical agitation in the presence of trypsin and DNAse results in a preparation of cells which secrete prolactin, produce cyclic AMP and possess functional active calcium channels. Moreover, the receptors for dopamine present in the intact anterior lobe remain functional and measurable by radioreceptor binding techniques on the dispersed cells.

In this experimental preparation, we found that depolarizing agents such as 50 mM K^+ and neurotensin (NT) stimulates prolactin release via activation of calcium channels. Exposure of the cells to high concentrations of K^+ for 15 sec leads to a marked increase of calcium influx and prolactin release both completely prevented by verapamil. Incubation of 100 nM NT for 10 min significantly increases $^{45}Ca^{++}$ incorporation by the cells without affecting cyclic AMP intracellular concentrations. The increased calcium influx elicited by NT was time- and dose-dependent reaching the maximal effect after 10 min of preincubation period at the concentration of 80 nM (+45% over basal incorporation). The changes in calcium permeability induced by NT well correlates with the increase in prolactin secretion elicited by the peptide.

Preincubation of 50 nM dopamine for 20 min inhibits the prolactin as well as the calcium influx induced by NT without affecting cyclic AMP intracellular levels. The concentration of dopamine required for half maximal inhibition of NT-induced calcium influx was 15 nM. The ability of dopamine to prevent the calcium entry changes induced by NT was mimicked by bromocriptine, lisuride and apomorphine and blocked by haloperidol and (-)sulpiride, respectively. Stimulation of dopamine receptors by dopaminergic agonists do not alter the increased calcium entry induced by K^+.

These results indicates a selective linkage between neurotransmitter-sensitive calcium channels and dopamine receptors.

84.7 **INTERACTION OF β-ADRENERGIC RECEPTORS WITH A GUANINE NUCLEOTIDE BINDING-PROTEIN IN cyc⁻ S49 LYMPHOMA CELLS.** **Stewart N. Abramson and Perry B. Molinoff. Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.**

A variety of agonist-specific effects have been observed in studies of β-adrenergic receptors. Guanine nucleotides, divalent cations, sulfhydryl reagents, and temperature all distinguish agonists from antagonists. Moreover, only agonists cause desensitization of receptor-mediated responses. In this study, the interactions of agonists with β-adrenergic receptors were investigated utilizing membranes prepared from S49 lymphoma cells. Direct radioligand binding assays were carried out with the agonist (^{3}H)-hydroxybenzyl-isoproterenol (^{3}H-HBI) or the antagonist (^{125})-iodopindolol (^{125}I-IPIN). The interaction of the receptor with a guanine nucleotide-binding protein (N) was assayed by determining the effects of GTP on the ability of agonists to interact with the receptor. (^{3}H)-HBI bound to receptors on membranes prepared from wild type (WT) cells with characteristics consistent with the formation of a ternary complex composed of agonist, β-adrenergic receptor, and an N protein. The binding of (^{3}H)-HBI was inhibited stereoselectively. The active (-)-isomers of propranolol and isoproterenol were 50 and 250 fold more potent than the inactive (+)-isomers. GTP (1 μM) inhibited the binding of (^{3}H)-HBI, while the same concentration of ATP was without effect. This result is consistent with the hypothesis that the agonist-bound β-adrenergic receptor interacts with an N protein, presumably the stimulatory N protein of adenylate cyclase (N_s). Saturation studies of the binding of (^{3}H)-HBI revealed a K_d of 224 pM and a B_{max} of 48.9 fmol/mg protein. These same membranes contained a 5 fold greater density of receptors as determined by saturation studies of the binding of (^{125}I)-IPIN. If N_s is required for high-affinity binding of (^{3}H)-HBI, then a limited number of available N_s proteins relative to that of receptors could explain this difference. (^{3}H)-HBI bound to receptors on membranes prepared from adenylate cyclase-deficient mutants (cyc⁻) with characteristics similar to those seen with WT membranes. The binding of (^{3}H)-HBI was stereoselectively inhibited by propranolol and isoproterenol, inhibited more by 1 μM GTP than ATP, and only 20% of the total population of receptors bound (^{3}H)-HBI with high affinity. In light of the absence of a functional N_s protein in cyc⁻ cells and the presence of a functional inhibitory N protein (N_i), it is possible that in cyc⁻ cells agonist-bound receptors form a ternary complex composed of agonist, β-adrenergic receptor, and N_i. It is also possible that such a complex mediates some of the agonist-specific effects observed in these and other cells.

(Supported by USPHS NS 18479 and the PMA Foundation)

84.8 AGONIST-INDUCED CHANGES IN THE PROPERTIES OF B-ADRENERGIC RECEPTORS ON INTACT S49 LYMPHOMA CELLS: SEQUESTRATION OF RECEPTORS AND DESENSITIZATION OF ADENYLATE CYCLASE. E.E. Reynolds, D. Hoyer, and P.B. Molinoff. (SPON. G. King) Dept. of Pharm., Univ. of Penn., Philadelphia, PA 19104

The binding of (^{125}I)-iodopindolol (IPIN) to B-adrenergic receptors on intact S49 lymphoma cells was studied. Experiments were carried out with wild-type cells (WT) and with mutant cells (cyc⁻) with a functional deficiency in the guanine nucleotide-binding protein (N_s) that serves to link receptor occupancy with activation of adenylate cyclase. In these experiments, cells were preincubated with a low concentration of an agonist or antagonist and the effects of these treatments on the properties of receptors on intact cells were determined. A rapid decrease in the affinity of the receptors for agonists was seen in both WT and cyc⁻ cells. Exposure to agonists also led to a rapid increase in the fraction of sequestered receptors from 20% to 50% in WT cells and from 20% to 34% in cyc⁻ cells. The extent of sequestration was measured as the fraction of specifically bound IPIN that was not displaced by hydrophilic ligands including isoproterenol and sotalol. In these experiments assays were carried out for 1 min after pretreatment of cells with isoproterenol for varying periods of time. The kinetics of agonist-induced desensitization of adenylate cyclase and sequestration of receptors were similar. In addition, several agonists which caused only partial sequestration also caused only partial desensitization. These observations support the hypothesis that desensitization and receptor sequestration are either causally associated or sequelae of a common cause. The time course of the change in the affinity of the receptor for agonists, however, was significantly slower than the other two events. Because the decrease in the affinity of the receptor for agonists and receptor sequestration occur in cyc⁻ cells, these events are unlikely to be related to cyclic AMP generation or the presence of a functional N_s.

84.9 [^{3}H]UK-14,304: CHARACTERIZATION OF BINDING TO RAT CORTICAL MEMBRANES BY A FULL α_2-ADRENOCEPTOR AGONIST D. Loftus*, R. Guchhait, G. Vantini*, J. Stolk* and D. U'Prichard. Maryland Psychiat. Res. Ctr., Univ. of MD Sch. of Med., Baltimore, MD 21228 and Nova Pharmaceutical Corp., Baltimore, MD 21228.

[^{3}H]Catecholamines are full agonists at α_2-adrenoceptors; however, they are chemically unstable and difficult to use in radioligand binding studies on brain tissues. We have investigated the kinetic and pharmacologic properties of rat cortex membrane sites labelled by a putative full agonist aryl-imidazoline, [^{3}H]UK- 14,304 (5-bromo-6-[2-imidazolin-2-yl-amino]-quinoxaline: tritiated at positions 4 and 5 of the imidazole ring) of high specific activity (84 Ci/mmol; Dr. S. Hurt, New England Nuclear).

[^{3}H]UK-14,304 specific binding, defined as that displaced by 0.1 mM (-)norepinephrine, was enhanced by Mn^{2+} and Mg^{2+}. Association and dissociation were moderately rapid, with $t_{½}$ values of 15 min and 34 min, respectively; the apparent K_D calculated from kinetic studies was 0.4 nM. Pharmacological studies revealed that α_2-agonists exhibited IC_{50} values in the 1-15 nM range, while the antagonist yohimbine (IC_{50}: 200 nM) was over 12 times more potent than prazosin in inhibiting [^{3}H]UK-14,304 specific binding. Scatchard plots derived from saturation isotherms (0.1-89 nM ligand) suggested heterogeneous binding sites; low concentrations of [^{3}H]UK-14,304 (0.1-10 nM) bound to brain membranes with an approximate K_D of 1.4 nM; higher ligand concentrations revealed at least one additional site with an approximate K_D of 20 nM. Sites with apparent high affinity for the ligand accounted for over 67% of the total sites. Divalent cations increased, and guanine nucleotides decreased, the density of sites labelled at lower ligand concentrations with no change in apparent K_D. These observations are compatible with the allosteric model of α_2-adrenoceptor regulation proposed by Lefkowitz, U'Prichard and colleagues.

Studies on human platelets revealed that UK-14,304 possesses full intrinsic activity at peripheral α_2-adrenoceptors negatively coupled to adenylate cyclase, confirming previous reports that UK-14,304 is a full agonist. These results suggest that [^{3}H]UK-14,304 is a useful new tool for studying α_2-adrenoceptor function. Unlike other full agonists, [^{3}H]UK-14,304 is chemically stable and relatively easy to use in routine binding assays. Unlike other available labelled imidazolines, such as [^{3}H]clonidine and p-aminoclonidine, [^{3}H]UK-14,304 behaves as a full, rather than as a partial agonist at α_2-adrenoceptors. (Supported by USPHS MH32842 and RSDA MH00018).

84.10 β-ADRENERGIC RECEPTOR PHOSPHORYLATION AND ADENYLATE CYCLASE DESENSITIZATION IN AVIAN ERYTHROCYTE MODEL SYSTEMS. D.R. Sibley, J.R. Peters*, P. Nambi* and R.J. Lefkowitz*. Duke University Medical Center, Durham, NC 27710.

Using avian erythrocyte model systems, we have demonstrated that phosphorylation of the β-adrenergic receptor (βAR) is stoichiometric and highly correlated with desensitization of adenylate cyclase activity. In intact [^{32}Pi]-labeled turkey erythrocytes there is, under basal conditions, 0.75 ± 0.1 moles PO_4 per mole βAR whereas after maximal desensitization with isoproterenol, this ratio increases to 2.34 ± 0.13 mol/mol. Throughout a variety of experiments including a dose-response to isoproterenol, time courses of desensitization and resensitization, and pharmacological characterization, the PO_4/βAR stoichiometry is shown to be tightly coupled with the level of adenylate cyclase desensitization. Incubation of turkey erythrocytes with cAMP analogs partially mimics catecholamines in promoting βAR phosphorylation as well as partially inducing adenylate cyclase desensitization. Using a turkey erythrocyte lysate system (Nambi et al., J.B.C. 259: 4629, 1984), we are also able to demonstrate βAR phosphorylation using [γ-^{32}P]ATP in response to isoproterenol stimulation. This latter reaction exhibits stereoselectivity and is attenuated by the specific inhibitor protein of cAMP-dependent protein kinase.

Studies with intact duck erythrocytes have shown that incubation with tumor promoting phorbol diesters, such as 12-O-tetradecanoyl phorbol-13-acetate, or catecholamines leads to attenuation of adenylate cyclase activity and a 3- to 4-fold increase in phosphorylation of the βAR. These processes are not elicited by other phorbol diesters which do not show tumor promoting capabilities and which do not activate protein kinase C. Phorbol diesters and catecholamines promote adenylate cyclase desensitization and βAR phosphorylation in duck erythrocytes in a nonadditive fashion suggesting a common mechanism or pathway of action.

These data indicate that in avian erythrocytes, desensitization of adenylate cyclase is highly correlated with βAR phosphorylation and further suggest roles for both cAMP-dependent protein kinase and protein kinase C in regulating βAR function.

D.R. Sibley is a recipient of NIH Postdoctoral Fellowship HL06631.

84.11 MODULATION OF BINDING BY GUANINE NUCLEOTIDE SUGGESTS THAT ANTAGONISTS AND AGONISTS BIND TO DIFFERENT FORMS OF AN ALPHA-2 ADRENERGIC RECEPTOR ON HUMAN PLATELETS. J.E. Piletz* and A. Halaris. Department of Psychiatry, UCLA and VA Medical Center Brentwood, Los Angeles, CA. 90073.

The alpha adrenergic receptor on platelets has previously been identified as an α-2 receptor, similar to a presynaptic receptor reportedly involved in feedback control of neurotransmitter release. Binding studies to membranes have revealed an agonist-specific state of this receptor which is sensitive to guanine nucleotides (J. Biol. Chem. 255: 4645, 1980). We report binding studies to intact platelets using the α-2 antagonist yohimbine and the agonist clonidine. Binding of ^{3}H-yohimbine to untreated platelets yielded a single (high affinity (H)) site with similar dissociation and association constants and Bmax; total = 185±53 receptors/platelet and Ka or Kd = 13.1±6.0nM (n = 16). Pretreatment with GTP did not affect the H-site, but resulted in the appearance of a second lower-affinity (L) site (474±127 rec./plat.; Ka = 76.5±15.2nM). Substitution of NaCl by 200mM sucrose also resulted in the appearance of L-sites. GTP did not affect the time course of yohimbine binding which rose to a maximum by 2 min. and was stable to 40 min. Binding of ^{3}H-clonidine to untreated platelets displayed an H-site of low capacity (16±8 rec./plat. and Ka = 8.0±6.3nM), as well as an L-site of high capacity (526±55 rec./plat. and Ka $\geq$ 500nM). In the presence of GTP, the L-site for clonidine was lost while H-sites were increased by 10-36 receptors/plat. (4 paired studies). Substitution of saline by 200mM sucrose also resulted in decreased L-sites. The time course of clonidine binding in the absence of GTP showed a decrease in both Bmax and Ka between 1-8 min., while in the presence of GTP clonidine binding rose to a maximum by 10 min. and was stable to 40 min. The increase in Bmax for yohimbine in the presence of GTP or Na may be due to the release of modified receptors from a GTP binding protein (G/F). The high affinity, low-capacity site for clonidine may represent a G/F-bound receptor complex. The rapid desensitization of clonidine binding in the absence of GTP may be due to a conformational change in the G/F-bound receptor complex yielding L-sites. These results help resolve the controversy in clinical studies of depressive illness where these ligands have been mistakenly used interchangeably. In those studies clonidine binding has been found to be increased during depressive illness. Our results suggest this may be due to increased G/F-bound receptor complex. (Supported by NIMH grant MH37664).

84.12 ADRENERGIC AND PEPTIDERGIC RECEPTORS IN THE RAT NTS: AN AUTORADIOGRAPHIC STUDY. D.P. Healy and M.P. Printz*. University of California - San Diego, La Jolla, CA 92093

The nucleus tractus solitarius (NTS) is an important integrative nucleus for central cardiovascular control. In addition to receiving primary baroreceptor afferents, the NTS has reciprocal connections with hypothalamic and limbic areas which are known to modulate the baroreceptor reflex. A large number of putative neurotransmitters have been localized by immunohistochemistry within the NTS; including amino acids, amines, and peptides. However, to satisfy the minimal requirements of a functional endogenous neuronal system in the NTS requires the co-localization of the putative neurotransmitter/neuromodulator with specific receptors and the ability to elicit a physiological response. In this study, we have begun to identify possible endogenous neuronal systems in the NTS by constructing comparative maps of the distribution of catecholaminergic and angiotensin II (Ang II) containing nerve terminals with adrenergic (α_1, α_2, β) and Ang II receptors visualized by autoradiography.

Receptors for each system were visualized by incubation of slide-mounted brain slices in vitro with the appropriate radiolabeled ligand followed by autoradiography with LKB Ultrofilm. Ligands used were: α_1-adrenergic receptors - 50pM [125I]-HEAT; α_2-adrenergic receptors - 4nM [3H]-p-aminoclonidine or [3H]-rauwolscine; β-adrenergic receptors - [125I]-iodocyanopindolol; Ang II receptors - [125I]-isoleu5-Ang II. Endogenous catecholamines were visualized by the glyoxylic acid histofluorescence technique and Ang II by immunohistochemistry.

α_1- and α_2-adrenergic receptors were concentrated within the medial and commissural subnuclei of the NTS as well as the dorsal vagal nucleus, whereas β-adrenergic receptors were not seen within the NTS. The distribution of α-adrenergic receptors closely matched the distribution of the endogenous catecholamines by histofluorescence. Ang II receptors and immunoreactive nerve terminals were also seen within these same NTS subnuclei. These results indicate that endogenously released catecholamines and Ang II, acting via α-adrenergic and Ang II receptors respectively, could be functionally active neurotransmitter/neuromodulatory systems within the NTS. The close overlap between catecholaminergic and Ang II systems within the NTS further suggests a possible interaction between these systems within the NTS. (This work was supported by HL25457, SCOR Hypertension.)

REGENERATION I

85.1 ANTISERUM DIRECTED AGAINST DAMAGED BRAIN INDUCES REGENERATION C.D. Alley* and E.E. Geisert (SPON: J.W. Brown). Department of Anatomy, University of Alabama in Birmingham, Birmingham, AL 35294.

Regeneration of the adult mammalian central nervous system does not normally occur (Ramon y Cajal, 1928). Central axons will grow into peripheral nerve grafts; however, at the distal end of the graft the regenerating axons will not invade the central neural tissue (Tello, 1911; David and Aguayo, 1981; Wendt et al, 1983). When a central tract (optic nerve) was grafted into peripheral nerve, the majority of axons grew around the central graft to reach the distal stump of the peripheral nerve (Aguayo, et al, 1978). These facts led us to hypothesize that the environment of the adult mammalian central nervous system is inappropriate for regeneration of damaged axons. Therefore, if the environment could be altered, then regeneration might occur. In the present study we used antiserum directed against damaged rat brain to test this theory.

The antiserum used in this study was produced by inoculating rabbits with tissue obtained from 4 rats that received lesions of the brain 7 days prior to sacrifice. Gamma globulin was isolate from the sera of immune rabbits by repeated ammonium sulfate precipitation.

Binding of antiserum was tested by indirect immunofluorescence microscopy. The rabbit-anti-damaged-rat-brain antiserum bound to neuropile, ependyma, hippocampal cell bodies, large cells surrounding the lesion and cells and fibers within the lesion. Controls included sections incubated with phosphate buffered saline, normal rabbit serum and rabbit-anti-rat-Thy 1.2 antibody.

When rats bearing brain lesions were treated intralesionally with the antiserum, they died during the first 14 days of treatment. Since certain antibodies, once attached to a cell membrane, can fix complement via their Fc portions causing lysis of the cell, we removed the Fc portion of the antibodies by pepsin digestion. The remaining portion, including the antigen binding region of the antibody has been termed the F(ab')2 fragment. Regeneration was observed in the lesioned brains of rats treated with the F(ab')2 fragments of rabbit-anti-damaged-rat-brain antiserum.

85.2 REGENERATION OF THE BRAIN INDUCED BY ANTISERUM, E.E. Geisert and C.D. Alley*. Department of Anatomy, University of Alabama in Birmingham, Birmingham, AL 35294.

In 9 of 17 F(ab')2-treated rats (see previous abstract), dense cellular bridges containing axons (acetylcholine esterase stain) spanned the lesion. No regeneration was seen in the 5 saline controls or 3 normal rabbit serum controls. The 4 animals treated with whole antiserum died during the first 14 days of treatment.

To insure that the cellular bridges were newly formed neural tissue and not unlesioned strands of normal brain, we made large lesions with a scalpel and a paper ring was lowered to the approximate location of the fornix. If the bridge passed through the inside of the paper ring, then the bridge must have formed after the lesion was made. In 3 of the 5 saline control animals the paper ring was in the appropriate position and no bridges passed through the paper ring. In 5 of the 11 F(ab')2 fragment-treated rats, the paper ring was in the appropriate position. All of these animals had dense cellular bridges passing through the center of the ring and axons were seen in all of the bridges (acetylcholine esterase stain and silver method). In a third set of experiments large lesions of the brain were made and animals were randomly selected for one of three treatments: saline treatment (5 rats), 1XF(ab')2 fragment treatment (5 rats), and 10XF(ab')2 fragment treatment (7 rats). Several types of connections were seen between the edges of the lesion: fusions (collagenous scar), loose cellular matrix (fibroblasts and collagen) and dense cellular bridges (containing axons). Axons were never seen to cross the lesions in the saline control animals, and there was no difference in the amount of fusion or loose cellular matrix between the saline control animals and the F(ab')2 treated rats. There were larger bridges in the 10X F(ab')2 animals than in the 1XF(ab')2. When the amount of dense cellular bridge was measured in 5 saline controls and 5 10XF(ab')2 animals, there was significantly more bridge ($p=0.004$, Mann-Whitney U) in the latter. A fortuitous section from one animal revealed individual axons spanning the entire bridge, beginning at the rostral edge and traversing the bridge to its caudal end. The animals in this third experiment were treated for only 14 days with an unknown concentration of the critical antibody. By extending the treatment duration and refining the antiserum, the limited regeneration we have demonstrated may be further enhanced.

85.3 REGENERATIVE RESPONSES ARE INDUCED BY SIGNAL OF EXTERNAL ORIGIN IMPLANTED INTO SEVERED OPTIC NERVES OF ADULT RABBITS. Schwartz, M., Hadani, M*., Harel, A*., Solomon, A*., Lavie, V*., Belkin, M* and Rachailovich, I*. Dept. of Neurobiology, The Weizmann Institute of Science, Rehovot, Israel and The Eye Research Institute, Sheba Medical School, Tel-Aviv Univ. Israel.

The mammalian CNS exhibits a limited capacity to regenerate following injury, whereas their lower vertebrates counterpart are endowed with a high ability to recover functionally. Recently we employed the visual system of adult rabbit as an experimental model to uncover whether the deficiency resides in the cell body response machinery or in the ability of the environment to provide an appropriate signal. Substances originating from optic nerves of fish (7 days post crush) or from optic nerves of neonatal rabbits (1-7 days postnatal) were implanted into the severed optic nerve of the adult rabbit. Implantation was carried out using a silicone tube filled with collagen onto which the diffusible substances originating from the various neuronal preparations were adsorbed. At various time periods following the injury and the implantations the retinae were dissected out and pulsed labeled with ^{35}S-methionine. The labeled proteins were then analyzed by SDS-PAGE.

It appeared that implantation of substances derived from regenerating optic nerve of fish or from optic nerve of neonatal rabbits caused changes in the pattern of the labeled proteins in the retinae. These changes were preferentially manifested in a few polypeptides. (Analysis was performed 7 days following the implantation). Preliminary results indicate that the soluble molecules within the implanted conditioned media, responsible for triggering the cell body response are at the molecular weight of 10 kDa The changes in the pattern of the synthesized proteins was accompanied by the ability of the retina of the implanted nerve to exhibit growth of neurites, in culture, whereas retinae of intact or injured optic nerves of adult rabbit lack such an activity. In addition ultrastructural analysis revealed the increased abundancy of new sprouts due to the implantation. Substances derived from the intact optic nerve of adult rabbit were also found to possess this inducing activity. In contrast, following the injury the nerve has lost its capacity to provide such active substances. It is therefore suggested that the inability of mammalian neurons to express regenerative response under ordinary conditions is the consequence of the failure of their non-neuronal cells to redevelop into signal producing cells.

85.4 GM_1 GANGLIOSIDE ENHANCES REGENERATION OF NORADRENALINE NERVE TERMINALS IN RAT CEREBRAL CORTEX AFTER A LOCAL NEUROTOXIN INDUCED DENERVATION. G.Jonsson*, A.Gorio, H.Hallman*, D.Janigro* and H.Kojima* (SPON: R.S. Lasher) Dept. of Histology Karolinska Institutet, Stockholm, Sweden and Fidia Research Laboratories, Abano Terme, Italy.

Exogenous administration of gangliosides has been observed to promote neuronal regrowth and functional recovery after various types of lesions. The present study was undertaken to investigate the effect of treatment with the monosialoganglioside GM_1 on regrowth of noradrenaline(NA) nerve terminals in rat cerebral cortex after a selective NA denervation induced by the catecholamine neurotoxin 6-hydroxydopamine(6-OH-DA). The effects were evaluated using neuro-and histochemical techniques. A local NA denervation was produced by intracortical infusion of 6-OH-DA(2mM; 1 ul/h for 3 or 7 days), which causes an almost complete degeneration of NA nerve terminals in a restricted area a few mm around the point of infusion, as reflected by very pronounced and long-lasting reductions of endogenous NA levels, NA nerve density and 3H-NA uptake in vitro. There is with time a slow, gradual recovery of all NA parameters due to a spontaneous regrowth of NA nerve terminals into the denervated area. GM_1 was not found to interfere with with the primary neurodegenerative actions of 6-OH-DA on NA nerve terminals. Post-treatment with GM_1 (30 mg/kg i.p.) for 7-14 days had very small effects on NA recovery after the lesion, while pre-treatment with GM_1 for 3 days and continuing the GM_1 administration for another 7-14 days significantly enhanced NA recovery, as observed both neuro-and histochemically. This effect was most pronounced in areas rostral to the point of 6-OH-DA infusion. The improving effect of GM_1 treatment was also observed after surgical sympathectomy demonstrationg that the enhanced NA recovery induced by GM_1 is related to regrowth of central NA neurons. Treatment with GM_1 alone was never observed to have any significant effects on intact NA nerve terminals. The present results indicate that GM_1 treatment has a regrowth improving effect on NA nerve terminals in cerebral cortex which might be due to a stimulation of regeneration and/or collateral sprouting. It is also possible that the apparent regrowth stimulatory effect of GM_1 treatment is at least partially related to protective actions of GM_1 against retrograde degeneration of NA axons following the initial 6-OH-DA induced degeneration of NA nerve terminals.

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85.5 EFFECT OF FORSKOLIN, DIBUTYRYL CYCLIC AMP AND THEOPHYLLINE ON SENSORY AND MOTOR NERVE REGENERATION IN HAMSTER. Suzanne L. Kilmer* and R. C. Carlsen (spon. James Lieberman). Dept. of Human Physiology, University of Calif., Davis, CA 95616.

We previously reported a 40% increase in the rate of sensory nerve regeneration in Rana pipiens injected with 10^{-4} M forskolin, a robust activator of adenyl cyclase (Nature 307: 455). The same treatment, however, did not influence the reinnervation of denervated muscle in the frog. In the present study, we tested the effect of forskolin, dbcAMP, and theophylline, all known to increase cyclic AMP concentration, on sensory and motor nerve regeneration in the hamster. Each agent was incorporated separately into an implantable pellet designed to release its contents at a constant rate over an interval of 21 days (Innovative Research of America). A single pellet was inserted near the site of a crush injury on the peroneal or sciatic nerves. Sensory nerve regenerative growth was measured using the pinch test. Motor nerve regeneration was determined by comparing twitch and tetanic tension generated by stimulating the motor nerve (indirect) to that obtained by direct electrical stimulation of the muscle (EDL). We also measured the resting membrane potentials of denervated EDL during the course of motor reinnervation. Both forskolin and dbcAMP at equivalent concentrations (2.3 mgm/kgm/day) increased the rate of sensory nerve regeneration, while theophylline at a concentration of 6.9 mgm/kgm/day had a slight inhibitory influence. Control nerves (placebo pellet) regenerated at a rate of 4.1 mm/day, while forskolin-treated nerves regenerated at 4.8 mm/day, dbcAMP at 4.4 mm/day and theophylline at 3.8 mm/day. All 3 agents, however, decreased the time between injury and the initiation of regenerative growth. This time interval was 2.3 days in control nerves, but 2.0, 1.6 and 1.2 days for forskolin, dbcAMP and theophylline respectively. In contrast, there was no difference in time of reinnervation of EDL in the presence or absence of forskolin. Nonetheless, there was a more rapid and substantial recovery of nerve-evoked muscle tension with forskolin. Indirect muscle twitch responses first appeared at 7 days and had recovered to 90% of direct responses by 9 days in forskolin-treated preparations. Control muscles had only recovered to 55% by 9 days. Muscle membrane potentials also recovered more rapidly in the presence of forskolin. Resting membrane potentials were 66.5 vs 59.8 mV at 5 days, 70.0 vs 65.0 mV at 7 days, and 71.3 vs 67.2 mV at 8 days, for forskolin vs control in denervated EDL. (Aided by a Grant from Innovative Research of America, Rockville, MD.)

85.6 BASAL LAMINA DIRECTS THE ACCUMULATION OF ACETYLCHOLINESTERASE AT SYNAPTIC SITES ON REGENERATING MUSCLES. L. Anglister & U.J. McMahan. Dept. of Neurobiology, Stanford Univ. Sch. Med., Stanford, California 94305.

If muscles are damaged in ways that spare the myofiber basal lamina (BL) sheaths, new muscle fibers regenerate within the sheaths and regenerating axons grow to the original synaptic sites on them. At the regenerating neuromuscular junctions, as at normal ones, acetylcholinesterase (AChE) is concentrated on the muscle cell surface. The experiments we describe, which were done on the cutaneous pectoris muscle of the frog, were aimed at learning whether the BL directs the accumulation of the enzyme at the synaptic sites.

Muscles were removed from the frog and the junctional region was crushed causing all cells at the neuromuscular junction to disintegrate (McMahan & Slater, J. Cell Biol., 98, 1984) while leaving large portions of the BL sheaths intact. At the same time, the damaged muscles were exposed to diisopropylfluorophosphate, which blocks all enzymatic activity detected by either histochemical or biochemical assays. The muscle was then replaced in the frog.Reinnervation was prevented. Four weeks later when the new myofibers had regenerated, the muscles were analyzed for AChE activity. Staining for the enzyme on the surface of the new myofibers revealed the arborized pattern characteristic of neuromuscular junctions; when we used Normarski optics to visualize empty BL sheaths of axons, the sheaths were traced directly to the arborizations leaving no doubt that they were at the original synaptic sites on the myofiber BL sheaths. Biochemical analysis of AChE activity in the regenerating muscle confirmed that the enzyme was in highest concentration in the junctional region.

We conclude that synaptic basal lamina directs the accumulation of AChE at synaptic sites on regenerating myofibers. These findings complement earlier studies in this laboratory showing that basal lamina components direct the formation of active zones in regenerating axon terminals and the aggregation of acetylcholine receptors and the formation of junctional folds in the postsynaptic membrane of the regenerating muscle fibers. Thus the synaptic portion of the myofiber basal lamina influence the development of all of the major constituents of the regenerating neuromuscular junction. (Sponsored by an MDA Postdoctoral Fellowship to L.A. & N.I.H. grant NS 14506.)

85.7 THE EFFECT OF 6-HYDROXYDOPAMINE ON THE SUPERIOR CERVICAL GANGLION IN CULTURE: AN IN VITRO MODEL FOR THE RETROGRADE RESPONSE. M.S. CANNELLA AND R.A. ROSS. Laboratory of Neurobiology,Dept. of Biol., Fordham Univ., Bronx, N.Y.10458.

The present study was undertaken to establish an in vitro model by which to study the neuronal response to axonal injury, referred to as the retrograde reaction. To do this, we have examined the response of sympathetic ganglia in culture to chemical neuritomy caused by 6-hydroxydopamine (6-OHDA). Two parameters were studied to characterize the response: neurite outgrowth and the activity of tyrosine hydroxylase (TH), the rate-limiting enzyme of norepinephrine synthesis.

Embryonic (E18) rat superior cervical ganglia (SCG) were placed in 35 mm dishes coated with a double layer of rat tail collagen. The cultures were fed with L-15 medium buffered with 20mM HEPES and supplemented with 10 ng/ml of 7S NGF, 0.1% fetal bovine serum and 100 mg% L-glucose. On the fifth day of culture the activity of TH reached a stable level at which it remained for the subsequent 10 days. On day 5, experimental explants were treated for 1 hour with either 0, 50 or 100 ug/ml of 6-OHDA, the untreated SCG served as controls. While no effect on neurite outgrowth was observed with 10 ug/ml of 6-OHDA, 50 and 100 ug/ml caused neuritic degeneration. By two days after drug treatment most of the neurites showed a beaded, degenerative appearance and, by the fourth day, few processes were observed in these cultures. However, new sprouts were seen six days after treatment and these sprouts continued to elongate and develop into the normal pattern of neuritic growth, as seen in the controls.

TH activity was measured at various times after drug treatment during the course of the experiment. Treatment of SCG with 10 ug/ml 6-OHDA has no effect on TH activity as compared to controls. However, 50 and 100 ug/ml caused a comparable and marked decrease in TH activity. By day 3, TH activity was reduced to 40% of control; it increased to 60% on day 6 and returned to control levels 9 days after treatment.

We can conclude that 6-OHDA initiates a retrograde reponse in cultures of E18 rat SCG. This response is characterized by a reversible reduction in neurite outgrowth and in TH activity. This in vitro system is a useful model to study the retrograde response and conditions which may promote regeneration.

Supported by grant NS 17738 from NINCDS.

85.8 POLYAMINES IN NERVE INJURY AND RECOVERY: ENHANCING REGENERATION. L.T. Kremzner*, S.L. Teitelbaum* and J.A. Downey* (SPON:L.J. Cote). Dept. Rehab. Med., Col. P&S, Columbia Univ., New York, NY 10032

In this study we investigated polyamine (PA) metabolism in the normal and experimentally crushed rat sciatic nerve (smooth Halsted hemostatic forceps were used), and the ability of the PA to accelerate axon regeneration. It is known that relatively high cellular levels of the PA, putrescine (PUT), spermidine (SPD), and to a lesser extent spermine (SPM) are observed in cells characterized by elevated rates of nucleic acid and protein synthesis, e.g. proliferating cells. Growth of cells in culture is enhanced by the addition of 10^{-8} M PUT (Nature 235:247,1972), SPM has also been reported to accelerate motor function recovery after axotomy (Exptl. Neurol. 70:507,1980), and PUT hastens recovery from periodontitis (Arch. Oral. Biol. 28:51,1983).

PA levels were determined using HPLC with post-column phthaldehyde flourescent detection. Normal sciatic nerve contains: PUT 300-500pm/mg protein, SPD 3900-4750, SPM 435-520. The SPD/SPM ratio of nerve is in marked contrast to that of other tissues: brain 1.1, pancreas 10, skeletal muscle 0.4. Following nerve injury PA elevations in the proximal segment were greater than those in the distal segments. The greatest change in all segments was the increase in SPM.

Based on these studies SPM-4HCl was injected (50umole/ Kg B.W.) i.p. daily and evaluated against control animals injected with saline, for its ability to modify axon regeneration rate. Functional recovery was determied by measuring the distance between digits 1 & 5 and 2 & 4, when the "spreading reflex" was activated by position change as recorded on an IBM photocopier. No difference in recovery rate was noted in the two groups between days 1 and 7 or 8, after which the SPM treated group showed an accelerated rate of recovery. Additional studies using varying levels of SPD or SPM and other parameters for measuring recovery are in progress.

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85.9 THE SOURCE OF POLYAMINES IS THE SKELETAL MUSCLE ITSELF AND NOT THE FIBROBLASTS. P.P. Deshmukh, A.M. Kaminska*, M. Sadeh*, D.H. Russell* and L.Z. Stern. Departments of Anatomy, Pharmacology and Internal Medicine, University of Arizona Health Sciences Center, Tucson, AZ 85724.

Polyamines (PA) and the activity of ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine synthesis have been shown to increase rapidly in tissues prior to onset of rapid growth or regeneration as well as in a variety of neuromuscular diseases (Kremzner, et al.,Adv. in Polyamine Res. Vol.2:241,1978). Recently, we have reported elevated polyamines in certain neuromuscular disorders and correlated these with morphological changes in biopsied muscle (Kaminska, et al.,Exp.Neurol.72:612,1981, Exp. Neurol. 78: 331,1982).

The experimental models of degeneration and regeneration using (1) neonatally denervated rats, (2) adult denervated rats and (3) tenotomized rats which have been utilized in our laboratory have generated interesting biochemical data showing alterations in PA levels. Under these conditions, since fibroblast proliferation is high, increase in PA levels could be due to skeletal muscle and/or fibroblasts. Hence we have used specific cytochemical markers to localize PA and ODC to study normal developing, denervated and regenerating muscle. Neonatal muscle, during the first week postpartum, contains groups of myotubes which fluoresce a brilliant bluish-green in a formaldehyde-induced fluorescamine reaction. In muscle denervated at birth, the neonatal fluorescence pattern is preserved. In muscle regeneration induced by bupivacaine injection, numerous intensely fluorescent fibers are seen. In adjacent sections, these fluorescent fibers were identified as regenerating muscle fibers using histological and histochemical techniques. The results of this study indicate that the source of elevated PA levels reported in previous biochemical studies, is indeed, regenerating muscle. This study underscores the potential usefulness of PA in monitoring human neuromuscular diseases.

85.10 LAMININ: ROLE IN GOLDFISH OPTIC NERVE REGENERATION. B.W. Agranoff, J.M. Hopkins*, R.E. Davis, T.S. Ford-Holevinski*, and J.P. McCoy* (SPON: G. Siegel). Neuroscience Laboratory and Department of Pathology, University of Michigan, Ann Arbor, MI 48109.

Affinity purified rabbit antibody to mouse EHS sarcoma laminin cross-reacts with basal lamina in frozen sections of goldfish kidney and other tissues, using either FITC-tagged goat antirabbit antiserum or a biotinylated goat antibody coupled to an avidin-biotinylated HRP complex. In rat optic nerve, laminin-reactive material is confined to the pia and vascular endothelium, while in rat sciatic nerve laminin appears to be present around nerve fascicles as well. The goldfish optic nerve shows a characteristic scalloped pattern with deep invaginations of laminin-reactive material around large nerve bundles. The amount of laminin present, as judged immunohistochemically, is sharply increased in the regenerating optic nerve. This finding is of particular interest in view of the observed effects of laminin on neurite outgrowth in culture. We had previously demonstrated neurite outgrowth from retinal explants, provided that the optic nerve was crushed 1-2 wks prior to explantation, and that when polylysine was the substratum, there was a characteristic clockwise directionality of outgrowth (Science 198:64-66, 1977). We now find that the addition of laminin to the polylysine substrate causes an intensification of the clockwise directionality. However, when explants are grown on a laminin substratum in the absence of polylysine, there is a striking radial growth pattern with loss of the clockwise pattern. The nerve growth index under these conditions is greatly increased. It is concluded that the extracellular matrix, including laminin, plays an important role in regrowth of the goldfish visual system. (Supported by NIH grant NS 13743.)

85.11 LAMININ GEL STIMULATES AXONAL REGENERATION IN VIVO. C. F. Da Silva, P. Dikkes*, R. Madison, D. Greatorex*, & R.L. Sidman. Departments of Neuroscience, Children's Hospital & Neuropathology, Harvard Medical School, Boston, Ma. 02115

Several laboratories have recently reported that laminin stimulates outgrowth of both CNS and PNS neurons in vitro. The following study was undertaken to determine if a gel containing 80% laminin and additional extracellular matrix components (gift of G. Martin & H.K. Kleinman, NIH) would stimulate sensory and motor neuron axonal growth in vivo.

The sciatic nerve of adult C57BL/6J mice was transected and proximal and distal nerve stumps sutured into a non-toxic, bioresorbable nerve guide 5-6 mm long, 0.75 mm inner diameter (D,L-polylactate) to give a final nerve gap length of 4-5 mm. Nerve guide lumens were empty or filled with laminin gel. At 2, 4, and 6 weeks following initial surgery, the distal stump was sectioned 5 mm beyond the nerve guide and sealed into a polyethylene tube filled with an HRP solution containing 40% free HRP and 10% lysolecithin dissolved in a conjugate of WGA-HRP (Vector). Three days later animals were perfused with fixative; the L3-L5 dorsal root ganglia attached to the sciatic nerve and the lumbar enlargement of the spinal cord were removed and processed for HRP histochemistry with TMB as substrate. Nerve guides with the enclosed regenerated nerve were processed for plastic embedding. HRP-positive spinal cord and DRG neurons were counted in serial 40 um frozen sections. The number of myelinated axons in 1 um cross-sections at the midpoint of the nerve guide was determined with a computer-controlled light microscope. Control values (mean±SEM) through initially empty tubes are:

Survival Time	HRP-Containing Cells in Spinal Cord	DRG	Myelinated Axons in Nerve Guide
2 Wk (N=7)	0	0	0
4 Wk (N=4)	385±20	933±50	1550±232
6 Wk (N=3)	527±19	1361±192	1491±591

Five additional animals that received laminin gel in the nerve guide were processed at two weeks (animals with longer survival times are currently under study), and had 49±19 spinal cord and 15±4 DRG HRP-labeled cells. No regenerated tissue cable was found at 2 Wk in any control animal, whereas all of the laminin gel animals demonstrated a nerve cable in the nerve guide at 2 Wk. These results suggest that laminin gel significantly speeds axonal regeneration in vivo. NIH grants EY05317(RM); NS20821, RR01393, HD18655 (RLS)

PEPTIDES: BIOSYNTHESIS AND METABOLISM I

86.1 SPECIFICITY OF DYNORPHIN CONVERTING ENZYME FROM RAT BRAIN. L. Devi* and A. Goldstein. Addiction Research Foundation, Palo Alto, CA 94304.

A thiol protease from rat brain membranes was shown to convert synthetic dynorphin B-29, "leumorphin") to dynorphin B (dyn B, "rimorphin"). This represents a "single arginine cleavage" at Thr^{13}-Arg^{14} of the substrate. The product was identified to be Dyn B by immunoprecipitation with highly specific Dyn B antiserum and by coelution with radiolabelled Dyn B on reverse-phase high-pressure liquid chromatography. This dynorphin converting activity displayed typical Michaelis-Menten kinetics with an apparent Km for the substrate of 5.8×10^{-7} M. This activity exhibited unusual specificity in that a synthetic peptide, Dyn B-29-(9-22), that contains the cleavage site did not inhibit the activity. Dynorphin A (Dyn A) inhibited the activity competitively with a Ki of 3.7×10^{-6} M. The converting activity was also inhibited by Dyn A-(6-17) and not by Dyn A-(8-17) suggesting the involvement of Arg^{6}-Arg^{7} in recognition by the enzyme. Peptides obtained by truncating Dyn A-(1-13) from its carboxyl terminus demonstrated that lysine-11, arginine-9 and arginine-7 play an important role in recognition of the peptide by the enzyme. In addition, substitution of serine for tryptophan-14 of Dyn A indicated the involvement of tryptophan-14 in recognition by the enzyme.

Bovine adrenal medulla peptide E and BAM 12P inhibited the converting activity substantially whereas metorphamide, [Met]enkephalin-Arg-Phe and [Met]enkephalin-Arg-Gly-Leu did not. These results suggest that the converting activity is due to a highly specific enzyme that requires a COOH-terminally extended enkephalin portion of the peptide for recognition.

86.2 IMMUNOCHEMICAL CHARACTERIZATION OF BOVINE PITUITARY CARBOXYPEPTIDASE E: A PROCESSING ENZYME FOR PEPTIDE HORMONE PRECURSORS. V.Y.H. Hook*, L.D. Fricker*†, R.M. Pruss*, R. Siegel*, E. Mezey*; S.H. Snyder, M.J. Brownstein*. (SPON: J. Deupree). Laboratory of Cell Biology, NIMH, Bethesda, MD. 20205, †Dept. of Biochemistry, Univ. of Oregon, Eugene, Oregon 97403, and ††Dept. of Neuroscience, Johns Hopkins University, Baltimore, MD. 21205.

Many peptide hormones are synthesized as large precursors which must be cleaved by specific peptidases to yield the small biologically active forms. Carboxypeptidase B-like activity involved in processing proenkephalin A has been identified in bovine adrenomedullary chromaffin granules, which has been previously referred to as enkephalin convertase. Such activity with identical properties has also been demonstrated in purified secretory granules from anterior, intermediate, and posterior lobes of rat pituitary, and in bovine pituitary and brain. The activities found in these areas may be involved in processing POMC, provasopressin, and perhaps other prohormones.

We have made specific rabbit antisera against purified bovine pituitary carboxypeptidase B-like enzyme. Western blots showed that the antisera stained one major protein band in rat pituitary and bovine adrenal medulla homogenates. Radioimmunoassay showed that the antisera had equivalent affinities for purified soluble (50,000 daltons) and membrane-bound (52,500 daltons) forms of the bovine pituitary enzyme, suggesting that these two forms may be structurally homologous. In contrast, the antisera showed no cross-reactivity with bovine CPB or CPN, demonstrating that the activity involved in prohormone processing appears to be immunologically distinct from other known carboxypeptidases. Immunohistochemical studies showed that the antisera stained bovine adrenal medulla but not adrenal cortex, and also stained rat pituitary. The immunochemical data further distinguishes the carboxypeptidase B-like enzyme(s) involved in processing prohormones from other carboxypeptidases. We hereon refer to this processing enzyme as carboxypeptidase E (CP Endocrine, or CPE) to designate this enzyme as being involved in the biosynthesis of many endocrine peptide hormones.

86.3 MECHANISMS OF NEUROTENSIN INACTIVATION BY RAT BRAIN SYNAPTIC MEMBRANES. P. Kitabgi*, F. Checler* and J.P. Vincent*(SPON: W.H. Rostène). Centre de Biochimie du CNRS, Faculté des Sciences, 06034 Nice Cedex, France.

Neurotensin (NT), a tridecapeptide (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu), is thought to function as a neurotransmitter in the central nervous system. By analogy with classical neurotransmitters, there might exist at neurotensinergic synapses mechanisms by which the interaction of the peptide with its receptors is terminated. In this context, we have studied the degradation of NT by purified rat brain synaptic membranes.

Incubation of NT with synaptic membranes at 37°C and separation of the degradation products by high performance liquid chromatography indicated that cleavage of NT occured at the Arg8-Arg9, Pro10-Tyr11 and Tyr11-Ile12 peptidyl bonds, thus leading to complete inactivation of NT. We have shown previously that endopeptidase 24.11 (also termed "enkephalinase") was entirely responsible for the cleavage of NT at the Tyr11-Ile12 bond and that cleavage of NT at the Arg8-Arg9 bond resulted partly from a conversion of NT(1-10) to NT(1-8) by angiotensin converting enzyme and partly from an endopeptidasic attack of NT between Arg8 and Arg9 (Checler, F., Vincent, J.P., and Kitabgi, P., J.Neurochem. 41: 375, 1983). Using a variety of general and specific protease inhibitors we have now established that : 1) Hydrolysis of NT at the Arg8-Arg9 bond was catalyzed by a recently characterized brain metalloendopeptidase (Orlowski, M., Michaud, C., and Chu, T.G., Eur.J.Biochem. 135 : 81, 1983). 2) Endopeptidase 24.11 contributed slightly (20-30 %) to the cleavage at the Pro10-Tyr11 bond of NT. 3) This cleavage resulted mainly from the action of an endopeptidase distinct from proline endopeptidase and other known brain peptidases. Purification and characterization of this peptidase are in progress in our laboratory.

Our results show that 3 distinct synaptic peptidases may separately or together participate to the inactivation of NT at the synaptic level. Further studies are needed to understand the exact role of each of these enzymes in neurotensin and more generally in neuropeptide metabolism.

86.4 IN VIVO DEGRADATION AFTER CYSTEAMINE OF L-(35S)-CYS-LABELED VASOPRESSIN, OXYTOCIN, SOMATOSTATIN-14, AND SOMATOSTATIN-28 IN RAT HYPOTHALAMUS AND NEUROHYPOPHYSIS.º R.E. Franco-Bourland*. (SPON: J. Villarreal). Departamento de Bioquímica, Instituto Nacional de la Nutrición, Salvador Zubirán, 14000 México, D.F. ºCONACYT-México: PCCBBNA-001716.

Given systemically to rats, cysteamine (CSH) rapidly depletes somatostatin-like immunoreactivity (SLI) in the hypothalamus, without affecting vasopressin (AVP) immunoreactivity; its effect on oxytocin (OT) immunoreactivity has not been examined. The depletion of hypothalamic SLI is not due to an enhanced secretion of the peptide, or to its loss of immunoreactivity, but might reflect the activation of somatostatin (SRIF) degrading mechanisms and/or the inhibition of its biosynthesis. I have shown (Franco-Bourland, R.E., Soc. Neurosci. Abstr., 9: 746, 1983), that CSH inhibits the incorporation of L-(35S)Cys into SRIF and OT, without significantly affecting AVP biosynthesis. I now report the effect of CSH on the in vivo degradation of L-(35S)Cys-labeled AVP, OT, SRIF-14, and SRIF-28 in rat hypothalamus and neurohypophysis after treatment with CSH.- Male rats (250 g) received 40 uCi L-(35S)Cys in the III ventricle via guide-cannulae. After 4h, paired rats were given, subcutaneously, 100 or 300 mg/kg CSH-HCl in neutralized H_2O. Controls received 1M NaCl. Rats were decapitated after 2h. Labeled AVP, OT, SRIF-14, and SRIF-28 were acid extracted from individual hypothalami and neurohypophyses, purified by HPLC, and quantitated by liquid scintillation.- The levels of the labeled peptides (dpm, corrected for recovery) in hypothalami (H) and neurohypophyses (N) of control (C) and CSH treated (100 & 300) animals from one experimental series were:

	H (dpm)				N (dpm)	
	AVP	OT	SRIF-14	SRIF-28	AVP	OT
C	953	1333	3555	3008	4104	15979
100	1322	819	395	401	9679	9642
300	1536	375	69	223	4149	2786

These results show, that CSH can induce SRIF-14 and SRIF-28, as well OT, degradation, without significantly affecting AVP.

86.5 ACTION OF RESERPINE ON MET^5-ENKEPHALIN UTILIZATION AND PRO-ENKEPHALIN mRNA CONTENT IN ADRENAL MEDULLA AND STRIATUM. J. R. Naranjo*, I. Mocchetti*, H. Kageyama*, A. Guidotti, J. P. Schwartz and E. Costa (SPON: J.R. Stevens). Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

In rats receiving reserpine (5 mg/kg daily for 2 days, i.p. or s.c.) and sacrificed 1,3 or 5 days later, the CNS and adrenal medullary content of enkephalins is increased. In striatum the reserpine-induced increase of enkephalin is associated with a marked increase in the content of proenkephalin mRNA, whereas in adrenal medulla the met^5-enkephalin content is increased by 1.5- to 2-fold but the proenkephalin mRNA content is decreased by 50%. While the increase in adrenal medulla met^5-enkephalin content could be observed on the second day, the change in specific mRNA content is delayed until three days: the changes in met^5-enkephalin and proenkephalin mRNA peak between the third and the fifth day following reserpine administration.

These data could suggest that in medulla, where enkephalin coexists in the same vesicles with catecholamines, reserpine decreases the output of met^5-enkephalin and this is followed by a reduction in the specific mRNA content (feedback inhibition?). The action of reserpine on striatal met^5-enkephalin stores was interpreted to consist of an increase in the turnover rate of met^5-enkephalin and was considered to be due to a lowering of the dopaminergic input, which transsynaptically attenuates the synthesis and utilization of enkephalins. In order to decide whether the action of reserpine on medulla was due to an intrinsic action of reserpine on the chromaffin cells, or to an indirect action via the transsynaptic regulation, experiments with primary cultures of chromaffin cells were carried out. In these experiments, reserpine (10^{-10}-10^{-7}M) increased the met^5-enkephalin content and lowered the proenkephalin mRNA content in a dose-related manner. Thus reserpine acts transsynaptically to change the dynamic state of met^5-enkephalin in striatum but acts intrinsically to change the dynamic state of enkephalin in medulla.

86.6 STUDIES OF THE REGULATION OF SYNTHESIS AND TRANSPORT OF SUBSTANCE P IN EXPLANTS OF GUINEA PIG NODOSE GANGLION - VAGUS NERVE. D. MacLean*, M. LaFave* and S.F. Lewis*. (SPON: J. Toole). Dept. of Medicine, Wake Forest Univ. Med. Ctr., Winston-Salem, NC 27103.

In previous studies, we have demonstrated that explants of guinea pig nodose ganglion-vagus nerve, maintained in vitro for up to 48 h, synthesize and transport substance P (SP) in quantities comparable to those measured in vivo using nerve crush and ligation techniques. Using [35S] methionine-supplemented medium, newly synthesized [35S]SP appears first in the NG and is present within nerve segments 2cm distal to the NG at 4 h; subsequent increases in [35S]SP are proportionate to immunoreactive-SP (IR-SP). Following incubation of explants in radiolabelled medium for 6-12 h, "chase" in cold medium demonstrates that within 6 h [35S]SP is in equilibrium within all nerve segments.

We have used this in vitro model to study factors regulating synthesis and transport of SP. Based on the above kinetic data, the accumulation of IR-SP following 18-48 h was measured in 3mm segments proximal to a ligation in the distal vagus (prox), intervening vagus, NG and a 1mm segment rostral to the NG (supranodose, SN). The addition of fetal calf serum (FCS) decreased transport and total nerve content vs. M199 medium alone (FCS, 949±330 vs. M199 1249±241, pg/3mm prox, mean±S.D., p<.02). Nerve growth factor (NGF) had no effect on SP transport (NGF, 1501±329 vs. control, 1601±252 pg/3mm prox). 2-deoxy-glucose decreased transport (p <.03), while supplemental glucose had no effect. Insulin, 50ng/ml, slightly increased efferent transport and increased the ratio of SP in efferent (prox) vs. afferent (SN) segments, (insulin, 6.35±1.5 prox:SN, vs. controls 3.96±1.3, p <.01). The addition of veratridine, $5x10^{-6}$M, to explant medium completely inhibited both synthesis and transport of IR- and [35S]SP; ouabain, 10^{-4}M, similarly inhibited synthesis but not transport. Tetrodotoxin reversed both the transport and synthesis effects of veratridine. Total [35S] incorporation into protein was reduced proportionately to [35S]SP.

These studies demonstrate the utility of this model in studying simultaneously neuropeptide synthesis and transport. The findings suggest that within this important sensory system, SP synthesis and transport are regulated by both neural and humoral mechanisms.

86.7 **CULTURED INTERMEDIATE PITUITARY CELLS REQUIRE ASCORBIC ACID FOR THE α-AMIDATION OF α-MELANOCYTE STIMULATING HORMONE.** C.C. Glembotski. Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Rat intermediate pituitary cells in primary culture display a time-dependent loss of the ability to produce carboxy-terminally α-amidated αMSH (Glembotski, C.C., Eipper, B.A., and Mains, R.E. (1983) J. Biol. Chem. 258, 7299-7304). Instead of des-, mono-, and diacetyl-ACTH(1-13)NH_2 the cells produce des-, mono-, and diacetyl-ACTH(1-14)OH. Since the pituitary secretory granule-associated α-amidation enzyme (peptidylglycine α-amidating monooxygenase or PAM) requires copper and ascorbic acid for optimal activity (Eipper, B.A., Mains, R.E., and Glembotski, C.C. (1983) Proc. Natl. Acad. Sci. USA 80, 5144-5148), these cofactors were added to cultures of intermediate pituitary cells in an attempt to reverse the loss of peptide α-amidation ability. When the cultures were supplemented with up to 100 μM copper (II) there was very little change in the ability to α-amidate αMSH. Ascorbic acid at concentrations of up to 500 μM resulted in a dramatic increase in the ability of the cells to form the α-amidated peptide. Various combinations of ascorbic acid and copper additions indicated that a relatively short exposure (hours) to ascorbic acid produced the maximal response. Ascorbic acid displayed a dose-dependent effect on the α-amidation ability with a half-optimal concentration of about 25 μM. Pulse-chase labeling experiments demonstrated the ascorbic acid-dependent conversion of labeled ACTH(1-14)OH-related peptides to ACTH(1-13)NH_2-related peptides. Partially purified pituitary PAM was shown to catalyze the copper and ascorbic acid-dependent conversion of ACTH(1-14)OH to ACTH(1-13)NH_2. These results correlate with the ascorbic acid requirement of pituitary PAM that has been characterized using synthetic D-Tyr-Val-Gly as the peptide substrate, and demonstrate that the direct precursors to ACTH(1-13)NH_2-related peptides are ACTH(1-14)OH-related peptides. Combined with our previous data, the present studies support the concept that a wide range of neuro- and endocrine peptides become α-amidated in a similar ascorbic acid-dependent manner.

86.8 **PROCESSING OF A NEUROPEPTIDE PRECURSOR IN THE R3-14 CELLS OF APLYSIA.** **Kaldany, R.-R., Schaeffer, M., Evans, C., Mak, G., and Scheller, R.H. (SPON: L. Ball) Depts. of Biological Sciences and Psychiatry, Stanford University, Stanford, CA 94305**

The Aplysia abdominal ganglion neurons R3-14 contain a prevalent 1.1 kb mRNA that is not expressed in other Aplysia neurons. This message encodes a 14 kd neuropeptide precursor protein. We have used pulse/chase experiments to delineate the sites of cleavage in an effort to determine the nature of the peptides which may be derived from the precursor. Whole abdominal ganglia were incubated with tritiated amino acids and single cells dissected. Alternatively, tritiated amino acids can be directly introduced into individual cells by pressure injection. Labeled proteins and peptides were acid extracted and separated by reverse phase HPLC. Peaks of radioactivity were sized by gel filtration or SDS-polyacrylamide gel electrophoresis. By a judicious choice of amino acids, specific parts of the precursor could be selectively labeled and followed. Further characterization was accomplished by subjecting peaks of radioactivity to NH_2 terminal sequence analysis.

By using these techniques we determined the putative cleavage recognition site for the signal peptidase. The two sets of paired basic residues in the precursor are believed to be cleaved yielding a 12 amino acid peptide, a 3.2 kd acidic protein and a 6.2 kd basic protein. The 6.2 kd protein is further processed at a Gly-Arg sequence yielding a 21 amino acid peptide with an amidated carboxy terminus. The complete delineation of cleavage sites should allow us to isolate or synthesize peptides and assay them for physiological activity. The R3-14 neurons in Aplysia are thought to use glycine as a modulator of cardiovascular physiology (Price and McAdoo, 1981, Brain Res.) The functional significance of the coexistance of multiple messengers in a single neuron is not clear; the relative simplicity of the Aplysia CNS affords the opportunity to address these issues.

86.9 METABOLISM OF THYROTROPIN-RELEASING HORMONE (pGlu-His-ProNH_2, TRH) IN HUMAN CEREBROSPINAL FLUID (CSF): CHARACTERIZATION OF PYROGLUTAMATE AMINOPEPTIDASE ACTIVITY. C. PRASAD, R.M. EDWARDS*, A. JAYARAMAN, T. FREDERICK*. Depts. of Medicine, Biochemistry and Neurology, Louisiana State University Medical Center, New Orleans, LA. 70112.

It is now well recognized that peptidases can not only terminate the actions of peptides, but also generate new biologically active molecules. TRH is ubiquitously distributed throughout the CNS and body fluids including CSF. The initial metabolism of TRH by tissue extracts is catalyzed by two separate enzymes: an amidase forming acid TRH (pGlu-His-Pro) and a pyroglutamate aminopeptidase (pGlu-peptidase) forming cyclo (His-Pro). However, the mechanism(s) through which CSF metabolizes TRH is yet unknown.

Incubation of ^{3}H-Pro-TRH with CSF led to the formation of radioactive proline, acid TRH, and cyclo (His-Pro). The cyclo (His-Pro)/ (proline + acid TRH) ratio was greater than five, suggesting pGlu-peptidase as the major pathway of TRH metabolism in human CSF. pGlu-peptidase activity was separated from amidase activity by gel (Sephadex G-200) filtration and the properties of the enzyme studied. The partially purified enzyme exhibited a broad pH optima between pH 6.0 and 7.4, and a K_m of 15.9±3.1 uM at 37 C. A number of potential competitive inhibitors of TRH metabolism were examined, of which LHRH and bombesin were the most effective. An examination of the structure and K_i of various peptides that inhibit pGlu-peptidase indicated that the enzyme shows a preference for peptides having an amino-terminal pGlu and blocked carboxy-terminal. Heavy metals, EDTA, and reducing agents inhibited the enzyme, whereas benzamidine, phenylmethylsulfonylfluoride, and iodoacetamide had little or no effect on the enzyme activity. A survey of several polypeptide hormones indicates that insulin and alpha-TSH, at high concentrations, inhibited the enzyme activity.

86.10 COPPER - AMINO ACID COMPLEXES STIMULATE THE RELEASE OF LUTEINIZING HORMONE RELEASING HORMONE (LHRH) FROM HYPOTHALAMIC NEURONS IN A CALCIUM-INDEPENDENT MANNER. A. Barnea and M. Colombani-Vidal. Dept. Ob/Gyn, Physiol, Cecil H. and Ida Green Center for Reprod. Biol. Sci., Univ. of Tx. Health Sci. Ctr., Dallas, TX 75235.

We have previously shown that chelated copper is an extremely potent stimulator of LHRH release from isolated hypothalamic secretory granules. This finding and the observation that copper, administered to female rabbits, is known to stimulate the release of LHRH into the hypophysial portal blood, led to our hypothesis that blood-borne copper can stimulate LHRH release by acting on the LHRH neuron itself. In this study, we wished to ascertain if copper, chelated to a putative circulating chelator, stimulates LHRH release from hypothalamic neurons and if so, does this release require extracellular calcium. Median eminence (ME) explants, obtained from 5-6 weeks old male rats, were incubated in a buffered medium (medium was changed every 15 min) and LHRH release into the medium was quantified by RIA. When ME were incubated with $CuCl_2$, histidine (His) or CuHis (50 μM each) for 15 min and then in buffer alone for an additional period of 75 min, LHRH release was (pg/ME/15 min):

Time (min)	0	15	30	45	60	75	90
$CuCl_2$ or His	3.0	3.3	3.6	3.2	3.2	3.3	3.5
CuHis	3.7	7.2	12.4	8.6	7.3	6.0	5.6

Thus, CuHis, but not $CuCl_2$ nor His, markedly stimulated LHRH release, maximal rate attained 30 min after initiation of exposure to CuHis. In addition, we found that CuHis and CuCysteine were equipotent in stimulating LHRH release, whereas complexes of Cu with BSA or the peptide Gly-His-Lys were ineffective. To examine the calcium requirement for this release process, ME were incubated with CuHis (50 μM) or KCl (60 mM) each in the presence or absence of calcium. In contrast to KCl, CuHis was as effective in stimulating LHRH release in the presence or absence of calcium in the incubation medium. It is known that blood is the source of copper in tissues and that in blood, copper is chelated by proteins, peptides and amino acids. Moreover, copper-amino acid complexes are presumed to be a component of the exchangeable pool of circulating copper. Our finding that Cu chelated to His or Cys markedly stimulated LHRH release is supportive of our hypothesis that blood-borne copper can interact with LHRH neurons, which leads to release of the peptide by a process that does not require extracellular calcium.

86.11 SYNTHESIS OF IMMUNOREACTIVE PROLACTIN IN THE RAT HYPOTHALAMUS. R.E. Harlan*, B.D. Shivers*, M. Kalamaridis* and D.W. Pfaff (Sponsor: P. Femano). The Rockefeller University, New York, N.Y. 10021.

Previous studies (Toubeau et al., J. Endocrin. 83: 261, 1979; Harlan et al., Science 219: 1451, 1983) demonstrated immunoreactive (ir)-prolactin in cell bodies in the hypothalamus, and in fibers in many regions of the hypophysectomized rat brain, indicating a non-pituitary origin. Prolactin messenger RNA is in the rat hypothalamus (Schachter et al., Endocrinol., in press), suggesting hypothalamic synthesis of prolactin. We report immunoprecipitation of a hypothalamic protein with the same molecular weight as pituitary prolactin.

In preliminary studies, fresh rat hypothalamic supernatants were immunoprecipitated (anti-prolactin immune serum from National Hormone and Pituitary Program, or non-immune serum), using SAC (heat-killed, formalin-fixed Staphylococcus aureus cells with cell-surface protein A) and centrifugation. The precipitates were boiled in SDS/mercaptoethanol to separate SAC, antibody and antigen. After removing SAC, supernatants were electrophoresed on 12% SDS polyacrylamide slab gels, which were then silver stained. In two experiments, we found an immunoprecipitated, hypothalamic protein with the same molecular weight as pituitary prolactin ($\sim$22 K). However, this did not exclude a pituitary origin.

To examine this question, we established explant cultures. Fresh hypothalami and pituitaries were minced in oxygenated Dulbecco's Minimum Essential Media deficient in methionine or cysteine. After incubation for 1 h, 35S methionine or cysteine was added, and the cultures incubated for 4 h. The cells were homogenized, and the supernatants immunoprecipitated, electrophoresed and fluorographed. Visual and densitometric analyses of two developed films revealed a newly-synthesized, immunoprecipitated, hypothalamic protein with the same molecular weight as pituitary prolactin. These results do not address the possibility that the anti-prolactin immune serum also bound peptides too small to be resolved on these gels, i.e. $<\sim$10 K.

The results indicate that a 22 K ir-prolactin is synthesized in the hypothalamus. This protein, or its derivative, is transported to terminals (Nishizuka et al., this meeting) where its release facilitates the estrogen-dependent behavior, lordosis (Harlan et al., Science 219: 1451, 1983). The finding that many ir-prolactin neurons concentrate estradiol (Shivers et al., this meeting) adds emphasis to the postulated role of ir-prolactin in the neural control of lordosis.

86.12 CARBOXYPEPTIDASE E: PURIFICATION AND CHARACTERIZATION OF A PEPTIDE HORMONE PROCESSING ENZYME FROM BOVINE, RAT, MOUSE, FROG, SHARK, AND APLYSIA NEURAL TISSUE. L.D.Fricker and E.Herbert* Dept. Chemistry, Univ. of Oregon, Eugene OR 97403

Many peptide neurotransmitters and hormones are initially synthesized as larger precursors. In these precursors the biologically active peptide is usually flanked by pairs of basic amino acids. The sequential action of trypsin-like and carboxypeptidase B-like enzymes would liberate the active peptide from the precursor. Prohormones with these basic amino acid pairs have been found in many diverse organisms, including yeast, aplysia, frogs, and various mammals. The similarity in the cleavage sites for the many different precursor proteins suggests that the processing pathway has been highly conserved during evolution. To investigate the possibility that the processing enzymes are conserved, we have isolated and characterized a carboxypeptidase B-like processing enzyme from several different organisms.

Carboxypeptidase E (enkephalin convertase), a peptide processing carboxypeptidase B-like enzyme has been previously purified and characterized from bovine brain, pituitary, and adrenal chromaffin granules (Fricker, L.D. and Synder, S.H. J. Biol. Chem. 258, 10950-10955, 1983). An enzyme with similar properties is present in rat, mouse, frog, shark and aplysia neural tissue. The various carboxypeptidase activities co-purify with bovine carboxypeptidase E (CPE) and are similarily affected by ions and inhibitors. Purified CPE from the various species migrate on SDS polyacrylamide gel electrophoresis with an apparent molecular weight of 50 kDa. The similarity of CPE isolated from these different species suggests that this processing enzyme has been highly conserved during evolution. We are currently screening other species for CPE-like enzymes.

86.13 NEUROPEPTIDE CONTENTS OF A PARTIALLY PURIFIED SECRETORY GRANULE FRACTION FROM THE BAG CELL NEURONS. S. Molloy* and S. Arch. Biological Laboratories, Reed College, Portland, OR 97202.

The bag cell neurons of Aplysia californica are committed to the synthesis and secretion of several peptides. Kinetic and molecular studies indicate that these peptides are produced initially in the form of a precursor that subsequently undergoes specific degradation. The signal-to-noise ratio for biosynthetically labeled (^{3}H-leucine) peptides is especially favorable for the egg-laying hormone (ELH) and the acidic peptide (AP). Our previous work indicates that these two species are not handled coordinately by the bag cell processing and transport apparatus. Moreover, a recent electron microscopic study of the bag cell terminal processes in our laboratory has disclosed two similar, but readily distinguished classes of granules. Since the distribution of these granules is not consistent with an hypothesis that they are different age classes of a single population, the possibility that they contain different peptide components of the common precursor deserves consideration. Consequently, we performed discontinuous density gradient centrifugation in isomolar sucrose-metrizamide solutions to determine if AP and ELH are covariant. A crude granule fraction was prepared from radiolabeled cells and formulated to serve as the 1.312 g/cc step. It rested on a 1.374 g/cc step and was overlayered with steps at 1.250, 1.189, and 1.127 g/cc. After centrifugation at 73,000xg for 60 min, each zone and interface was taken for analysis by isoelectric focusing electrophoresis. Both ELH and AP were found at peak radiochemical specific activity at the 1.127/1.189 interface. However, their distribution throughout the gradient differed and the ELH:AP ratio at the first interface differed from that predicted if the peptides were enclosed in granules according to their biosynthetic stoichiometry. Much of the AP "missing" from the putative granule fraction appears in the sample zone. Since it is not in a particulate association, we infer that AP is in a more labile association with secretory granules than is ELH. Either ELH and AP are in a single granule class having different permeabilities for the two peptides, or the peptides are enclosed in distinct granule classes. The fact that another, as yet unidentified, peptide appears to codistribute with AP and not with ELH lends some support to the latter possibility.
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87.1 CONVERGENCE OF THALAMOCORTICAL PROJECTIONS IN RETROSPLENIAL CORTEX FROM ANTERIOR AND LATERAL THALAMIC NUCLEI. R.T. Robertson, S.M. Thompson*, and L. A. Tengelsen*. Department of Anatomy, College of Medicine, University of California, Irvine, CA 92717.

A number of reports have described the convergence of thalamocortical projections from two separate thalamic nuclei onto a single area of cerebral cortex. These examples have been either (1) cases of convergence of "specific" and "nonspecific" thalamocortical projections or (2) cases of convergence from two closely related (functionally and regionally) thalamic nuclei. We report here the areal and laminar convergence of thalamocortical projections from two quite distinct thalamic nuclei: the anterior dorsal and the lateral dorsal nuclei.

Data come from experiments using experimental neuroanatomical tract tracing techniques with hooded or albino rats. Small injections of horseradish peroxidase (HRP) in ventral retrosplenial cortex (VRS) result in retrograde labeling of neuron somata in the anterior dorsal (AD) and the lateral dorsal (LD) nucleus. Projections from LD to VRS were demonstrated by anterograde transport techniques utlizing HRP histochemistry or autoradiography following appropriate injections into LD. These experiments demonstrate that ventral LD projects strongly to layers I and III of VRS. Cortical projections of AD were demonstrated by the placement of small lesions and Fink-Heimer impregnation of degenerating axon terminals in cortex. Small electrolytic lesions placed in AD result in degenerating axon terminals in layer I and III of VRS, and also result in a correlated decrease in the staining of the enzyme acetylcholinesterase (AChE) in these layers of VRS.

These data demonstrate that thalamic nuclei AD and LD both project to layers I and III of VRS. This convergence is remarkable because AD and LD appear to be quite distinct thalamic nuclei, based on the system of subcortical afferents to each. AD receives major inputs from the mammillary bodies and appears to relay to VRS information related to the classic Papez circuit of the limbic system. LD receives input from the pretectal complex and probably relays polysensory information to VRS. Thus, the convergence of these thalamocortical projections may form a basis for the convergence of sensory and limbic information in the brain.

Supported by NIH grant 14267.

87.2 TOPOGRAPHIC ORGANIZATION OF CERTAIN PARAHIPPOCAMPAL CORTICAL PROJECTION NEURONS IN THE MONKEY USING RETROGRADE TRACERS. D.R. Brady and G.W. Van Hoesen, Depts. of Anatomy and Neurology, Univ. of Iowa, Iowa City, IA 52242.

The posterior parahippocampal cortex in the monkey sends axons to all types of cortex (allocortex, periallocortex, proisocortex and isocortex). However, this information is based largely on anterograde studies and little is known about the laminar origin and topography of its projection neurons. Our aim has been to ascertain whether the neurons that project to such diverse cortical regions are segregated, or whether they are intermixed. The posterior parahippocampal gyrus can be subdivided into three areas: TF_1, TF_2 and TH. Area TF_1 forms the medial bank of the occipitotemporal sulcus, while area TF_2 occupies the crown of the gyrus medial to area TF_1. Area TH is found medial to area TF_2 and lateral to the parasubiculum. The efferent projections of this cortex were determined with autoradiography in 6 monkeys that received tritiated amino acid injections. Retrograde tracers (HRP, fast blue or nuclear yellow) were injected in cortical regions of 24 monkeys. We have observed that neurons primarily in area TF_1 project to the isocortical areas 7 and 19, while those to isocortical area 22 are found in area TH and the medial parts of area TF_2. A still different topography was observed in the cells of origin for projections to the proisocortical cingulate gyrus. For example, area TF_2 projects principally to cingulate areas 23 and 24 while area TH projects primarily to cingulate areas 24 and 25. For both isocortical and proisocortical projections labeled neurons occupy layers III, V and VI. In contrast, projections to periallocortical area 28 arise almost exclusively from layer III of areas TF_1, TF_2 and TH, with the heaviest labeling in areas TF_2 and TH. Double labeling studies provided an opportunity to assess these populations of neurons in the same preparation and to compare their diversity and communality. The cells of origin for isocortical, proisocortical and periallocortical projections revealed two zones of overlap. These occurred where areas TF_1 and TF_2 merge and where areas TF_2 and TH merge. The results indicate a complex topography. For example, cells giving rise to cingulate projections overlap those that send axons to the parieto-occipital and temporal isocortices whereas the entorhinal cortex receives input from cells whose topography overlap the neurons projecting to the other studied regions. (Supported by Grant NS 14944 to G.W.V.H.)

87.3 TEMPORAL POLE PROJECTIONS TO VENTROMEDIAL TEMPORAL LOBE STRUCTURES IN THE MONKEY. C.L. Barnes, K.P. Maskey* and G.W. Van Hoesen. Depts. of Anatomy and Neurology, Univ. of Iowa, Iowa City, IA 52242.

The cortex that forms the temporal pole is a major component of the limbic lobe and is thought to play a functional role in emotion and autonomic regulation. It is known that this cortex receives substantial input from the frontal association cortices and auditory and visual association cortices along the lateral parts of the temporal lobe. However, its efferent connections have not been studied systematically in primates using newer tracing methods. We have initiated such a study and focus here on projections to structures that form the ventromedial parts of the temporal lobe like the amygdala, entorhinal cortex and hippocampal formation. The brains of 14 old-world monkeys were studied using autoradiograph for anterograde tracing and fluorescent dyes and HRP for retrograde tracing. Two distinct cyto- and myeloarchitectonic divisions of the temporal pole have been identified. A more lateral division (LTP) is characterized by an accentuated layer II, a discontinuous layer IV and a dense staining fiber plexus corresponding to layer IV. A more medial division (MTP) has a thin and discontinuous layer II, an incipient layer IV and diminished fiber staining in layer IV. Autoradiograhic results reveal that the temporal pole has topographically organized projections to the amygdala, entorhinal cortex and hippocampal formation. The lateral, accessory basal and central nuclei of the amygdala all receive strong projections. Afferents to the medial portion of the lateral amygdaloid nucleus arise from MTP, while afferents to the lateral portion of this nucleus arise from LTP. Similarly, MTP projects strongly to layers I, II, III of lateral entorhinal cortex and moderately to intermediate entorhinal cortex, while LTP projects to layers I, II, III of intermediate entorhinal cortex. The heaviest projections are to layer III. Complimentary studies using fluorescent tracers and HRP reveal that the cells of origin of the entorhinal afferents are located primarily in layer III with a lesser number in layer V. LTP also projects to the superficial layers of the parasubicular region of the hippocampal formation. These projections provide a structural basis for information from the various associational regions of frontal and temporal lobes to influence limbic structures implicated in emotion, memory and autonomic regulation. Supported by Grants 5T32MH15172-07 to C.L.B. and NS 14944 to G.W.V.H.

87.4 QUANTITATIVE STUDIES OF MITOSES IN CEREBRAL HEMISPHERES OF FETAL RAT. Stephen Zamenhof. Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

In the continuation of the study of brain development of the fetal rat (twenty 14½ and 15 day-old fetuses, parasagittal sections 10μ; Zamenhof and van Marthens, Dev. Brain Research 3:657, 1982), evidence was obtained of a uniform single layer of mitoses, which, in contrast to numerous ventricular mitoses, are situated deep inside the wall of the cerebral hemispheres. These mitoses, called here "deep mitoses", occur before the appearance of a subventricular zone (Boulder Committee, Anat. Rec. 166:257, 1970). The number of these deep mitoses is 17.1 $\pm$ 2.6% of the ventricular mitoses, and they are situated away from the ventricular surface, at the depth of 72.7 $\pm$ 2.8% of the wall thickness of cerebral hemispheres. Unlike the ventricular mitoses, of which 88 $\pm$ 5% have their spindles parallel (tangential) to the ventricular surface, in the deep mitoses 75 $\pm$ 13% of the spindles are perpendicular to the ventricular surface (i.e. perpendicular to ventricular mitoses).

As can be seen above, the standard deviations are low, and there are no significant differences between right and left cerebral hemispheres, between littermates, and between different litters (fetal age difference ½ day).

The distribution (density) of ventricular mitoses along the ventricle (future ependyma) follows a characteristic pattern, but the locations of peaks and depressions of densities are different from animal to animal, even in the same litter. This speaks against the possibility that this specific pattern of distribution serves the general pattern of morphological changes (enlargements) of cerebral hemispheres. The locations of peaks and depressions of mitotic densities of deep mitoses are not correlated with those of ventricular mitoses, even in the same hemisphere of the same animal.

Since there are no mitoses situated between deep mitoses and the ventricular surface, there is no gradient of mitoses; thus, lower numbers of deep mitoses than ventricular mitoses cannot simply result from low penetration of nutrients and/or mitogens. It appears more likely that the cells in deep mitoses have specific receptors for special mitogens that may arrive through the ventricle. The fate and destination of these cells after mitosis is at present unknown.

87.5 ORGANIZATION OF ASCENDING DORSAL RAPHE PROJECTIONS IN THE RAT. B.E. Kosofsky and M.E. Molliver. The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

At a transverse level through the trochlear nucleus the dorsal raphe nucleus can be fractionated into four subdivisions: a dorsomedial cluster of cells below the aqueduct, a ventromedial cluster of cells forming a "fountain" between and above the Median Longitudinal Fasciculi (MLF), and two lateral clusters of cells. Each subdivision exhibits distinct cytologic features such as soma size, shape, dendritic orientation and specific neurotransmitter; the predominant phenotype in each of these subnuclei is serotonergic. The existence of these raphe cell clusters suggests connectional heterogeneity; the targets of individual raphe subgroups may be non-identical. To explore this possibility by a sensitive anterograde transport method, we iontophoresed the lectin PHA (Vector Labs) into discrete subdivisions of the dorsal raphe of adult rats (Gerfen and Sawchenko, Brain Res. 290 (1984) 219-238). After three week survivals the brains of these animals were processed for immunocytochemistry to visualize the injection site, the pathway, and terminal projections of selected cell clusters. One such cluster that we injected, a division of the ventromedial group, is situated just dorsal to the MLF (SMLF=SupraMLF). The axons of these small (15μ)-medium (25μ) cells pass medially between the MLF's and then arch rostrally as they ascend towards the ventral surface of the midbrain joining the Median Forebrain Bundle. This small cluster of cells sends divergent yet discrete projections to multiple subdivisions of the forebrain. The divergence of this projection is evident in the targets of the SMLF cells: the amygdala, olfactory bulb, striatum, and cerebral cortex. In each of these structures there is a restricted distribution of fibers. In the neocortex the SMLF fibers are most prominent frontally where both tangential and radial fibers are seen in all cortical layers. Fewer fibers are present in parietal cortex, and fibers are virtually absent from occipital cortex and hippocampus. Complementary patterns of fiber distributions in the forebrain have been observed after discrete iontophoretic injections of PHA into other rostral raphe sites. From these experiments we conclude that the mesencephalic raphe cell groups are organized in discrete clusters which selectively innervate particular forebrain sites. These organizational features would allow the multi-targeted raphe neuronal population to influence differentially multiple neuronal systems throughout the rostral neuraxis. (Support: BEK-GM7303, MEM-NS15199)

87.6 CORTICAL AFFERENT INPUT TO THE BANKS OF THE PRINCIPALIS SULCUS OF THE RHESUS MONKEY. H. Barbas and M-M. Mesulam. Dept. of Health Sciences and Anatomy, Boston University and School of Medicine, and the Harvard Neurological Unit, Beth Israel Hospital, Boston, MA 02215.

The sources of ipsilateral cortical projections to the banks of the principalis sulcus in the prefrontal cortex were studied with horseradish peroxidase (HPR) in macaque monkeys. The results show that the peri-principalis cortex receives a substantial proportion of its cortical afferent input from neighboring prefrontal regions. However, differences were noted in the distribution of labeled cells projecting to specific sites within the banks of the principalis sulcus. For example, only the ventral bank of the middle third of the principalis received a substantial proportion of its afferent projections from premotor (area 6), and somatosensory areas (parietal operculum and posterior insula). Furthermore, a greater proportion of the total cortical input to the anterior third of the peri-principalis cortex originated in auditory association regions, when compared with other principalis sites. Input from visual and visuomotor association regions was preferentially directed to the ventral bank of the caudal third of the principalis sulcus. There seemed to be a consistent trend, whereby the proportion of labeled neurons in limbic cortical regions was higher the more rostral the HRP injection site was within the banks of the principalis sulcus, irrespective of whether the injection was in the ventral or dorsal bank. These findings suggest that monosynaptic input from the modalities of vision, audition, somatic sensation, and from the limbic cortex is differentially distributed within the banks of the principalis sulcus.

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87.7 CELLS OF ORIGIN OF THE CORTICO-PONTINE PROJECTIONS IN THE RAT. C.R. Legg* and M. Glickstein. (SPON: C. Elbaum). The City University of London, Northampton Square, London EC1V OHB and MRC Unit of Neural Mechanisms of Behaviour, 3 Malet Place, LONDON WC1E 7JG

The neocortex is a major source of input to the mammalian cerebellum via a relay in the pons. In monkeys some cortical areas project only sparsely to the pons and some not at all. Rats may be different. We studied the cortico-pontine projection in rats by filling the pontine nuclei or control areas of 16 animals with horseradish peroxidase (HRP) and reacting cortical sections to reveal retrogradely labelled cells. After one to three days survival, the animals were perfused under deep anaesthesia. The brains were cut and sections reacted with tetramethylbenzidine to reveal the extent of the injection and the distribution of retrogradely labelled cells.

In the four cases in which the pons was completely filled, retrogradely labelled cells were found confined to layer V of the cortex. There were high densities of labelled cells in all cortical areas except for two temporal areas (Zilles, K.B., et al., Anat. Embryol., 159, 335, 1980). TE1 (auditory cortex) contained a relatively low density of labelled cells while TE2 was even more sparsely labelled. The distribution of labelled cells in the remaining cortex was quite uniform with a high density of labelled cells in primary and secondary visual areas including the regions representing the central visual field.

Control injections were made in the corticospinal tract caudal to the pons, and in the mesencephalic and pontine reticular formation dorsal and rostral to the pontine nuclei. The corticospinal tract injections produced labelled cells restricted to motor and somatosensory cortex, while reticular formation injections resulted in labelling of only a few cells near the frontal pole.

Thus, in contrast to monkeys, both striate and extra striate visual areas project heavily to the pons in the rat. In rats there appears to be no frontal association area devoid of pontine projections like that which is found in monkeys.

There is in rats the hint of a temporal area with few pontine-projecting cells, perhaps analogous to a much larger area of temporal association cortex with no cortico-pontine projections in the monkey.

87.8

WITHDRAWN

88.1 SELECTIVE BREEDING FOR CHOLINERGIC SUPERSENSITIVITY: IMPLICATIONS FOR ANIMAL MODELS OF DEPRESSION. D.H. Overstreet. School of Biological Sciences, The Flinders University of South Australia, Bedford Park, S.A. 5042, Australia.

Over the past several years we have developed, through selective breeding procedures, a line of Sprague-Dawley rat which is more sensitive to the anticholinesterase agent, DFP (the Flinders S-line). A second line of rats (the Flinders R-line) does not appear to differ from normal rats in its sensitivity to DFP, but is relatively more resistant than the S-line. Recent studies indicate that the Flinders S-line of rats is also more sensitive to muscarinic agonists than is the R-line and has a higher concentration of muscarinic receptors (mAChR) in the striatum and hippocampus (Overstreet et al, Brain Res. 294, 327, 1984). Thus, a cholinergic supersensitivity may underlie the increased sensitivity of S-line rats to DFP.

Most previous clinical studies of human depression have focussed on the involvement of catecholamines and indoleamines, but recent work suggests the possibility of cholinergic supersensitivity in depressive disorders. Similarly, most animal models have focussed on changes in catecholamines or indoleamines; in addition, these models have generally lacked a genetic component. In the present paper I wish to review some findings on the Flinders S-line which suggest that it might be a useful animal model of depression.

The present studies were carried out on male and female rats of the S15 and S22 generations of the selectively bred S- and R-lines. The S-line of rats had lower body weights, reduced locomotor activity, and increased sensitivity to muscarinic agonists, thus resembling findings with depressed humans. To explore the question of behavioural immobility in greater detail, the rats were placed in an open field immediately after exposure to a 1 mA footshock (2 sec.) or swum in 25°C water for 15 min. The S-line of rats exhibited a significantly greater reduction in locomotor activity and remained immobile for longer periods of time than did the R-line. Thus, in both situations a stressor has produced a greater degree of immobility in the S-line of rats.

These findings indicate that the S-line of rats are more behaviourally depressed that the R-line of rats and become even more immobile when exposed to stressors. These results are therefore consistent with the growing body of human literature suggesting that stress may precipitate depression and indicate that the S-line of rats may be a useful animal model of depression.

88.2 MULTIPLE [3H]IMIPRAMINE BINDING SITES ON HUMAN PLATELET MEMBRANES: CLINICAL IMPLICATIONS. J.R. Ieni, S.R. Zukin, and H.M. van Praag. Dept. of Psychiatry, Albert Einstein Col. of Med., Bronx, N.Y. 10461.

High-affinity binding of the antidepressant drug imipramine has been demonstrated in rat brain, human brain, and human platelets. Several laboratories have reported that densities of platelet [3H]imipramine sites are reduced in depressed patients when compared to controls. However, change in [3H]imipramine binding does not always accompany clinical recovery from depression. While Raisman et al. (1981) found no change with recovery, Suranyi-Cadotte et al. (1982) found a delayed normalization of high-affinity binding to platelet membranes following clinical recovery.

Recently, a second apparent [3H]imipramine binding site of lower affinity has been demonstrated in rat brain by Reith et al. (1983) and Conway and Brunswick (1983). The present study was performed to determine whether low-affinity [3H]imipramine binding sites exist on human platelet membranes and to elucidate their potential role in depression.

Human platelet samples were homogenized in 50 mM TRIS buffer containing 120 mM NaCl and 5 mM KCl (pH = 7.5 at 7° C). Homogenates (100 µl) were incubated (3 hr, 0-4° C) with 0.2-250 nM of [3H]imipramine in the presence of 100 µM desipramine or buffer. Binding was terminated by rapid filtration through GF/B filters. Data analysis was performed using the LIGAND program (Munson, 1979; Teicher, 1982)

Non-linear scatchard analysis showed the data to fit a two-site model, with apparent Kd values of 0.78 and 425 nM and Bmax values of 1024 fmol and 11.22 pmol/mg protein for the apparent high- and low-affinity sites, respectively. These results are consistent with our findings in rat brain of two apparent sites, with Kd values of 7.0 and 692 nM and Bmax values of 455 fmol and 8.68 pmol/mg protein. An apparent low-affinity binding site (Kd = 97-356 nM; Bmax = 6.46-21.40 pmol/mg protein) as well as a high-affinity site was observed in each of four platelet samples obtained from the W.H.O. Collaborative Study of [3H]Imipramine Platelet Binding Sites and Endogenous Depression.

The demonstration of low-affinity imipramine binding to human platelet membranes may be important for clarifying the active uptake system for 5-HT. Knowledge of whether this low-affinity component is systematically altered in affective disorders may help elucidate the etiology and treatment of these conditions.

88.3 EFFECT OF ANTIDEPRESSANT ADMINISTRATION ON SEROTONIN UPTAKE AND ^{3}H-IMIPRAMINE BINDING SITES IN PLATELETS FROM DEPRESSED AND NORMAL SUBJECTS. B. Suranyi-Cadotte*, R. Quirion, N.P. V. Nair, F. Lafaille* and G. Schwartz*. (SPONSOR: M. Beaulieu). Douglas Hospital Research Centre, Verdun, Québec H4H 1R3

Decreased density of ^{3}H-imipramine binding sites and decreased uptake of serotonin (^{3}H-5HT) in platelets have been observed in depressed patients. A functional relationship between uptake and binding sites have been proposed by some investigators. To determine the extent to which clinical state and antidepressant drug treatment may influence these two processes, we monitored platelet ^{3}H-imipramine binding and ^{3}H-5HT uptake in depressed patients and normal volunteers during treatment with imipramine. In 8 normal volunteers, imipramine administration (125 mg/day) for 3 weeks produced no significant change in the mean density (B_{max}; fmol/mg protein) or the mean transport rate of ^{3}H-5HT (V_{max}; fmol/10^{5} platelets/2 min) between baseline (B_{max} ± SEM = 767 ± 49; V_{max} ± SEM = 57 ± 7) and the third week of treatment (B_{max} = 788 ± 58; V_{max} = 66 ± 11). This suggests that at this dosage level imipramine does not affect B_{max} of ^{3}H-imipramine binding nor V_{max} of ^{3}H-5HT uptake in platelets. In 10 unmedicated depressed patients meeting Research Diagnostic Criteria for Major Depressive Disorder, mean B_{max} values increased significantly from baseline (B_{max} = 569 ± 42) to normal levels with successful antidepressant treatment during remission (B_{max} 755 ± 82; $p < 0.05$). B_{max} levels continued to rise even after antidepressants had been discontinued for 4 weeks (827 ± 69). V_{max} of ^{3}H-5HT uptake showed no significant changes during the study period. These findings suggest that decrease in the density of ^{3}H-imipramine binding sites in depressed patients is not likely to be a direct drug effect, and that normalization of this variable may be associated with clinical remission. Furthermore these data indicate no association between the ^{3}H-imipramine binding site and ^{3}H-5HT uptake in platelets.

88.4 CATECHOLAMINE TURNOVER WITH E.C.T. A. Khan*, A. Nies*, G. Johnson*, and J.T. Becker (SPON: M. Oscar-Berman), Dept. Psychiatry, Univ. of Connecticut, Farmington, CT 06032

Electroconvulsive therapy (ECT) is an established treatment for depression, although its exact mechanism of action is unknown. There is evidence from animal experiments that there is considerable change in catecholamines in ECT. We report our preliminary findings of the quantification of the acute and cumulative turnover of serum catecholamines during several ECTs in a depressed patient.

The patient (73 y.o. male) was hospitalized for major depression with melancholia (DSM-III). He was severely withdrawn and needed parenteral nutrition. He was given 9 ECTs during a 4 week period and showed improved clinical status. The ECTs were given unilaterally to the right hemisphere, at about 10 AM, and were preceeded by IV thiopental and succinyl choline. Several blood samples were drawn during each of ECT 1,2,3 and 9. The first sample was drawn prior to the thiopental, and the second one just after the succinyl choline. Two to three samples were drawn during the 15 minutes following the ECT until the patient was fully awake. The serum from each of the samples was assayed for serum epinephrine (EP), norepinephrine (NEP), and dihydroxyphenylglycol (DOPEG) by an investigator who was unaware of the nature of the experiment. ECTs 2 and 9 were good clinical seizures, whereas ECTs 1 and 3 did not lead to a convulsion.

The IV drugs had minimal effects on serum catecholamine levels. During ECT 2 and 9, serum NEP levels peaked at more than 5.5 times their baseline values before dropping during the 15 minutes after the electrical stimulus. Serum DOPEG peaked by at least 47% and serum EP fifteen-fold. During the nonconvulsive ECTS (i.e., 1 & 3), serum NEP rose to less than 85%, serum DOPEG less than 22%, and serum EP less than 15.5 times their baseline values. These findings cannot be attributed to non-specific arousal effects since the patient was anesthetized.

While the clinical measures of depression and serum cortisol levels (from dexamethosone suppression test) fell during the four weeks of treatment, the baseline levels of catecholamines rose.

These data supoort the finding that ECT leads to marked shifts in peripheral catecholamines. Similar findings in the CNS may have implications on the mode of action of ECT.

88.5 MONOAMINE OXIDASE B LOCALIZATION IN HUMAN BRAIN USING A SPECIFIC MONOCLONAL ANTIBODY. K.N. Westlund, R.M. Denney*, J.D. Coulter, R.M. Rose, and C.W. Abell* Depts of Anatomy, Human Biol Chem and Genetics, Psychiatry and Beh Sci, and Physiol and Biophysics. The University of Texas Medical Branch, Galveston, TX 77550.

MAO-B-positive cells were localized immunocytochemically in a human brain fixed (4% paraform.) 5 hr post mortem. MAO B was identified with a monoclonal antibody (MAO-1C2) which was produced against human platelet MAO and is specific for the B form of the enzyme (Denney et al., Science 215: 1400, 1982). Immunocytochemical controls included omission of one or more of the reagents in the PAP protocol, dilution of the antibody, competition of the antibody with antigen, and incubations with ascites fluid from mice injected with vehicle only. Specific staining for MAO B was not seen in control stains or when the antibody (1:2,000) was preabsorbed with 9.3 µg/ml of antigen. Alternate sections were stained for serotonin and the catecholamine synthetic enzyme, dopamine- β -hydroxylase (DBH). As expected from biochemical studies, cell staining for MAO B was localized in cytoplasmic structures which appeared to be mitochondria and was absent from the nucleus. Neurons and some glia were stained in specific regions of the brain. Of the regions thus far studied, staining for MAO B was widely distributed among cells in regions which contained cells staining for monoamines in alternate sections. MAO-B-positive neurons were localized in serotonergic cell groups including raphe obscurus (cell group B2 of Dahlstrom and Fuxe), raphe pallidus (B1), raphe magnus (B3), raphe pontis (B5), raphe dorsalis (B6, B7), and in the nucleus centralis superior (B8, B9). Catecholamine cell groups were identified by staining of alternate sections for DBH as confirmed by comparison with reported localization of tyrosine hydroxylase in humans (Pearson et al., Neurosci. 8:3, 1983). MAO-B-positive neurons were localized in noradrenergic cell groups of the lateral tegmental group (A1, A2, A5, A7) and in the locus coeruleus (A6). Neuronal staining for MAO B was localized in regions corresponding to dopaminergic cell groups A8 laterally in the midbrain, in the pars compacta of the substantia nigra (A9), and in the periventricular region of the caudal hypothalamus (A11). A few scattered glial cells were also stained for MAO-B in the regions detailed above, near ventricular spaces, and around some of the large blood vessels. In addition to neuronal staining in known monoaminergic cell groups, another region of intense neuronal staining was identified in the caudal hypothalamus. Multipolar and some fusiform-shaped neurons (20-40 µm dia.) with prominent nuclei were observed in the nucleus tuberomammillaris just lateral to the medial mammillary nuclei.

88.6 TREATMENT OF PARKINSON'S DISEASE WITH A DOPAMINE PARTIAL AGONIST. G.U.Corsini, R.Horowski°, E.Rainer°, M.Del Zompo*, Clinical Pharmacology, University of Cagliari, 09100 Cagliari, Italy; ° Dept.of Neuroendocrinology and Neuropsychopharmacology, Schering AG, Berlin - Milan.

The use of a DA partial agonist should be of interest in the treatment of P.D. since such a compound might predominantly stimulate hypersensitive receptors without affecting others which are of no interest in the neurological improvement. Transdihydrolisuride (TDHL), a 9,10 dihydrogenated analogue of lisuride, which proved to be a partial agonist of DA receptors in animal experiments, was considered in order to test this hypothesis. In fact, TDHL elicited hypokinesia, catalepsy and antagonized apomorphine-induced stereotypies and hyperactivity in rats, but produced a contralateral turning in 6-hydroxydopamine lesioned animals, indicating that the weak agonistic action of TDHL at the striatal level is unmasked only at supersensitive postsynaptic DA receptors. In healthy volunteers, TDHL (0.2 - 1 mg orally) effectively lowered prolactin levels with similar potency but with a markedly longer duration of action than lisuride (24h after 0.5mg og TDHL). In contrast to the side effects after acute lisuride treatment, no comparable adverse reactions such as nausea, emesis or postural hypotension, were observed when using effective prolactin-lowering doses of TDHL. As chronic studies with TDHL were available, indicating that this compound does not produce any toxic reactions over a period of treatment of one month, we studied the effects of this compound in P.D. patients. We report here only the preliminary results obtained in 5 untreated patients. TDHL at increasing dosage from 0.2 up to 0.8-1.2mg daily in two divided doses induced a general improvement of more than 50% of all the neurological disability. During the treatment, no major side effects were noted and the patients complained variably of poliuria or transient headache during the first few days of treatment only. These preliminary results, even though limited by the difficulty in finding an appropriate dosage schedule, indicate that TDHL is effective in improving Parkinson's Disease.

88.7 CLINICAL AND NEUROCHEMICAL CORRELATES OF NEURONAL LOSS IN THE CHOLINERGIC BASAL FOREBRAIN SYSTEM IN ALZHEIMER'S DISEASE. P. J. Whitehouse, J. C. Hedreen, A. W. Clark, R. M. Zweig*, B. E. Jones*, R. D. Terry*, P. G. Antuono*, J. T. Coyle, P. F. Davies* and D. L. Price. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Neuronal loss in the cholinergic basal forebrain system (Ch system) appears to be the anatomical basis for loss of presynaptic cholinergic markers in the telencephalon in Alzheimer's disease (AD). The Ch system includes neurons in the medial septum, nucleus of the diagonal band of Broca, and nucleus basalis of Meynert, which project to neocortex, hippocampus, and amygdala. Several important questions remain unresolved concerning the anatomical extent and clinical-pathological-chemical correlations of this neuronal loss. For example, does the neuronal loss vary in relation to the anatomical division of the Ch system, the age of the patient, or the reduction of cortical choline acetyltransferase (ChAT) activity We have assessed the number of Ch system neurons in 50 patients with AD (age at death ranging from 52-97 years), 10 patients with mixed dementia including multiinfarct and Parkinson's disease, and 20 age-matched controls using 12-µm cresyl violet-stained, paraffin-embedded sections. A microprojector system was used to count all large (>20 µm diameter), darkly stained neurons at five standard levels of the Ch system. In 15 patients, ChAT activity was measured in 5-8 standard cortical regions dissected from frozen material, usually taken from the hemisphere contralateral to the side in which Ch neurons were counted. In neuronal assessment studies, interrater reliabilities usually exceeded 0.85 but varied depending on the anatomical level assessed. Statistically significant (54-76%) neuronal loss occurred throughout the entire extent of the Ch system in the AD cases and to a lesser degree in the mixed dementias. At every level assessed, neuronal loss was more severe in the younger (<70 years of age at death) AD cases than in older cases. Correlations between cortical ChAT activity and Ch neuronal counts are difficult to interpret because of considerable individual case variability and the complex corticotopic organization of the Ch system. Our preliminary correlations ranged from 0.35 to 0.50. The present study confirms that neuronal loss involving all components of the Ch system is characteristic of AD and occurs in greater magnitude in the younger cases.

88.8 INVOLUNTARY OROFACIAL MOVEMENTS IN RELATION TO NEUROLEPTIC TREATMENT, INTELLECTUAL IMPAIRMENT AND AGEING IN SCHIZOPHRENIC PATIENTS AND SENESCENT ANIMALS. J.L.Waddington, H.A.Youssef¶ K.M.O'Boyle* & A.G.Molloy¶ Dept.Clinical Pharmacology, Royal College of Surgeons in Ireland, Dublin 2 and St. Davnet's Hospital, Monaghan.

'Tardive' dyskinesia is believed to be a late-onset side effect of long-term neuroleptic treatment that is characterised by involuntary orofacial movements and which derives from supersensitivity of striatal dopamine receptors.

We have found that in a sample of 68 schizophrenic patients, the 28 with abnormal, involuntary orofacial movements had received neither longer nor more aggressive neuroleptic treatment. Rather, the syndrome was associated ($p < 0.001$) with ageing and progression to the 'defect state'(The Type II syndrome) of intellectual impairment and negative symptoms, where structural brain change(s) have been demonstrated on CT scan. We report 3 older schizophrenic patients who had never received neuroleptics; 2 showed involuntary orofacial movements indistinguishable from their medicated counterparts, with negative symptoms or intellectual impairment.

In senescent (22 month) and young (4 month) rats given monthly i.m. depot injections of 2.5 mg/kg fluphenazine decanoate, 25 mg/kg haloperidol decanoate or vehicle for 3 months, oral dyskinesia over controls emerged ($p < 0.05$) beyond the 1.5 month point in young neuroleptic groups. In senescent animals, oral dyskinesia was more common in all groups, principally in the control age-related baseline ($p < 0.05$) with little additional effect of medication. There was a significant decrease ($p < 0.01$) in the B_{max} for striatal ^{3}H-spiperone binding to D_2 dopamine receptors across all aged groups; oral movements were dissociable from dopamine receptor supersensitivity in neuroleptic groups.

In both clinical and animal populations, ageing/disease and presumed medication effects could not be distinguished; such orofacial dyskinesia did not appear to relate to dopamine receptor supersensitivity.

We thank MRCI, RCSI, RCPI, Sanity, the Mason Medical Research Foundation, Janssen and Squibb for their support.

88.9 ABNORMAL FORM OF 5-HYDROXYTRYPTOPHAN AND SEROTONIN IN CEREBROSPINAL FLUID (CSF) OF ALZHEIMER PATIENTS. L. Volicer, P. Langlais, W.R. Matson*, K.A. Mark* and P.H. Gamache*, E.N. Rogers Mem. Vet. Hosp., GRECC, Bedford, MA, McLean Hospital, Belmont, MA, and ESA, Inc., Bedford, MA.

Despite recent advances in our understanding of dementia of Alzheimer type (DAT) its etiology is still unknown. It is not known if the cholinergic deficit, detected in most DAT patients, is the primary pathological process or a consequence of other processes. There is considerable evidence indicating involvement of neurotransmitters other than acetylcholine in DAT. We have found a strong evidence for involvement of the serotoninergic system.

A recently developed three electrode coulometric detector has been used to verify the purity of sample peaks obtained through liquid chromatographic separation by comparing the ratio of the peak's signals at two different detector voltages with those observed in the analysis of an authentic standard. Using this system we have found that in a large proportion of CSF from DAT patients the serotonin (5-HT) peak gives an aberrant ratio. This indicates the presence of a co-eluting compound with a reduction wave different from that of authentic 5-HT. Investigation with a 6-sensor system showed that both 5-hydroxytryptophan (5-HTP) and 5-HT are oxidized in two steps, presumably through an intermediary quinone compound, each resulting in two oxidation waves of similar magnitude. In contrast the second oxidation wave of 5-HT and 5-HTP observed in CSF from most DAT patients was significantly larger than the first one. This indicates presence of 5-HTP and 5-HT in a form which had already undergone oxidation of the 5-hydroxy group, presumably to the quinone. This assumption is supported by electrochemical behavior of partially oxidized 5-HT and 5-HTP, prepared by electrochemical oxidation, which produce waves identical to the second waves observed in CSF.

The presence of an abnormal ratio of 5-HTP waves indicates an abnormal tyrosine hydroxylation process. Unlike 5-HT, the second oxidation wave of 5-HIAA was roughly equal to the first 5-HIAA wave, suggesting that the partially oxidized 5-HT (5-HT-e-) in DAT CSF is not metabolized by monoamine oxidase. This could explain the aberrant relationship of 5-HT and 5-HIAA levels in rostral fraction of DAT CSF found by us previously. In this work we used a single sensor amperometric detection which does not differentiate unchanged 5-HT and 5-HT-e-. It is possible that 5-HT-e- participates in pathogenesis of DAT, since the neurotoxic effect of 6-hydroxy-dopamine is mediated by formation of a quinone intermediate. However, it remains to be determined if increase of the second oxidation wave, observed in CSF from DAT patients, is due to a quinone form of 5-HT, if this compound actually exists in the brain, and if it has a neurotoxic effect.

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88.10 ANTIGENIC DETERMINANTS SHARED BY NICOTINIC ACETYLCHOLINE RECEPTOR AND PEPTIDES IN BACTERIA. K. Stefansson,* M.E. Dieperink,* D.P. Richman* and L.S. Marton* (SPON: M.R. O'Shea). Dept. of Neurology and The Brain Research Institute, University of Chicago, Chicago IL 60637.

Myasthenia gravis (MG) is a disease that is characterized by defective neuromuscular transmission. More than 90% of patients with MG have circulating antibodies directed against the nicotinic acetylcholine receptor (AChR) and it is believed that these antibodies play a key role in the pathogenesis of the disease. It is, however, not known what causes the patients to raise these anti-AChR antibodies. In our search for environmental components that share antigenic determinants with AChR, we stained Western blots containing electrophoresed peptides from homogenates of several bacterial species with 24 well-characterized monoclonal antibodies (mAbs) against AChR. One of them (BK57) bound to two peptides of E. coli; one tentatively identified as being the outer membrane protein OmpC. OmpC is a receptor for the outer membrane and is therefore in its function somewhat homologous to AChR. The other protein did not co-purify with the outer membrane and had a larger molecular weight (mw) than OmpC. BK57 also bound to a protein in P. vulgaris that had the same mw as the larger protein identified in E. coli. A second antibody (77F) bound to a protein in P. vulgaris with the same relative mobility as the one identified by BK57 but it bound to nothing in E. coli. We have therefore shown that the AChR shares at least one antigenic determinant with two peptides in E. coli and at least two antigenic determinants with a peptide in P. vulgaris. We want to point to the possibility that these peptides which are found on members of normal human bacterial flora may under certain circumstances sensitize people against determinants shared by them and the AChR and thereby cause MG.

88.11 THE RELATIONSHIP BETWEEN VIRUS DISTRIBUTION AND NEUROCHEMICAL CHANGES IN THE MOUSE AFTER DIFFERENT ROUTES OF CENTRAL NERVOUS SYSTEM INFECTION WITH HERPES SIMPLEX VIRUS TYPE I. S.P. Neeley*, A.J. Cross*, T.J. Crow*, J.A. Johnson* and G.R. Taylor* (SPON: Dr. R.G. Hill). Division of Psychiatry, Clinical Research Centre, Watford Road, Harrow, England.

Mice (4-6 week, male TO) were infected by intracranial (IC) innoculation with herpes simplex virus type I (Justin, 30 p.f.u. in 30 µl MEM) or by intranasal (IN) innoculation (Justin, 100 p.f.u. in 10 µl MEM). Brains were taken on day 5 for neurochemical and immunohistochemical analysis.

IC HSV-1 encephalitis resulted in no changes in the activity of choline acetyltransferase, or in the level of muscarinic cholinergic receptors in any brain area. Similarly, no changes were observed in glutamic acid decarboxylase activity, or in the level of GABA receptors. The levels of a broad range of monoamine receptors also remained unchanged. While the concentrations of the monoamines dopamine (DA) and serotonin (5HT) were unaffected by HSV-1 encephalitis, the concentrations of the DA metabolite homovanillic acid (HVA) and the 5HT metabolite 5-hydroxyindoleacetic acid (5HIAA) were markedly increased in all brain areas after HSV-1 infection. HVA concentrations were increased, however, to a greater extent in cortical and limbic areas than in striatum, while 5HIAA concentrations were increased to a similar extent throughout the brain. IN HSV-1 encephalitis also resulted in increased concentrations of HVA and 5HIAA in all brain areas examined. After this treatment however, the increases in the concentrations of these metabolites were of greater magnitude in brainstem than in forebrain areas.

Immunohistochemical staining for HSV-1 antigen in brain sections from mice infected by IC and IN HSV-1 innoculation demonstrated distinct patterns of virus distribution for the two treatments. IC HSV innoculation resulted in a widespread infection, with heavy concentrations of antigen positive cells in the DA containing nuclei of the substantia nigra, the 5HT containing dorsal raphe nucleus, and the norephinephrine containing nuclei locus coeruleus, as well as in forebrain areas. IN HSV-1 innoculation resulted, on the other hand, in virus antigen being confined almost exclusively to cells in the brain stem, with concentrations of antigen-positive cells again being found in brainstem monoamine containing nuclei.

88.12 SEROTONIN-IMMUNOREACTIVITY IN THE SPINAL CORD OF RATS WITH EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE). S. R. WHITE D. VYAS*, D. BIEGER AND L. OWEN*. FACULTY OF MEDICINE, MEMORIAL UNIV. OF NEWFOUNDLAND, ST. JOHN'S, NFLD. A1B3V6.

Experimental allergic encephalomyelitis (EAE) is an autoimmune disease which can be induced in laboratory rodents by inoculation with central nervous system tissue or myelin basic protein emulsified in complete Freund's adjuvant (CFA). This disease has been used extensively as a model for human demyelinating diseases such as multiple sclerosis. Most attention has focused on demyelination in both EAE and multiple sclerosis, however, the correlation between severity of demyelination and severity of clinical signs is not very good for either disease. We have recently reported that the acute paralytic stage of EAE is associated with apparently extensive damage to bulbospinal catecholamine-containing axons. Fluorescent microscope observations indicate that these fine diameter, unmyelinated axons become markedly swollen as they course near inflammatory foci in the spinal cord; and terminals in the gray matter of the caudal spinal cord appear depleted of catecholamine-fluorescent intensity. Furthermore, we have found that spinal cord terminals are depleted of noradrenaline as measured by radioenzymatic assay of samples obtained using the micropunch technique. The purpose of the present study was to examine the state of fine-diameter, unmyelinated bulbospinal serotonin-containing axons during the paralytic stage of EAE.

EAE was produced in Lewis rats by inoculation with Lewis rat spinal cord in CFA. Control animals were inoculated with CFA only or received no injection. When they developed complete hindlimb paralysis, EAE rats were anesthetized with pentobarbital and perfused through the abdominal aorta with 4% paraformaldehyde in PBS. The brain and spinal cord were removed, cut into blocks and sectioned on a vibratome (40 µm thick sections). The sections were preincubated with normal goat serum, then incubated with antibody to serotonin obtained from ImmunoNuclear, and processed with the PAP technique as modified for free-floating section. Serotonin-like immunoreactivity was clearly visible in terminal plexuses in the dorsal horn, in the gray matter around the central canal, in the region of the intermediolateral nucleus and around large somata in the ventral horn in spinal cord sections from CFA and from uninjected rats. Fine-diameter immunoreactive axons were also observed coursing longitudinally in the ventral and lateral funiculi and sending branches perpendicularly into the gray matter. In EAE-paralyzed rats, however, serotonin immunoreactive axons near inflammatory foci in the lateral and ventral funiculi of the cervical spinal cord were grossly swollen. These very large, distorted axons were quite numerous and could be found around nearly every inflammatory lesion. The density of serotonin immunoreactive terminals in the spinal cord gray matter of the paralyzed rats appeared to be decreased compared to controls; and many of the terminals which remained in the EAE rats appeared to be enlarged. Fine-diameter, unmyelinated axons may be particularly vulnerable to damage at inflammatory foci in the central nervous system, resulting in depletion of neurotransmitter at sites distal to the damage. This research was supported by the Multiple Sclerosis Society of Canada.

89.1 ROLE OF AN INCREASE IN PROTEIN SYNTHESIS IN THE EFFECT OF SEROTONIN ON A CIRCADIAN PACEMAKER. J.S. Yeung*, A. Eskin, and M.R. Klass*. Biol. Dept., Univ. of Houston-Univ. Park, Houston, TX 77004.

Anisomycin, an inhibitor of protein synthesis, blocks the phase shifting action of serotonin (5-HT) on the ocular circadian rhythm of Aplysia. This implies that protein synthesis is either directly or indirectly involved in mediating the effect of 5-HT on the rhythm. Since the synthesis of at least one protein should be increased by 5-HT if protein synthesis is directly involved, we have begun a search for proteins whose synthesis is increased by 5-HT.

Isolated experimental eyes were treated for 6 h with 5-HT and incubated with labeled amino acids during the last 4 h of the 5-HT treatment. Two-dimensional gel electrophoresis of eye homogenates consistently revealed a spot of increased label incorporation on experimental versus control gels. This protein (protein A) has an apparent MW of 67,000 daltons and a pI of 7.2. This effect was observed when eyes were incubated with either L-[^{35}S] methionine or L-[4,5-^{3}H] leucine. In another series of experiments, experimental eyes were exposed to label before exposure to 5-HT. No difference in incorporation of label into protein A between experimental and control eyes was observed. This suggests that the increased label incorporation into protein A is not due to an effect of 5-HT on some posttranslational process but is due to an effect of 5-HT on the synthesis of protein A.

Two types of experiments are in progress to investigate the involvement of protein A in mediating the effect of 5-HT on the rhythm. Forskolin, an activator of adenylate cyclase, mimics the action of 5-HT on the rhythm and appears to mimic the effect of 5-HT on protein A. The magnitude of the phase shift in the rhythm produced by 5-HT is phase-dependent, and the effect of 5-HT on protein A also seems to be phase-dependent.

The tissue specificity of protein A was investigated by examining abdominal and pleural ganglia for protein A. Label was incorporated into a protein with the same apparent MW and pI as protein A, but 5-HT had no noticeable effect on incorporation of label into this protein. Thus, the effect of 5-HT on protein A is to some degree tissue specific. The evidence gathered so far leads us to believe that protein synthesis is directly involved in mediating the effect of 5-HT on the circadian oscillator in the eye and that protein A may be an important element of the 5-HT phase-shifting pathway.

89.2 CIRCADIAN PACEMAKER COUPLING IN BULLA: WINDOWS OF INTERACTION AND PHASE RESPONSE CURVE. M. H. Roberts and G. D. Block, Dept. of Biology, Univ. of VA, Charlottesville, VA. 22901.

The two Bulla ocular circadian pacemakers are mutually coupled and their interaction can be observed in vitro (Science, 221:87; 1983). A 5 hour ocular rhythm separation can be induced by phase shifting one eye rhythm with a seawater solution containing manganese (Mn) substituted for calcium from 1500 to 2100 EST (CT 6-12). This separation is reduced to approximately 3 hours after one day of ocular interaction.

During the 24 hours immediately following the Mn pulse (2100-2100 EST), the untreated ocular rhythm delays approximately one hour by virtue of pacemaker interactions. In order to determine the time that the information causing this phase shift is transfered from eye to eye, the 24 hour period after the Mn pulse was divided into four 6 hour quadrants. (Quad 1: 2100-0300, CT 12-18; Quad 2: 0300-0900, CT 18-24; Quad 3: 0900-1500, CT 0-6; Quad 4: 1500-2100, CT 6-12). The Mn treated eye produces compound action potentials during quadrants one and four. However, information that phase delays the contralateral ocular rhythm could be present during any one of the four quadrants.

After blocking synaptic transmission in the eye with HiMg-LoCa seawater (125mM Mg, 0.1mM Ca) for the entire 24 hour period, the unshifted eye has an average phase of 8.75 hours (1.13 SD, n=6). Allowing interaction only during Quad 1 results in a phase shift of -2.92 hours (n=3); Quad 2: +0.25 hours (n=3); Quad 3: +0.42 hours (n=3); Quad 4: -1.41 hours (n=6). Only interaction during Quads 1 and 4 cause significant phase shifts (delays) of the ocular rhythms ($p<.05$, t-test). This suggests that the time of impulse production is the time of information transfer.

Different initial Mn induced phase separations result in impulse activity occuring in Quads 2 and 3. Activity during Quad 2 causes phase advances, while activity during Quad 3 results in no phase shift. If the magnitude of all impulse induced phase shifts are plotted with respect to the circadian time of the interaction, a coupling "phase response curve" (PRC) is generated. This curve shows advances from CT 18-24, and delays from CT 6-18. This is the first report of a coupling PRC derived from the experimental manipulation of two pacemakers. NS15264

89.3 CIRCADIAN PACEMAKER COUPLING IN BULLA: EFFERENT IMPULSES GENERATE DEPOLARIZATIONS IN PUTATIVE CIRCADIAN PACEMAKER NEURONS. G.D. Block and M.H. Roberts, Dept. of Biology, Univ. of Virginia, Charlottesville, VA. 22901

The eye of the molluac, Bulla, contains a circadian pacemaker which can be phase shifted by light (Block & Wallace, 1982, Science 217) and by the circadian pacemaker in the contralateral eye (Roberts & Block, 1983, Science 221). A study of the timing of the signals which couple the two eyes reveals that phase shifts of each pacemaker occur when the eyes are spontaneously producing compound action potentials (Roberts & Block, 1984, this volume). These compound potentials are conducted through the cerebral ganglia and appear as efferent impulses in the contralateral optic nerve.

We have begun a study of the effects of efferent signals on retinal neurons. In order to obtain electrophysiological recordings, the central ganglia along with both attached eyes were pinned out in a Sylgard lined dish. Extracellular recordings were obtained en passant from both optic nerves by means of auction electrodes. The lens of the eye and part of the connective tissue capsule was removed in order to provide access to retinal cells. Intracellular recordings from selected retinal neurons were obtained with glass microelectrodes (80 - 120 MΩ) Recordings from R-type photoreceptors and H-type cells failed to indicate any effect of efferent signals on membrane potential. In contrast, efferent impulses generated large (5mV), prolonged (3-5 S) postsynaptic potentials in basal retinal neurons (BRNs), the putative circadian pacemaker neurons in Bulla. These postsynaptic potentials occasionally induced impulses in the impaled BRN.

We suspect that it is the repeated depolarizations of BRNs by efferent impulses from the contralateral eye which generate the phase shifts in the ocular pacemakers. Light pulses which also depolarize BRNs cause phase shifts in the ocular pacemaker. The role of membrane potential in phase shifting is a critical issue which has been addressed in Aplysia (Eskin, 1977, J. Neurobiol. 8) and, more recently, in Bulla (McMahon & Block, 1984, this volume). Supported by NS15264 and RCDA NS00714.

89.4 CIRCADIAN PACEMAKER ENTRAINMENT IN BULLA: LIGHT AND SEROTONIN AFFECT THE MEMBRANE POTENTIAL OF PUTATIVE CIRCADIAN PACEMAKER NEURONS. D.G. McMahon and G.D. Block, Dept. of Biology, Univ. of Virginia, Charlottesville, Va. 22901

The basal retinal neurons (BRNs) of the Bulla eye are putative circadian pacemaker neurons and exhibit circadian rhythms in membrane potential and action potential frequency which underly the ocular circadian rhythm in compound action potential frequency. (McMahon and Block, Soc. Neuroaci. Abstr., 1983.). The membrane potential rhythm in the BRNs is an output of the circadian pacemaker. In addition, membrane potential changes play a role in the entrainment of the ocular circadian pacemaker of Aplysia (Eskin, 1977, J. Neurobiol., 8).

We have now measured directly the effects on BRN membrane potential of the phase-shifting agents light and serotonin. To obtain BRN recordings eyes were removed from the animal and maintained in sterile artificial seawater at 15°C. BRNs were impaled with glass microelectrodes (80-120MΩ) filled with 3M KCl. Light (1000 lux) produced a phasic depolarization of 20 mV followed by a tonic depolarization of 5 mV which persisted as long as recordings were sustained (up to 2 hr). Both the phasic and tonic depolarizations increased impulse frequency. Bath application of 10^{-5}M serotonin produced an initial depolarization of 12 mV, followed by a tonic hyperpolarization of 10 mV which persisted as long as recordings were sustained (up to 4 hr). The initial depolarization increased impulse frequency, while the tonic hyperpolarization decreased impulse frequency compared to baseline. Net membrane conductance in the BRN measured with constant current pulses increases during the phasic depolarization to light, and decreases during the initial depolarization to serotonin. This suggests that these two phase-shifting agents act through different ion channels.

Light and serotonin produce opposing tonic effects on the membrane potential of BRNs. If membrane potential is a critical element in entrainment then the phase-shift produced by these two treatments should be different. Light delivered from CT 20-2 advances the Bulla ocular circadian rhythm, while application of serotonin during this same interval produces a phase delay (McMahon and Block, unpublished). Thus, BRN membrane potential may play a critical role in the entrainment of the Bulla ocular circadian pacemaker. NS15264.

89.5 THE SUPRACHIASMATIC NUCLEI OF THE HOUSE SPARROW, PASSER DOMESTICUS. Vincent M. Cassone and Robert Y. Moore. Departments of Neurology and Neurobiology and Behavior, State University of New York, Stony Brook, N.Y. 11794

As in mammals, the avian suprachiasmatic nuclei (SCN) appear to participate in the expression of circadian rhythmicity. Although much work has focussed on the anatomy and physiology of the mammalian SCN, very little is known about its avian homologue. In this study, we have investigated the anatomy of the house sparrow (Passer domesticus) SCN using autoradiographic, immunohistochemical and cytoarchitectonic techniques. Birds were injected intraocularly with 10 uCi 3H-proline, and, after one day survival, their brains were processed for autoradiography. Brains from other birds were processed for immunohistochemical analysis of avian pancreatic polypeptide (APP), glutamic acid decarboxylase (GAD), 5-hydroxytryptamine (5HT), substance P (SP), vasoactive intestinal peptide (VIP) and vasopressin (VP). Data from these studies were analyzed within the framework of a cytoarchitectonic study of the avian hypothalamus obtained from Bouin's fixed, paraffin-embedded and Nissl-stained material.

From this study, we have identified an area in the anterior hypothalamus which we believe may be the avian homologue of the SCN. This contains nuclei which are located in close apposition to the optic chiasm and nestled for much of its rostrocaudal extent between the supraoptic decussation (SOD) and the ventral lateral geniculate nucleus (vLGN). The nuclei are composed of small, tightly packed neurons which can be divided into two groups: a medial group characterized by small multipolar cells and a lateral group composed of small fusiform cells. The nuclei are about 600 um long, extending from the caudal preoptic area, rostral to the SOD, to a point where the vLGN apposes the SOD. Autoradiographic labeling from the contralateral eye is present in the nucleus. Cells in the medial group contain VIP-like immunoreactivity; cells in the lateral group contain SP- and VP-like immunoreactivity. GAD reactive cells and fibers are present throughout the nucleus. 5HT-like and APP-like immunoreactive fibers are present in the medial group. The APP fibers appear to arise from somata in the perirotundal area of the thalamus. This organization is similar to that of the mammalian SCN; the avian lateral group appears to correspond to the mammalian dorsomedial division while the medial group is similar to the ventrolateral group. Whether these nuclei are true homologues and/or circadian oscillators remains to be established.

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89.6 **REDEFINING THE DURATION OF THE CIRCADIAN RHYTHM OF PHOTOPERIODIC PHOTOSENSITIVITY (CRPP) IN MALE AND FEMALE SYRIAN HAMSTERS.** J.S. Ferraro and C.E. McCormack*. Dept. of Physiol. Biophys., The Chicago Medical School, N. Chicago, IL 60064.

Feedback lighting (LD_{FB}) (Ferraro and McCormack Soc. Neurosci. Abst. 9(2):1075, #313.14, 1983) which illuminates the cage in response to active wheel running, exposes only the photosensitive portion of the circadian cycle to light. In the hamster, the photoinducible zone of the CRPP is believed to occur during an interval beginning 0.5h before and extending to 11.5h after activity onset (Elliott 1981). Since this interval roughly coincides with the photosensitive portion of the circadian cycle, and since LD_{FB} exposes the proposed photoinducible zone almost as much as does constant light (LL), we predicted that LD_{FB} would maintain gonadal function just as LL does. Surprisingly, 5 male hamsters exposed to LD_{FB} for 8 wks. had regressed testes similar to those of hamsters in continuous darkness (DD) but significantly smaller than hamsters exposed to LL ($P<0.01$). Testis weights (mg/100g body wt) for DD, LD_{FB}, and LL were 385.6 ± 110, 574.2 ± 126, and 2511.0 ± 201 respectively. All females exposed to LD_{FB} and all exposed to DD ceased showing cyclic signs of ovulation after 20 days; whereas most hamsters exposed to LL continued to show signs of cyclic ovulation for 8 wks. 6 of the 8 hamsters exposed to LL had ova in their oviducts at autopsy, and also had significantly larger uteri ($P<0.01$) than hamsters exposed to DD or LD_{FB}. None of the latter two groups (N=6 and 9 respectively) had oviductal ova at autopsy ($P<0.01$). Adrenal weights of hamsters in DD, LL, or LD_{FB} did not differ significantly indicating that exposure to LD_{FB} was no more stressful than DD or LL. The evidence presented here indicates that the duration of the CRPP must be redefined. We propose that the Syrian hamster has two temporally discrete photoinducible zones; one occurs during a short interval (1h) just prior to activity onset and one occurs during a short interval just after activity offset. Supported by 1-R01-HD13131.

89.7 ESTABLISHMENT OF THE ENDOGENOUS CIRCADIAN NATURE OF THE DAILY VARIATION AND PROESTROUS SURGE OF PROLACTIN HORMONE RELEASE FROM FEMALE HAMSTERS (MESOCRICETUS AURATUS). K.L. Horwath*, J. Swann, and F. Turek. Dept. Neurobiology and Physiology, Northwestern University, Evanston, IL 60201.

To document the temporal profile of circulating prolactin (PRL) hormone titres from female hamsters and to investigate whether it is controlled by an endogenous circadian clock the following experiments were performed. First, 4d cycling females held in running wheel cages on an LD 16/8 (lights on 0800-2400h) were fitted with intra-atrial cannula. Beginning the next day serial blood samples were taken at 90min intervals for the entire 4d estrous cycle. The serum was assayed for PRL using an homologous RIA for hamster PRL. A clear proestrous(PE) surge in PRL levels was observed for each animal. Compared to basal PRL values averaging 20ng/ml, the PE surge went to over 350ng/ml. The timing of the surge occurred from 1700-2300h with maximal responses observed at the 1830 and 2130h sampling times. Locomotor activity onset occurred at 2400h, therefore, the peak PE surge occurred an average of 4-5h prior to activity onset. Furthermore, rhythmic PRL levels were observed on the other 3 days of the cycle with elevated PRL titres(100-200ng/ml)only occurring from 1700-2130h. These daily peaks in PRL levels were further investigated with ovariectomized-estrogen (E) treated animals held in 16L/8D. Serial blood samples were taken every 60min between 1400-2300h from d1-d7 after cannulation and E implant. All animals receiving E displayed daily surges of PRL release at a time coincident with that of the PE surge(1830-2130). Furthermore, from d4-d7 the magnitude of the daily peaks reached levels comparable to those measured on PE. Animals receiving empty capsules failed to display daily surges.

Finally, to establish the circadian nature of the rhythmic daily increases in PRL levels intact females were held under constant light conditions(LL). After 100d in LL each animal was cannulated and bled at 90min intervals throughout their entire estrous cycle. Rhythmic increases in PRL titres for every day of the cycle persisted. Furthermore,the PRL peak maintained a fixed phase relationship to activity(just prior to activity onset). In fact, there is some evidence to suggest that among animals whose activity rhythm had split into 2 distinct components the daily PRL rhythm also split and was associated with each bout of activity. These combined results strongly suggest that the temporal profile of circulating PRL levels and presumably the neuroendocrine events involved with PRL release are regulated by an endogenous circadian clock.

89.8 GLUCOSE UTILIZATION OF THE SUPRACHIASMATIC NUCLEI IN THE GOLDEN HAMSTER: USE OF ANESTHESIA. W.J. Schwartz. Neurology Svc., Mass. Gen. Hosp. & Harvard Med. Sch., Boston MA 02114

The rate of glucose utilization (measured by the ^{14}C-labeled deoxyglucose method) has provided an effective *in vivo* marker for the functional activity of the circadian clock located in the suprachiasmatic nuclei (SCN) of fetal & adult rats and squirrel monkeys. Here we report preliminary observations on measuring SCN metabolic activity in golden hamsters (*Mesocricetus auratus*).

Adult hamsters were entrained to a 12h:12h light:dark (LD) cycle. 25 uCi 2-deoxy-D-[1-^{14}C] glucose (DG; s.a. 60 Ci/mole) was administered by either cardiac puncture or intravenous injection through a previously implanted intra-atrial silastic catheter. After 45 min, animals were sacrificed, brains removed & frozen, & serial 20 um coronal sections cut and autoradiographed.

When unrestrained, unanesthetized hamsters were injected during the L phase of the LD cycle, the SCN could not be visualized on the autoradiographs; the location of the nuclei was indistinguishable from adjacent hypothalamus. Results were similar when brains were cut in the horizontal and sagittal planes.

The rodent circadian clock is known to oscillate during general anesthesia. Since pentobarbital anesthesia globally depresses brain glucose utilization, we thought this treatment would improve the metabolic "signal-to-noise ratio" between SCN and surrounding hypothalamus. Hamsters were given 12 mg pentobarbital I.P. and were fully anesthetized for both the DG injection and the ensuing 45 min. Under these conditions, the SCN were easily discernable from the now homogenous, depressed background activity. The nuclei were relatively active during the L phase; optical density (OD) of SCN/OD of adjacent hypothalamus = 1.24 ± 0.03 (S.E.M.). Activity remained high during the "subjective day" in the absence of environmental lighting (1.21 ± 0.02). The nuclei were relatively inactive and not visible on autoradiographs during the D phase (1.02 ± 0.01). Exposure to light during the D phase increased activity to daytime levels (1.26 ± 0.05).

Thus, the DG method measures a metabolic oscillation in the hamster SCN which persists during general anesthesia. The circadian system of these animals is of special interest since its formal properties, including photoperiodism & "splitting" of expressed rhythms in constant light, have been well characterized.

89.9 ABLATION OF THE VENTRAL LATERAL GENICULATE NUCLEUS (vLGN) ALTERS THE PHASE SHIFTING EFFECTS OF LIGHT ON THE MAMMALIAN CIRCADIAN SYSTEM. G. E. Pickard and M. R. Ralph*. Inst. of Neuroscience, University of Oregon, Eugene, OR 97403.

The mammalian circadian system receives input from the retina which serves to entrain the circadian clock to the 24 hr cycle of light and dark. At least two different neural pathways carry photic signals to the circadian system; a monosynaptic projection from retinal ganglion cells via the retinohypothalamic tract (RHT) to the suprachiasmatic nucleus (SCN), and an indirect, multisynaptic pathway to the SCN which includes the vLGN. The RHT is sufficent to maintain entrainment under standard laboratory light:dark (LD) cycles when the remainder of the optic system is destroyed. The vLGN may be involved in light-induced phase shifting or entrainment, but its role has not been determined. We have examined the ability of animals both to phase delay and phase advance in response to brief light pulses following bilateral destruction of the vLGN.

Male hamsters (8 weeks of age), housed individually in running wheel cages, were entrained to a 14:10 LD cycle for 14 days. Some animals then received bilateral lesions aimed at the vLGN (N=15) or sham lesions (N=6). Lesions were verified histologically. The remaining animals served as unoperated controls (N=5). After recovering for 14 days in LD the animals were released into constant darkness (DD). After 7 days in DD, each animal was given a 15 minute light pulse (515 nm; total fluence = 3.0×10^{14} photons$\cdot cm^{-2} \cdot sr^{-1}$) calculated to produce a half maximal phase shift at either phase advance (CT 18) or phase delay (CT 13.5) time points.

Ablation of the vLGN had significant effects on light-induced phase delays and advances. Phase advances (CT 18) were significantly smaller in vLGN lesioned animals compared to controls (0.66±0.08 hr vs 0.91±0.08 hr, $P<0.05$). However, phase delays at CT 13.5 were significantly larger in vLGN lesioned animals (0.67±0.04 hr vs 0.42±0.03 hr, $P<0.01$). Sham and unoperated controls were not different and results have been combined.

These results suggest that the vLGN influences the sensitivity of the circadian system to light, but that the influence on phase advances and delays is in opposite directions. The vLGN may serve to modify the relative sensitivity of the system to light at different time points and may therefore contribute to the entrainment of the circadian system.

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89.10 ELECTROPHYSIOLOGICAL BASIS FOR BIORHYTHMIC ACTIVITY IN THE SUPRACHIASMATIC NUCLEUS OF THE RAT: AN IN VITRO STUDY. M. Sugimori, S. Shibata* and Y. Oomura. Dept. Physiology & Biophysics, New York Univ. Med. Ctr., 550 First Ave., New York, NY 10016; Dept. Physiology, Kyushu Univ. Faculty of Medicine, Fukuoka 812, Japan.

Intracellular recordings from suprachiasmatic nucleus neurons were obtained from a single horizontal slice containing the totality of the nucleus. The optic nerve was left attached to the slice such that contralateral optic nerve stimulation evoked graded excitatory postsynaptic potentials (EPSPs) on suprachiasmatic nucleus neurons. Reversal of these EPSPs was achieved with depolarizing current injections on the order of 2 nA. The input resistance and resting potential of these neurons ranged from 110 to 140 Mohms and had a resting potential of -65 to -70 mV. TTX-sensitive fast action potentials had after-hyperpolarizations comprised of 70 to 80 mV amplitude. The after-hyperpolarization had two components: an early fast component blocked by TEA or 4-aminopyridine, and a later slow component blocked by Co^{++}. Hyperpolarizing current pulses of higher than 0.1 nA produced clear anomalous rectification with rebound depolarization which followed immediately the termination of the hyperpolarizing current pulse. This anomalous rectification was removed by replacing Ca^{++} with Mg^{++}, Mn^{++} or Cd^{++} ions. Membrane hyperpolarization by more than 10 mV by either D.C. current or by square current pulses of more than 50 msec produced low threshold spikes similar to those described in the inferior olive (Llinás & Yarom, J. Physiol. 315: 569-584, 1981). This potential was blocked by Co^{++}. The above set of conductances provided the necessary ionic mechanisms to generate intrinsic oscillatory electroresponsiveness in the suprachiasmatic neurons, the basic oscillatory rhythm being close to 5 Hz with minimal depolarization. F-I plots showed, beyond this basic rhythm, two ranges of repetitive firing, the first with a 50 Hz/nA gain and the second 120 Hz/nA. Supported by NIH grant NS13742 from NINCDS and by National Science Foundation Cooperative Grant INT-8211161.

89.11 CIRCADIAN RHYTHMS OF CIRCULATING CORTICOSTERONE AND α_2-NORADRENERGIC RECEPTORS IN DISCRETE HYPOTHALAMIC AND EXTRAHYPOTHALAMIC AREAS OF RAT BRAIN. Leibowitz, S.F., Jhanwar-Uniyal, M.*, and Roland, C.R.* Rockefeller Univ., NY 10021.

The paraventricular nucleus (PVN) α_2-noradrenergic system and the glucocorticoid corticosterone (CORT) are known to modulate feeding and to exhibit circadian patterns which may be related to the natural periodicity of feeding. The present study examined, in relation to serum CORT, the circadian rhythm of α_2-noradrenergic receptor number in brain areas.

Male albino rats on a 12:12 light-dark cycle (with lights off at 1800 hr) were sacrificed at 0600, 1200, 1800, 2100 and 2400 hr, by decapitation within 20 sec of removal from their cages. The brains were quickly removed, and discrete hypothalamic (8) and extra-hypothalamic (6) areas were micropunched. To assay for α_2-receptor sites, standard radioligand binding procedures were employed with the α_2-noradrenergic agonist [^{3}H]para-aminoclonidine ([^{3}H]PAC, 3.0 nM), with nonspecific binding determined in the presence of phentolamine (50 μM). Serum CORT was measured by RIA.

The results demonstrate that, of the 14 brain areas examined, 3 hypothalamic areas showed a circadian rhythm of α_2-noradrenergic binding sites. The PVN exhibited the most dramatic changes, with a significant peak of α_2-receptor number (183 fmoles/mg protein) occurring at the onset of the dark cycle (1800 hr), in contrast to low levels (96 fmoles/mg protein) occurring at 0600, 1200 and 2400 hr. The suprachiasmatic nucleus showed a similar but attenuated peak at dark onset, in contrast to the supraoptic nucleus which showed the reverse pattern. Other hypothalamic areas (i.e., dorsomedial nucleus, ventromedial hypothalamus, medial preoptic nucleus, perifornical lateral hypothalamus and arcuate-median eminence) showed no periodicity of their α_2-receptor binding sites. The well-known serum CORT circadian rhythm was in phase with the α_2-adrenoceptor rhythm observed in the PVN, with CORT levels elevated to 7.0 μg% at 1800 hr but low (0.2-2.7 μg%) at other time points.

These findings correlate the circadian rhythms of PVN α_2-adrenoceptor number with the circadian rhythm of circulating CORT. This study is interesting in light of evidence that the onset of normal circadian feeding in the rat occurs at dark onset and that feeding may in part be regulated by an interaction between PVN norepinephrine and CORT. Feeding induced by PVN α_2-noradrenergic stimulation is abolished by adrenalectomy, and α_2-receptor binding ([^{3}H]PAC) in the PVN is strongly down-regulated by adrenalectomy (Jhanwar-Uniyal et al.). (Research supported by grant MH-22879)

89.12 CIRCADIAN ACTIVITY RHYTHMS IN RATS WITH SURGICALLY "SPLIT" SUPRACHIASMATIC NUCLEI: REPORT ON WORK IN PROGRESS. A.M. Rosenwasser, J.A. Yanovski* J. Levine* N.T. Adler. Dep't. of Psychology, Univ. of Pennsylvania, Phila. PA 19104.

We have been conducting a long-term study on the effects of separating the rat suprachiasmatic nuclei (SCNs) by a midparasagittal knife cut between the SCNs and throughout the rostral-caudal extent of the optic chiasm (S-SCN). This knife cut is designed to sever the direct neural connections between the SCNs (and restrict retinal input to each SCN to that arising from the ipsilateral eye). This project has led to two earlier presentations of preliminary results (Soc. Neurosci. Abstr. 7, 857, 1981; 9, 1070, 1983). The present report describes our more recent results on wheel running activity rhythms in S-SCN rats. The objective of this project is to elucidate the nature of coupling pathways in the circadian activity pacemaker system, especially with respect to the possible functional significance of the bilateral anatomical organization of the SCNs.

Histological observations reveal that about one third of the knife cuts were accurately placed on the midline, one third were slightly lateral and thus damaged the SCN unilaterally, and one third were more substantially lateral and thus isolated both SCNs on the same side of the cut. However, nearly all knife cut animals showed normal rhythms under entrained and free-running conditions, regardless of the exact localization of the cut.

We have also studied several animals with S-SCN combined with either pinealectomy (PX), lesions of the median raphe (RX), or unilateral blinding by enucleation (UE), as well as with each of these procedures individually, and some sham operated animals. While most of these animals are still being tested, indications are that about one third of the S-SCN+UE and S-SCN+PX animals show impaired ability to sustain coherent free-running (but not entrained) activity rhythms. We have not yet identified the critical histological characteristics which distinguish these animals from similarly treated animals displaying intact rhythms. However, preliminary evidence suggests that accurate midline placement of the knife cut is at least not sufficient to produce rhythmic disturbances.

In general, the circadian activity rhythm appears to be largely resistant to disruption by surgical manipulations designed to interrupt possible coupling pathways in the underlying oscillator system. These results may imply that multiple and possibly redundant integrative pathways exist. SUPPORTED BY NSF GRANT BNS 82-17281 (NTA & AMR).

89.13 SUPRACHIASMATIC NUCLEI: STATE-DEPENDENT MODULATION OF ACTIVITY. T.S. Kilduff, H.C. Heller* and F.R. Sharp. Dept. Biol. Sci., Stanford Univ., Stanford, CA 94305; Dept. Neurosci., Univ. Calif. School Med., La Jolla, CA 92093.

The suprachiasmatic nucleus (SCN) undergoes a circadian rhythm of glucose metabolism in rats, cats and squirrel monkeys as shown through use of the 2-deoxyglucose (2DG) technique. A brief report suggested the absence of such a rhythm in a hibernating species, the 13-lined ground squirrel. This was puzzling in the light of our observations of relatively high levels of 2DG uptake in the SCN during hibernation in the golden-mantled ground squirrel (GMGS). The current study was undertaken to (1) extend observations of 2DG uptake to entrance and arousal from hibernation and (2) to assess whether there was a circadian rhythm of glucose metabolism in the SCN of the non-hibernating GMGS.

GMGSs were kept on an LD 12:12 photoperiod at 5°C. At least 1 week prior to an experiment, each animal was implanted with a jugular catheter and subcutaneous thermocouple reentrant tube. Experiments were performed in a metabolism chamber in darkness at 5°C. A thermocouple was placed into the reentrant tube, and the jugular catheter was connected to a syringe on the outside of the metabolism and surrounding temperature-controlled chambers. Body temperature (T_b) and metabolism were continuously measured. Injection (150 uCi/kg) and incubation of the [^{14}C]2DG occurred at six points in the circadian cycle during euthermia and during the following phases of the hibernation cycle: entrance (T_b=30-25°C, 25-20°C, and 20-15°C), deep hibernation (T_b=6-7°C) and arousal. The incubation period was 45 min for all euthermic experiments but was adjusted for hibernation experiments to correct for lowered body temperature. The animal was then sacrificed, its brain frozen, sectioned in a cryostat, and autoradiographed.

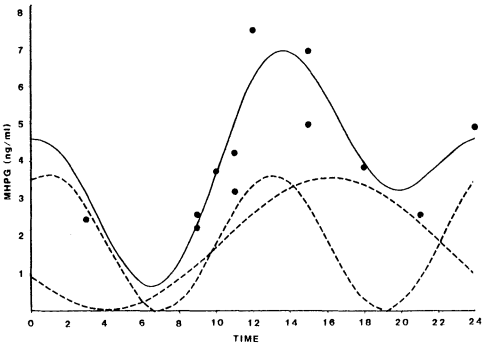
During entrance to hibernation, we observed a progressive increase in the 2DG uptake of the SCN relative to adjacent hypothalamic structures. In the T_b range between 30°-25°C, the SCN was not distinguishable on the autoradiographs from the surrounding hypothalamus. In the range from 25°-20°C, the medial aspect of the SCN was labelled. In the T_b range between 20°-15°C, the entire SCN was labelled, as it was during deep hibernation at T_b=6-7°C. The SCN was not discernible from adjacent nuclei during arousal. We found no evidence of a circadian rhythm of 2DG uptake in the SCN in the non-hibernating state.

These results demonstrate a state-dependent change in the activity of the SCN throughout the hibernation cycle and suggest that the metabolism of the SCN is more temperature-compensated than that of surrounding neural structures, implying an important role for the SCN during hibernation. Relative maintenance of SCN metabolism during hibernation in the absence of a circadian rhythm of 2DG uptake in euthermia may indicate a unique function for the SCN in hibernating species. (Supported by NIH NS10367 to H.C.H.)

89.14 TRICYCLIC ANTIDEPRESSANTS REDUCE ULTRADIAN MHPG RHYTHM IN DEPRESSION. E.M. DeMet and A.E. Halaris. University of California, Irvine, California 92717 and University of California, Los Angeles, California 90024.

Plasma levels of the norepinephrine metabolite 3-methoxy-4-hydroxyphenyl glycol (MHPG) are believed to reflect changes in noradrenergic activity. Previous studies from our laboratory, and others, have shown that circadian MHPG rhythms are phase advanced by 2-3 hrs. in depressed patients vs. normal controls ($p < 0.05$), and that the rhythms tend to normalize after treatment with tricyclic antidepressants (TAD's).

The present study examines the shape of the MHPG rhythm in patients and controls. Normal circadian MHPG rhythms were well fit by a simple COSINOR model, whereas pre-treatment patient rhythms were not. Patient rhythms were well fit by a two cosine model: MHPG = BASELINE + AMPL#1 x COS(PHASE#1 + TIME/24 HRS) + AMPL#2 x COS(PHASE#2 + TIME/12 HRS). A comparison of 10 patients before and after 4 weeks of TAD treatment showed that the amplitude of the 24 hour component was not altered by treatment, and was indistinguishable from a normal MHPG rhythm. The 12 HR. component was significantly reduced ($p < 0.025$) after treatment, which was evident in 7 out of 10 patients. Addition of the two components was found to produce an apparent phase advance.



SUBCORTICAL VISUAL PATHWAYS II

90.1 INTEROCULAR INTERACTIONS IN THE LATERAL GENICULATE NUCLEUS (LGN) OF THE UNANESTHETIZED AND UNPARALYZED CAT. W. L Salinger, W. Guido*, and C. E. Schroeder*. Dept. of Psychology, Univ. North Carolina-Greensboro, NC 27412.

Previous work on visually mediated interocular interactions in the LGN of the cat has involved the use of anesthetized and systemically paralyzed preparations. Recently, we have observed that the use of anethesia may severely attenuate the intensity of interocular interactions in the LGN which are produced by brief periods of monocular paralysis (Schroeder et al.,ARVO 1984). We have continued our analysis of interocular interactions in an unanesthetized, unparalyzed preparation by examining the effects of sectioning the optic nerve in the mobile eye of cats which had undergone a brief period of monocular paralysis.

The optic nerve was exposed and sectioned using a ventral approach which left intact the boney covering of optic chiasm and the orbit. Extracellular single unit recordings were made in the right and left LGN prior to and immediately following unilateral optic nerve section. A standard battery of receptive field tests together with axonal conduction velocity measurements were used to classify LGN cells as X or Y. In the layers which continued to receive direct retinal input from one eye the removal of retinal output from the other eye produced an immediate and marked increase in the encounter rate for X-cells and a related decrease in that for Y-cells. This result was evident in the data obtained from each individual subject. This effect was shown not to arise as an artifactual sequel to the surgical transection of the optic nerve. Rather, it appears that LGN cells are influenced by the removal of retinal input arising from the nondominant eye. Further, it appears that the influence of input arising from the nondominant eye is strong enough and selective enough that its removal actually affects the recordability of X-and Y-cells. The contrast between the strength of interocular interactions observed here, and the more subtle interocular effects which have been observed in the LGN of anesthetized and systemically paralyzed preparations, suggests that anesthetic and/or paralytic agents may alter neural processes in a way which masks the physiology of interocular interactions in the LGN.

90.2 PROPRIOCEPTIVE CONTRIBUTIONS TO VISUALLY MEDIATED INTERCULAR INTERACTIONS IN THE LATERAL GENICULATE NUCLEUS (LGN) OF THE CAT. W. Guido, W. L. Salinger, and C. E. Schroeder. Dept. of Psychology, Univ. North Carolina-Greensboro, NC, 27412.

When subtle interocular interactions in the cat LGN are enhanced by a brief period of monocular paralysis, such interactions become powerful enough to reduce the encounter rate for X-cells and increase that for Y-cells (Garraghty et al., 1982). In order to explore whether proprioceptive signals from extraocular muscles (EOM) are involved in such interactions, these signals were eliminated from the mobile eye of monocularly paralyzed cats by section of the ophthalmic branch of the fifth cranial nerve (V).

Using a ventral approach, V and the semilunar ganglion were exposed and V was sectioned. The orbit and its contents remained intact and shielded by bone. Extracellular single unit recordings were made in both the right and the left LGN prior to and immediately following unilateral section of V. LGN cells were classified as X or Y on the basis of a standard battery of receptive field tests and axonal conduction velocity. In comparison to preoperative measures, the removal of extraocular proprioceptive input from the mobile eye produced an immediate, sustained, and substantial increase in the encounter rate for X-cells and a similar decrease in that for Y-cells. This change was observed in all principal layers of each LGN whether the EOM's of the innervating eye were able to provide proprioceptive input or not. This effect was observed in results from each individual cat. Control experiments ruled out contributions by surgical trauma or residual effects from surgical anesthesia. Thus proprioceptive output from a single orbit influences LGN cells innervated by either eye. Further, it appears that proprioceptive input to the LGN is more pervasive than previously recognized, and is strong enough to modulate the actual recordability of large proportions of LGN cells. Although some investigators have reported proprioceptive responsivness in LGN cells and others have reported visually mediated interocular interactions among LGN cells, this study demonstrates that most LGN cells which are involved in interocular interactions also exhibit a sensitivity to the removal of proprioceptive signals.

90.3 DIFFERENCES IN THE TIMING AND SENSITIVITY OF RESPONSES FROM CELLS ACROSS SINGLE LAYERS OF THE LATERAL GENICULATE NUCLEUS IN THE CAT. Douglas B. Bowling, Departments of Medical Physiology & Anatomy, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

In the lateral geniculate nucleus of the cat the two dorsal cell laminae (A and A_1) are each about half a millimeter thick. Thus at a given retinotopic location there are a number of cells through the depth of a layer whose receptive fields represent roughly the same place in the visual field. The aim of these experiments is to ask what physiological differences, other than scatter in receptive field position, may exist in this vertical distribution of cells. To examine this question I am simultaneously recording from two cells at different depths within the same layer. In anesthetized cats tangential penetrations are made through layers A or A_1 with two, closely spaced microelectrodes. In this way pairs of cells are compared that are separated vertically in the layer by several hundred microns but whose receptive fields have nearly identical positions in the visual field.

Results from these experiments show that the timing and sensitivity of responses at a given retinotopic location varies between cells across the depth of a layer. Two cells representing essentially the same place in the visual field may have differences in treshold sensitivity (threshold luminance) as large as six tenths of a log unit. When such pairs of cells are stimulated separately or simultaneously by spots or bars of light that impinge on the centers of their receptive fields the latencies of the onset of their responses may differ by twenty milliseconds or more. Qualitatively the differences in timing are consistent with the differences in sensitivity i.e., for a given stimulus, the more sensitive cell of a pair has the faster response. Furthermore, collected results from 82 pairs of cells shows a tendency for the faster cells to be located ventrally in the layers. This work is supported by the Alberta Heritage Foundation for Medical Research and by MRC (Canada) Grant MA7612.

90.4 VISUAL MOTION DISPLACEMENT THRESHOLDS OF X, Y, AND W CELLS IN THE LOWER VISUAL PATHWAYS OF THE CAT AT VARIOUS DURATIONS OF MOVEMENT. P.L.E. van Kan* and R.P. Scobey (SPON: A.J. Gabor). Dept. of Neurology, Univ. of Calif. Sch. of Med., Davis, CA 95616.

Single neurons recorded in the lateral geniculate nucleus and optic tract of anaesthetized, paralysed cats were tested for their involvement in the detection of movement by measuring displacement thresholds in response to a narrow line stimulus along a diameter of their receptive field. A displacement threshold was defined as the distance that an initially stationary line must move from a location A to a location B to evoke a criterion response from the cell. On successive trials the A-B distance was adjusted by computer according to a variable staircase paradigm. The average of 8 reversals at the minimum stepsize defined each displacement threshold. For each cell, the receptive field location that was optimal for the detection of small amplitude stimulus movements was identified from a plot of displacement threshold measurements as a function of visual field position. This location formed a plateau region of the displacement curve at which the threshold values reached a minimum and were independent of small positional changes of the stimulus. The center of this plateau region was selected as the site at which the displacement threshold was measured as a function of the duration of stimulus movement. Displacement thresholds were found to be constant for durations between 10 and 640 ms for all cells tested. This result is consistent with the psychophysical finding that a constant displacement is the principle factor underlying motion thresholds at these durations. It does not correspond to a constant velocity prediction for determination of threshold.

90.5 Y-, X- AND W-LIKE CELLS IN THE LATERAL GENICULATE NUCLEUS OF THE OPPOSUM. P. D. Wilson and M. A. Kirby. Psychology Depart., Univ. California, Riverside, CA 92521

Cell activity in the lateral geniculate nucleus (LGN) of the opossum was recorded extracellularly while the animal was anesthetized, paralyzed and mechanically respired. Three classes of cells were evident, and a fourth class perhaps distinguishable, on the basis of several receptive field characteristics and the latencies to electrical stimulation of optic nerve (ON), optic chiasm (OX) and visual cortex (VC). Conduction velocities were computed to the basis of ON - OX latencies.

Type 1 cells gave phasic responses to light-on or light-off, had relatively large receptive field centers (8^{o}-16^{o}), and responded well to rapidly moving stimuli (cutoff 40 to >120 deg/sec). Type 1 cells tested with sinusoidally reversed gratings showed response doubling (Y-like). Type 1 cells had short ON-latencies (3.0-4.4 ms) and OX-latencies (1.2-2.2 ms) and short antidromic latencies to VC stimulation (1.3-3.8 ms). The average afferent conduction velocity was 16.5 m/s.

Type 2 cells gave tonic on- or off-center responses, had small receptive field centers (3^{o}-10^{o}) with responsive antagonistic surrounds, responded to intermediate stimulus velocities (cutoff 20-70 deg/sec), and showed linear spatial summation (X-like). The Type 2 latencies were longer (ON, 4.0-6.2 ms; OX, 1.8-3.1; VC, 2.2-4.0 ms) than Type 1 latencies. Afferent conduction velocities averaged 13.4 m/s. Particularly notable was the high proportion of Type 2 cells (>25%), all with clearly X-like properties, encountered in the opossum LGN.

Type 3 cells were phasic on, off, or on-off type with relatively large receptive field centers (4.5^{o}-21^{o}). The Type 3 cells did not respond well to rapidly moving stimuli (cutoff velocity typically <30 deg/sec). Type 3 latencies were longer than Type 1 but overlapped Type 2 (Type 3, ON, 3.6-16.4 ms; OX, 1.7-3.0 ms; and VC-antidromic, 3.2-6.2 ms). Conduction velocities averaged 8.5 m/s. Type 1 and 3 cells seemed Y- and W- like, but only a few Type 1 and none of the Type 3 had responsive antagonistic surrounds.

A small number of tonic cells may constitute a subset of Type 2 or a fourth class of LGN cells. They have inhibitory, but not antagonistic surrounds, longer afferent latencies (ON, 5.3-11.2 ms), slower mean conduction velocity (10.5 m/s), and in some cases larger receptive fields than the X-like Type 2 cells (8^{o} to 13^{o}). The antidromic VC-latencies (3.5-4.2 ms) did not differ from Type 2 cells, and these cells exhibited linear spatial summation, as did Type 2 cells.

90.6 FUNCTIONAL CELL CLASSES IN THE MACAQUE'S LGN. S.M. Sherman, R.A. Schumer* and J.A. Movshon. Dept. Neurobiol. Behav., SUNY, Stony Brook, NY 11794 and Dept. Psychol., NYU, New York, NY 10003.

We studied the responses of cells in the LGNs of Cynomolgus monkeys to achromatic sinusoidal gratings. We were interested in the functional cell groups that could be identified by an analysis of spatial and temporal tuning properties, contrast sensitivity and spatial summation, and in the relationship of these groups to the conduction velocity of retinal input and the disposition of the cells in the magnocellular (M) and parvocellular (P) divisions of the nucleus.

P cells resolved higher spatial frequencies of drifting gratings than M cells; the difference, however, was much less than between X and Y cells in cats. P cells rarely showed evidence of strong antagonistic surrounds. We studied cells' spatial summation characteristics using stationary contrast-modulated gratings of different spatial frequencies and phases. The degree of nonlinearity was continuously distributed for both P and M cells; only a small minority of M cells showed strongly nonlinear summation. M cells had consistently higher contrast sensitivity (measured by the slope of the initial segment of the contrast-response function) than P cells.

The most striking differences between M and P cells were in the temporal domain. M cells resolved higher temporal frequencies than P cells, and the "steady-state latency" given by the slope of the function relating response phase to temporal frequency was consistently less for M cells than for P cells. As stimulus contrast increased, the phase of M cell responses usually advanced, while that of P cells tended to remain constant; thus only M cells showed a "contrast gain control". M cells received a faster-conducting retinal input that P cells.

Apart from previously-reported differences in the distribution of cone inputs to different P cells, we have no evidence for significant functional subtypes within the magno- and parvocellular divisions of the LGN. We conclude that the macaque's LGN contains only two major functional classes of cells. While there are similarities between these classes and the X and Y classes described in the cat, the qualitative and quantitative differences between LGN cells in cats and monkeys make the precise homologies between these species unclear.

90.7 THE ELECTRICAL PROPERTIES OF A X-CELL IN THE CAT'S LGN: DOES THE SPINE-TRIAD CIRCUIT SUBSERVE VISUAL ATTENTION?
C. Koch. Center for Biological Information Processing, MIT, Cambridge, MA 02139 USA.

Two EM studies (Wilson, Friedlander & Sherman 1984 Proc. Roy.Soc.Lond.B in press and Hamos et al. 1983 Neurosci.Abst. 9,814) of relay cells in the lateral geniculate nucleus have shown that the retinal input of X-cells is associated with a special synaptic circuit, termed the spine-triad complex: the retinal afferents make an asymmetrical synapse (RLP)with both a dendritic appendage of the X-cell and a presumed interneuron. The interneuron contacts in turn the same dendritic appendage with a summterical profile (F2). In Y-cells the retinal input is predominant found on dendritic shafts without any triadic arrangements. This study addresses the functional significance of this synaptic architecture in terms of the differential roles of the X- and Y-system for visual behavior. The basis for our analysis is the solution of the cable equation for an arbitrary branched dendritic tree with a known somatic imput impedance. Retinal and extraretinal inputs are described by conductance changes distributed throughout the dendritic tree according to the known sunaptic architecture. HRP-stained X- and Y-cells of an adult cat were proveded by M. Sherman and S. Bloomfield.

Following our earlier suggestion that the conjunction of an excitation with a shunting inhibition on the same spine could lead to a circuit implementing a very localized veto-like operation (Koch & Poggio 1983 Proc.Roy.Soc.Lond.B 218, 455), we have investigated the function of the spine-triad complex If the interneuron is assumed to mediate a post-synaptic conductance increase with a reversal potential close to the resting potential of the cell, then activation of this synapse can reduce efficiently the excitatory post-synaptic potential induced by the retinal afferent without affecting the electrical activity in the rest of the cell. This is true over a wide range of parameters. Because Y-cells receive almost all of their input directly onto the dendritic stem, reducing the retinal evoked epsp invariably dampens the general activity of the whole neuron.

We propose that the spine-triad circuit is responsible for mediating intrabeniculate inhibition specific to the X-system like lateral inhibition or saccadic suppression. We suggest in particular that the very specific shunting of the retinal afferent could be one operation possibly underlying visual attention, varying for instance the extent of the receptive field or the strength of the synaptic transmission of X-cells.

90.8 A MONOCLONAL ANTIBODY THAT MAY IDENTIFY Y-CELLS IN THE CAT LATERAL GENICULATE NUCLEUS.
M. Sur, S. Hockfield, M. MacAvoy, P. Garraghty, M. Kritzer and R. McKay. Sec. of Neuroanatomy, Yale Medical School, New Haven, CT 06510 and Cold Spring Harbor Lab., Cold Spring Harbor, NY 11724.

A monoclonal antibody (CAT-301) made from homogenized cat spinal cord recognizes a surface antigen on subsets of neurons in many parts of the vertebrate CNS (McKay and Hockfield, PNAS '82; Hockfield and McKay, PNAS '83). One group of neurons identified by CAT-301 lies in the cat lateral geniculate nucleus (LGN). The antibody labels large cells (mean soma size 525 um^2, range 200-1050 um^2) in geniculate laminae A, A1 and C, in interlaminar zones, and in the medial interlaminar nucleus (MIN). Nearly every cell in the LGN over 400 um^2 in size is antibody positive. Labelled cells are concentrated near interlaminar zones in the A- and C-laminae, and include most cells in the MIN. Labelled cells in the A-laminae are significantly larger than a population of A-laminae X-cells intracellularly filled with HRP (Friedlander et al., J. Neurophysiol. '81; $p<.001$, Mann-Whitney U-test) but are comparable in size to Y-cells in laminae A and A1. Antibody labelled cells in the C-laminae are significantly larger than W-cells in the C-laminae (Stanford et al., J. Neurophysiol. '83; $p<.001$) and are similar in size to C-laminae Y-cells. While fine morphological detail of antibody labelled neurons is lacking, the orientation of proximal dendrites on many LGN neurons is typical of cells with class I morphology, a distinguishing feature of LGN Y-cells.

Double labelling experiments involving retrograde transport of HRP from area 18 (a cortical target of A-laminae Y-cells and C-laminae Y- and W-cells) to the LGN and antibody labelling indicate that many neurons in the A-laminae that project to area 18 are antibody positive. In the C-laminae, large cells filled retrogradely with HRP are antibody positive while small HRP-filled cells are not.

These results indicate that a functional and morphological class of cells in the cat LGN, Y-cells, can be distinguished by molecular characteristics as well. We are continuing experiments to further define the anatomical and functional specificity of cells that express the antigen recognized by CAT-301.

Supported by NIH Grants EY05241 (M.S.), NS18040 (S.H.) and NS17556 (R.M.).

90.9 FINE STRUCTURAL MORPHOLOGY OF A PHYSIOLOGICALLY IDENTIFIED W-CELL IN THE CAT'S LATERAL GENICULATE NUCLEUS.
D. Raczkowski, J.E. Hamos and S.M. Sherman, Department of Neurobiology and Behavior, SUNY at Stony Brook, NY 11794.

In an effort to further understand the functional significance of the parallel pathways in the cat's geniculocortical system, we have examined the pattern of synaptic input onto a physiologically identified W-cell in the lateral geniculate nucleus. After recording and identifying the neuron, it was impaled with our recording micropipette, and horseradish peroxidase was injected into it. This permitted subsequent morphological analysis of its soma, dendrites and axon at the light and electron microscopic levels. This neuron was located in lamina C1 and had class 4 morphological features, possessing an intermediate sized soma and many thin, smooth, straight dendrites. The long axis of the dendritic arbor was oriented parallel to the geniculate layer of origin. This neuron had an axon that projected into the optic radiations.

Representative labeled portions of the neuron were examined with the electron microscope to obtain an estimate of the type and distribution of synapses contacting the cell. Virtually the entire soma and about 20% of each of the proximal (within 100μm) of the soma), intermediate (100-200μm), and distal (beyond 200μm) dendritic arbors were surveyed. From this analysis, several conclusions were drawn. First, this W-cell received relatively few retinal inputs. We estimated that only 60 retinal terminals (RLP) contacted this cell. This represents about 1-2% of all synapses innervating this neuron. (In contrast, X- and Y-cells receive about 300-600 RLP synapses, representing approximately 10-15% of the total number of synaptic contacts.) All retinal inputs on this W-cell were located on smooth portions of proximal and intermediate dendrites. Only one retinal terminal formed a triadic arrangement with an F terminal. Second, presumed cortical terminals (RSD) formed the predominant innervation on this W-cell, representing about 84% of the total synaptic contacts. These RSD inputs were uniformly distributed along the entire dendritic arbor. Third, the distribution of synaptic input on the dendritic arbor of this W-cell was discontinuous. Indeed, several long dendritic segments, 5-10μm in length, were devoid of any synaptic input.

The generality of these conclusions depends on similar analysis of other W-cells, and a second such neuron is presently being analyzed. These observations indicate differences in synaptic inputs among W-, X-, and Y-cells. The paucity of retinal inputs to this W-cell might account for its poor visual responsiveness. (Supported by USPHS Grant EY03038)

90.10 W-LIKE RECEPTIVE-FIELD PROPERTIES OF INTERLAMINAR CELLS IN PRIMATE LATERAL GENICULATE NUCLEUS.
G.E. Irvin*, T.T. Norton, M.A. Sesma and V.A. Casagrande. Dept. of Physiological Optics, Univ. of Ala. in Birmingham, Birmingham, AL 35294 and Depts. of Anatomy and Psychology, Vanderbilt Univ., Nashville, TN 37232.

In the prosimian primate, galago, the small-celled (koniocellular) layers of the LGN contain cells with W-like physiological properties (Norton and Casagrande, '82). The remaining parvo- and magnocellular layers contain X-like and Y-like cells, respectively. In terms of morphology and connections, the koniocellular cells resemble the cells that lie in the interlaminar zones (ILZs) of the galago and other primate species. It therefore becomes important to determine whether the ILZ cells have W-like properties as would be predicted from their anatomical features.

To date, we have compared the physiological properties of 16 histologically verified ILZ cells with the properties of 16 W-like cells that were located in the koniocellular laminae using a battery of tests employed previously. Overall, the results showed that ILZ and W-like cells were very similar and distinct from X-like and Y-like cells. As was the case with W-like cells, ILZ cells sometimes had ON-OFF centers, large receptive-field centers (~2°) and long latencies to optic chiasm (OX) and striate cortex (VC) stimulation (4 - 6 ms). Interestingly, we could demonstrate antidromic activation from VC in fewer ILZ cells than W-like cells, suggesting that some ILZ cells might be interneurons. ILZ and W-like cells were often sluggish or hard to drive using spots of light or sine-wave gratings. Also, ILZ cells were W-like, and unlike X- and Y-like cells, in their response latency to visual stimuli (86 ms onset, 113 ms peak). There was, however, a suggestion that ILZ cells within different parts of the nucleus might be distinct from one another. For instance, ILZ cells adjacent to the magnocellular layers exhibited shorter latency to OX stimulation.

Taken together, these data show that ILZ cells in galago exhibit W-like response properties. Thus, in simian primates that lack specialized layers of W-like cells, the ILZ cells may constitute the relay pathway of W-like information from retina to cortex.

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90.11 IMMUNOCYTOCHEMICAL LOCALIZATION OF SEROTONERGIC FIBERS IN THE LATERAL GENICULATE COMPLEX OF THE CAT. R.R. Mize, M.P. Payne*, L.E. Faulkner*, and L.H. Horner*, Department of Anatomy, Division of Neuroscience, University of Tennessee Center for the Health Sciences, Memphis, TN 38163.

We have studied the serotonin (5-HT) innervation of 4 subdivisions of the cat lateral geniculate complex: the magnocellular (A, A_1, C) and parvocellular (C_1, C_2) laminae of the dorsal lateral geniculate nucleus (DLGN), the medial interlaminar nucleus (MIN), and the ventral lateral geniculate nucleus (VLGN). Vibratome sections were cut through the thalamus of 12 cats, incubated in varying dilutions of 5-HT antibody (Immuno-Nuclear), and reacted using the avidin-biotin technique. Serotonin immunoreactivity was found throughout the geniculate complex. On gross inspection, it was most dense in VLGN, moderately dense in MIN and the parvocellular C laminae of DLGN, and least dense in the magnocellular laminae of DLGN. Some of the labeling was due to diffuse staining of the tissue. Densely filled fibers were also visible. The stained fibers varied in diameter and had prominent varicosities at irregular intervals along the fiber.

To determine whether the different staining densities in different regions were due to diffuse staining or fiber innervation density, we examined fiber density quantitatively. To quantify the density of fibers, we measured the total length of all fibers within selected fields in each of the 4 geniculate regions using a computer digitizer. Our results illustrate that some of the difference in staining intensity is due to the number of fibers innervating the region. Mean fiber density (length per unit area) was highest in the VLGN (.058 per um^2), moderate in MIN and the parvocellular C laminae (.044 per um^2), and lowest in the magnocellular laminae of DLGN (.026 per um^2). In MIN, fiber innervation was particularly dense along the medial edge of the nucleus, a region called the geniculate wing.

We conclude that the innervation density of serotonin fibers varies with the functional subdivisions of the lateral geniculate nucleus. Coupled with other results demonstrating that the serotonin innervation is densest in the superficial gray layer of the cat superior colliculus, we suggest that visual system structures receiving input from W type retinal ganglion cells receive a denser serotonergic input than do other visual system structures.

(Supported by USPHS Grant EY02973).

90.12 CONTRIBUTIONS OF Y- AND W-CELL PATHWAYS TO RESPONSE PROPERTIES OF SUPERIOR COLLICULUS NEURONS: COMPARISON OF ANTIBODY- AND DEPRIVATION-INDUCED ALTERATIONS. John W. Crabtree*, Peter D. Spear, Maureen A. McCall, Kim R. Jones, and Steven E. Kornguth. Depts. of Psychology and Neurology, Univ. of Wisconsin, Madison, WI 53706

The superior colliculus (SC) of the cat receives input from Y- and W-cell pathways but little or no input from the X-cell pathway. The W input arises directly from retinal W-cells (W-D input), while the Y input comes either directly from retinal Y-cells (Y-D input) or indirectly via a geniculocortical loop (Y-I input). In high doses, antibodies to large ganglion cells virtually eliminate retinal Y-cells and have little or no effect on retinal W-cells (Crabtree et al., 1983). We used such antibodies to study the contribution of the Y inputs to response properties of SC neurons. These effects were compared to those of binocular deprivation (BD), which have been attributed to a loss of Y-I input.

Ten adult cats received binocular intravitreal injections of antibodies; 4 received low concentrations and 6 received high concentrations of the antibodies (330 or 1000 µg/0.1 cc injection). These animals were studied 1-21 months later. Five cats were binocularly deprived by eyelid suture for 21-35 months. Seven normal cats also were studied. SC cells were classified as receiving Y-D, Y-I, or W-D inputs on the basis of response latencies to electrical stimulation of the optic chiasm and optic tract. In addition, the cells' responses to visual stimuli were examined, including ocular dominance, preference for moving or stationary stimuli, velocity sensitivity, and direction sensitivity.

Initial results indicate the following. At low doses, the antibodies have no effect on inputs to the SC and little or no effect on responses to visual stimuli. At high doses, the antibodies produce a 75% reduction in both Y-D and Y-I inputs. In addition, there is a 55% reduction in direction selective cells, a 40% reduction in cells that respond to high velocity stimuli, and a 17% reduction in cells that respond to the ipsilateral eye. BD produces a 50% reduction in the Y-I input, an 80% reduction in direction selective cells, and a 25% reduction in cells that respond to the ipsilateral eye.

Together, these results suggest that Y-D input provides SC cells with high velocity information, Y-I input provides information for direction selectivity and response to the ipsilateral eye, and W-D input provides low velocity information and response to the contralateral eye. W-D input also may contribute to direction selectivity and response to the ipsilateral eye. (Supported by EY01916 and EY02545)

90.13 TEMPORAL DETERMINANTS OF MULTIMODAL INTERACTIONS IN SUPERIOR COLLICULUS CELLS J.W. Nemitz, M.A. Meredith and B.E. Stein, Depts. Physiol. and Anat., Med.Col.Va., Richmond, VA 23298.

Visual, auditory and somatosensory inputs are known to converge on deep laminae superior colliculus (SC) cells and responses to simultaneously presented multisensory stimuli interact to enhance the activity of some SC cells and to depress others. Yet under natural conditions sensory stimuli occur in various temporal relationships. Thus, in the present experiments we examined the effects of such 'staggered' inputs and found that by varying the timing between two different sensory stimuli we could always vary the magnitude of the activity they evoked. In some cells we could produce both response enhancement and response depression by staggering sensory inputs appropriately.

Experiments were conducted on cats chronically prepared (anesthetized, paralyzed, and respired on 75% N_2O/ 25% O_2) for extracellular recording and 47 multimodal cells were tested in the following manner: 1). separate-modality stimuli (e.g. visual only, auditory only) were repeatedly (n=16) presented to establish response latency, duration, mean impulse frequency and number, and 2). combined-modality (e.g. visual and auditory) stimuli were repeatedly (n=16) presented (always in spatial register) simultaneously and at predetermined (10-500 ms) temporal intervals. The results of the simultaneous and staggered combined-modality tests were compared to those obtained from the separate-modality tests to establish the magnitude of response interaction.

Response interactions were elicited by combined-modality stimuli presented simultaneously as well as by stimuli presented up to 500 ms apart. While cues presented in close temporal sequence (0-100 ms) consistently evoked the largest degrees of response interaction (either enhancement or depression), the magnitude of that response interaction decreased as the temporal interval (100-500 ms) between the stimuli increased. However, in 11/47 cells, as the interstimulus interval was increased, the degree of enhancement not only decreased but was replaced by significant ($p < 0.05$) levels of response depression.

These data coupled with those regarding the influence of spatial register indicated that the spatial and temporal coincidence of two different sensory stimuli are critical factors in determining the enhanced or depressed activity of deep laminae SC cells. Presumably, the nature and magnitude of these response interactions are directly linked to the likelihood of SC-mediated orientation responses.

Supported by Grant EY 04119.

90.14 MULTIMODAL ENHANCEMENT AND DEPRESSION IN SUPERIOR COLLICULUS CELLS IS DETERMINED BY STIMULUS LOCATION M.A. Meredith and B.E. Stein, Depts. Anat.&Physiol. Med.Col.Va.,Richmond, VA.

Visual, auditory and somatosensory inputs converge on deep laminae superior colliculus (SC) cells and combined-modality stimulation (e.g.simultaneous visual and auditory cues) elicits response interactions that can enhance or depress the activity of a given SC cell. The present study shows that the relative positions of these stimuli in space is a critical factor in determining the nature of these interactions.

Forty-two multimodal neurons were identified in 9 cats (prepared chronically, anesthetized and respired on 75/25% N_2O/O_2) and their receptive fields (RF) were mapped for each effective modality. The multimodal RFs of individual SC cells generally overlapped one another, although their outer borders were rarely in exact correspondence. The functional significance of the topographical register of multiple sensory RFs was examined in 28 of these cells by varying the spatial register as follows: 1) two stimuli (e.g. visual and auditory) were presented at the same location in space and within the region of their RF overlap, 2) within their respective RFs but separated in space, and 3) one stimulus (e.g. visual) in its RF and one (e.g. auditory) beyond the excitatory borders of its RF. The results of these combined-modality tests were compared with those from appropriate separate-modality tests.

In the majority of cells (18/28) the greatest degree of response enhancement was elicited when stimuli were presented in spatial register and within the area of RF overlap. The degree of enhancement resulting from multimodal integration decreased as the stimuli were positioned progressively out of spatial register with one another and response enhancement was ultimately replaced by response depression when one stimulus fell outside its RF. Exceptions to these patterns were present in 4 cells where the RFs of the two modalities showed only a minimum area of overlap. In these cases, the greatest response enhancement was elicited with spatially separate stimuli placed in the most effective (greatest discharge frequency) regions of their respective RFs.

These data demonstrate that a given SC cell can integrate multiple sensory cues to exhibit either response enhancement or response depression, depending upon the relative positions of the sensory stimuli involved. Thus, in more 'natural' circumstances, if sensory cues change their spatial relationship, the evoked activity of deep laminae SC cells can vary from vigorous excitation to profound depression.

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91.1 CHANGES IN SYSTEMIC BLOOD PRESSURE ALTER NOREPINEPHRINE RELEASE AND SEROTONIN TURNOVER IN NUCLEUS TRACTUS SOLITARIUS AS MEASURED BY IN VIVO ELECTROCHEMISTRY. D. Bhaskaran* and C.R. Freed, Depts. of Medicine and Pharmacology, Univ. of Colo. Sch. Med., Denver, CO 80262.

Serotonin (5-HT) and norepinephrine (NE) are known to play a role in blood pressure (BP) regulation and the two transmitters have been shown to interact in brain stem nuclei. Previous work in our laboratory has demonstrated changes in NE release and 5-HT turnover in dorsal raphe nucleus following vasopressor and vasodilator-induced changes in systemic blood pressure (Echizen and Freed, Life Sci. 34: 1581-1587, 1984). We have extended these studies to the nucleus tractus solitarius. Urethane anesthetized male Sprague-Dawley rats had femoral arterial and venous catheters implanted and then had a carbon paste in vivo electrochemical electrode placed in nucleus tractus solitarius. Signals were measured by linear sweep voltammetry at a rate of 10 mv/sec every 5 min using a DCV-5 cyclic voltammetry amplifier with semiderivative signal processing. Phenylephrine was infused intravenously to produce a 50 mm/Hg increase in BP. In other experiments nitroprusside was infused to lower BP 20 mm/Hg. Results showed that during phenylephrine induced hypertension, the NE peak declined (25 ± 10%). When the infusion was stopped, there was an increase in the NE signal (15 ± 5%); however, the signal remained low compared to the baseline values. By contrast, the 5-HIAA peak showed a significant increase soon after the infusion was started and remained elevated (25 ± 5%) even after the infusion was discontinued. In animals made hypotensive by nitroprusside, the NE peak also declined (20 ± 5%) during the hypotensive phase. When the infusion was stopped, animals had a rebound increase in BP and during this time the NE signal fell even lower than during the drug infusion (10 ± 3%). 5-HIAA showed no change during the infusion but increased when the infusion was stopped in parallel with the rebound increase in BP (40 ± 8%). The control group maintained under similar conditions with saline infusion failed to show any change in NE and 5-HIAA levels over the same experimental time course. These results show that drug induced hypertension and hypotension lead to changes in NE and 5-HT metabolism. Increases in 5-HIAA occur only when BP increases. 5-HIAA rises both in vasopressor induced hypertension and in reflex hypertension. Reductions in NE release are more complex and occur both during elevations and reductions in BP.

91.2 BIOCHEMICAL AND IMMUNOCYTOCHEMICAL EVIDENCE FOR INTRINSIC GABA NEURONS IN THE C1 AREA OF THE ROSTRAL VENTROLATERAL MEDULLA OF THE RAT. M.P. Meeley, D.A. Ruggiero, T. Ishitsuka*, M. Anwar* and D.J. Reis, Lab. of Neurobiol., Cornell Univ. Med. Coll. NY, NY 10021

The C1 adrenaline area of the rostral ventrolateral medulla (RVL): (1) contains neurons which excite sympathetic fibers and mediate vasodepressor responses to baroreceptor stimulation (Ross et al., J. Neurosci. 4:474-494, 1984); and (2) is innervated by projections, mostly unilateral, from the nucleus tractii solitarii (NTS) (Ruggiero et al., Neurosci. Abstr. 209.10, 1982). The facts that locally applied GABA in the C1 area lowers, while bicuculline elevates, arterial pressure (AP), and that endogenous GABA is released in a Ca^{2+}-dependent manner (Meeley et al., Neurosci. Abstr. 77.10, 1983), suggest that GABA in the RVL inhibits tonic sympathetic discharge. We sought to determine whether the GABA in C1 is from the NTS-C1 pathway or from local GABAergic neurons.

For cytochemistry, rats were treated with intraventric. colchicine (COL) (150 ug/10 ul) and, after 24 hr, perfused, medulla sectioned (40 um) and stained using GAD antibody, 1:1000 (provided by Dr. W.H. Oertel et al.). Following COL treatment, GAD-containing cell bodies were identified in NTS and RVL. GABA neurons were in n. parasolitarius and regions primarily lateral or dorsomedial to the caudal portions of NTS which project to RVL. In the C1 area, numerous GAD-stained perikarya were localized ventral, ventromedial or rostral to PNMT-containing neurons of the C1 group.

The effect of NTS lesions on biochemical markers for GABA in C1 area of RVL was examined. A unilateral electrolytic lesion (1 mA anodal DC, 15 sec) was placed in NTS, 0.5 mm rostral to obex; controls were unoperated. After 6-7 days, brains were removed, sliced (1 mm) and fast-frozen. Bilateral micropunches (1 mm) were taken from C1 area of RVL, other medullary areas, and from NTS in control animals. Tissue was sonicated and assayed by HPLC for endogenous amino acid content, or for GAD activity. Unilateral lesion of NTS did not modify the content of GABA (contra, 40.3 ± 2.7; ipsi, 40.2 ± 2.3 nmol/mg protein; n=15) or 18 other amino acids, nor did it affect GAD activity (contra, 38.9 ± 3.9; ipsi, 38.6 ± 5.0; unoperated controls, 38.2 ± 3.8 nmol/mg protein/60 min; n=6) in C1 area of RVL.

We conclude: (a) GAD-containing neurons are present in both NTS and the C1 area of RVL; (b) the projection from NTS to RVL is not GABAergic; (c) intrinsic GABAergic cells in RVL may provide tonic inhibition of vasomotor, possibly C1 adrenaline, neurons to regulate AP.

91.3 THE ROSTRAL VENTROLATERAL MEDULLA: IMMUNOCYTOCHEMISTRY OF INTRINSIC NEURONS AND AFFERENT CONNECTIONS. D.A. Ruggiero, C.A. Ross, M. Anwar* and D.J. Reis. Lab of Neurobio., Cornell Univ. Med. Coll., NY,NY 10021

The rostral ventrolateral medulla (RVL), an area containing C1 adrenaline neurons, is innervated by the cardiovascular nucleus of the solitary tract (NTS), projects to autonomic spinal neurons, is a component of the baroreceptor reflex and tonically controls arterial blood pressure (Granata et al., Hypertension 5:V80-84, 1984; Ross et al., J. Neurosci. 4:474-494, 1984).

In this study, we sought to: (a) localize several neurotransmitters of intrinsic perikarya within RVL; and (b) map the central distribution of afferents to the RVL. For immunocytochemical studies, adult Sprague-Dawley rats injected with colchicine (150ug/10ul) were formalin (4%) perfused after 24 hrs. Alternate sections were stained by the PAP-technique for phenylethanolamine-N-methyltransferase (PNMT - the enzyme synthesizing adrenaline), glutamic acid decarboxylase (GAD; courtesy of Drs. Oertel, Schmechel; Tappaz and Kopin), substance P (SP), 5 hydroxytryptamine (5HT), leu-enkephalin (Enk), ACTH and neurotensin (NT). Afferents to RVL were traced by microinjection of 5-15 nl WGA-HRP (1-2%) into RVL or into structures shown to project into RVL. Tissues were processed with the Mesulam procedure. RVL CELLS: Neurons in RVL lying between the rostral inferior olive and trigeminal complex express PNMT, GAD, Enk and SP. The majority stain for PNMT and are organized into 2 principal groups: (a) a medial cell sheet and (b) a lateral and caudal cell column. Enk neurons were abundant and intermixed with both these PNMT populations. Most GAD, SP and 5HT cells were ventral, medial and rostromedial to the PNMT group although some overlap was observed especially at the level of retrofacial nucleus. Few or no cells stained for NT or ACTH. RVL AFFERENTS: Projections to RVL, some reciprocal derive from: (a) a cell column in caudal ventrolateral medulla overlapping the A1 norepinephrine area; (b) nucleus retroambiguus; (c) nucleus of Koelliker-Fuse; (d) a dorsal superficial lamina of the lateral parabrachial nucleus; (e) central grey; (f) posterior-lateral and dorsal parvocellular divisions of paraventricular nucleus; (g) caudolateral nucleus of stria terminalis.

We conclude: (a) several populations of cells synthesizing adrenaline, GABA, Enk and SP may contribute to cardiovascular functions attributed to RVL; (b) afferents to RVL, an autonomic nucleus, derive from a restricted and highly specific set of central autonomic substructures. (Supported by NIH Grant HL18974.)

91.4 DYNAMIC REGULATION OF ANGIOTENSINOGEN AND ANGIOTENSIN II (ANG II) IN CEREBROSPINAL FLUID (CSF). KB Brosnihan, J Pierzga*, A Husain*, M Schiavone*, FM Bumpus* and CM Ferrario. Cleveland Clinic, Cleveland, OH 44106.

Several studies have demonstrated that the levels of the brain neuropeptide Ang II and its precursors, renin substrate (RS) and angiotensin I (Ang I), are altered in experimental models of hypertension. Basic knowledge of the factors controlling the release of these components from the brain and their presence in CSF is lacking. Two stimuli known to release other neuropeptides by different mechanisms were employed to study the dynamic regulation of brain renin angiotensin components during constant, non-recirculating perfusion of the dog brain ventricular system. Melittin has been shown previously to augment the release of bradykinin into CSF and prolactin and ACTH from pituitary by activating membrane-bound enzymes, while excess K^+ is known to evoke release of peptides via cell membrane depolarization.

Perfusion from the lateral ventricle to the cisterna magna with artificial CSF (194 µl/min) with or without either melittin (20 µM) or excess K^+ (65 mM) was carried out in dogs anesthetized with morphine (2 mg/kg) and pentobarbital (30 mg/kg b.w.). The protocol consisted of three control, four experimental, and four recovery periods, each lasting for 15 min. Both RS and Ang II were constantly released into the CSF perfusate since their concentrations were relatively stable during the 45 min control period (39.2 ± 17.0 ng Ang I/ml and 9.8 ± 3.8 pg/ml, respectively); Ang I was not found in the CSF perfusate. Within 30 minutes after the addition of melittin into the CSF perfusate (n = 7), there was a significant increase in CSF Ang II (51.3 ± 16.4 pg/ml) with no change in CSF RS. This effect was associated with sustained hypertension (114 ± 3 vs 137 ± 4 mmHg) and tachycardia (70 ± 10 vs 148 ± 16 b/min). On the other hand, the introduction of excess K^+ into the perfusate (n = 6) did not affect CSF Ang II even though the increases in MAP and HR were comparable to those seen with melittin. Neither of these treatments altered the concentration of RS, Ang I and Ang II in the plasma.

These studies showed that the constant levels of RS and possibly Ang II during the perfusion are consistent with a sustained brain contribution to these components in CSF. The stimulatory effects of melittin upon CSF Ang II but not upon CSF RS or Ang I would suggest the possibility of separate localization of these components in the brain, at sites not accessible to K^+-evoked depolarization. Lastly, the actions of melittin upon the dynamic regulation of RS and Ang II cannot be explained by either hemodynamic effects or plasma contamination. (Supported by NHLBI grant, HL-6835).

91.5 CARDIOVASCULAR AND DRINKING RESPONSE INTEGRATION: DISRUPTION FOLLOWING PHARMACOLOGICAL MANIPULATIONS OF THE VENTRAL TEGMENTUM AND NUCLEUS ACCUMBENS. D. L. Jones. Departments of Physiology and Medicine, Faculty of Medicine, University of Western Ontario, London, Ontario, Canada N6A 5C1.

I have previously found that a neural circuit involving dopaminergic projections from the ventral tegmentum to the nucleus accumbens, which influence a GABAergic output and in turn receives a GABAergic input, contributes to the initiation of drinking elicited by central injections of angiotensin II. Although central administration of angiotensin II also elicits pressor responses, it is unknown if these same projections influence the elicited pressor responses. The present experiments investigated the effects of pretreating the ventral tegmentum with GABA and the nucleus accumbens with spiperone on cardiovascular and drinking responses elicited by central injections of angiotensin II. Male Wistar rats were prepared with four bilateral guide cannulae implanted above the lateral ventricles and either the ventral tegmental area or the nucleus accumbens. The descending aorta was cannulated via the femoral artery. Drinking and cardiovascular responses were elicited by injecting 25 ng of angiotensin II into a lateral ventricle. Pronounced drinking ensued averaging 5 ml in 15 min with latencies of less than 3 min. Pressor response peaks averaged 25% (20 mmHg) in 5 to 10 min. Injections of spiperone into the nucleus accumbens attenuated the water intake responses to angiotensin II but did not alter the cardiovascular response. However, injections of GABA into the ventral tegmentum blocked drinking in most sites and blocked or attenuated pressor responses in approximately half the sites. These findings suggest that dopaminergic projections to the nucleus accumbens primarily influence mechanisms responsible for the skeletomotor components of drinking induced by central angiotensin II. On the other hand the ventral tegmentum is involved in both the cardiovascular and drinking responses elicited by central angiotensin II administration. Further there may be some topographical organization within the ventral tegmentum which selectively influences the skeletomotor component alone or the integration of behaviour and cardiovascular responses.

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D. L. Jones is a London Life Research Scholar.

91.6 CARDIOVASCULAR EFFECTS OF VARIOUS AGONISTS INJECTED INTO THE DORSAL RAPHE NUCLEUS OF SPONTANEOUSLY HYPERTENSIVE (SHR) AND WISTAR-KYOTO (WKY) RATS. S.E. Robinson and W. Davidson, III*
Dept. of Pharmacology&Toxicology, Medical College of Virginia Richmond, VA 23298.

Electrical stimulation of the dorsal raphe nucleus (DRN) elevates blood pressure (BP) in the anesthetized rat, with SHR being more sensitive than WKY to electrical stimulation of this area (Wolf et al, Brain Res. 208:192, 1981). Noradrenergic and GABAergic neurons innervate this area. Injection of norepinephrine or phenylephrine(PE) and injection of muscimol (M) into the DRN of Sprague-Dawley rats increases and decreases, respectively, both BP and heart rate (HR) (Robinson, Brain Res. 295:249, 1984; Robinson, et al, The Pharmacologist 25:146, 1983). We now have compared cardiovascular actions of DRN injections of PE, clonidine (CL), and M in 13-week old SHR and WKY. Drugs were injected in 0.5 µl of artificial CSF into the DRN, using a 28-gauge internal cannula passed through a stereotaxically-implanted guide cannula. BP was measured by femoral artery catheters in urethane-anesthetized rats.

Injection of PE (15nmol) elevates BP by similar amounts in WKY and SHR (+12.8±3.5/+18.2±3.4, n=6, and +15.2±1.3/+18.6±2.9, n=7, respectively). However, SHR respond with a significantly smaller increase in HR (+39±6) than WKY (+88±10). The smaller increase in HR in SHR may result from the fact that resting HR in SHR is significantly higher than in WKY. Intra-DRN injection of CL (10nmol) appears to reduce BP and HR equally in urethane-anesthetized SHR and WKY. However, similar treatment in unanesthetized, freely-moving rats results in a significantly larger decrease in BP in SHR than in WKY. The decrease in HR in SHR is also larger than in WKY, but does not reach statistical significance. Intra-DRN injection of M (2nmol) causes similar reductions in BP and HR in SHR and WKY (-13.2±5.1/-11.8±4.8 and -29±10 bpm, n=5, vs. -12.6±1.3/-8.6±1.5 and -55±19 bpm, n=5). In both cases, methylatropine (1 mg/kg,i.v.) reduces the decrease in HR without significantly affecting the depressor response. WKY, but not SHR, exhibit tachycardia after the injection of methylatropine alone.

Thus, anesthetized SHR and WKY exhibit similar BP responses to DRN injection of PE, CL, and M and similar changes in HR in response to CL and M. The lack of tachycardia after methylatropine and the smaller increase in HR after PE in SHR may reflect an excessive stimulation of HR by the sympathetic nervous system. Finally, anesthetized and unanesthetized rats respond differently to certain treatments. (Supported by the American Heart Association, Virginia Affiliate, Inc.)

91.7 EVIDENCE FOR A SEROTONERGICALLY-MEDIATED SYMPATHOEXCITATORY RESPONSE TO MEDULLARY RAPHE STIMULATION. R.B. McCall. Cardiovascular Diseases Research, The Upjohn Co., Kalamazoo, MI 49001.

A previous study in this laboratory (R.B. McCall, Brain Res., 289:121, 1983) indicates that microiontophoretically applied serotonin (5-HT) excites sympathetic preganglionic neurons (SPNs) and that this effect can be blocked by the 5-HT antagonists methysergide (UML) and metergoline (MET). In addition, UML and MET decreased the spontaneous firing of SPNs in control but not in spinal transected animals. These data suggest that raphe-spinal 5-HT pathways to SPNs subserve a sympathoexcitatory function. In contrast, electrical stimulation of areas of the medulla and spinal cord containing descending 5-HT pathways typically results in an inhibition of SPN firing. The present study was designed to resolve these apparent contradictory findings. I found that high frequency electrical stimulation of nucleus (n.) raphe (r.) pallidus, n.r. obscurus and n.r. magnus (i.e. the nuclei containing 5-HT cell bodies which innervate SPNs) produced both pressor and depressor responses. Depressor responses were predominate in the sampling, but a clear distinction between pressor and depressor zones could not be identified. Single shock stimulation of pressor sites produced an excitatory evoked potential of sympathetic nervous discharge (SND) recorded from the inferior cardiac nerve. Conversely, single shock stimulation of vasodepressor sites resulted in a computer-summed inhibition of SND. The mean conduction velocity in the sympathoexcitatory raphe-spinal pathway to SPNs was 1.24 M/S. The 5-HT antagonists UML (0.2-0.8 mg/kg, i.v.) and MET (0.05-0.2 mg/kg, i.v.) blocked the excitation of sympathetic activity evoked from medullary raphe nuclei. In contrast, these agents failed to alter the sympathoexcitatory response to electrical stimulation of lateral medulla pressor sites or the sympathoinhibitory response elicited by raphe depressor site stimulation. The 5-HT uptake inhibitor chlorimipramine (0.3-1.0 mg/kg, i.v.) increased the duration of the sympathoexcitatory responses evoked from the raphe but not from the lateral medulla. Finally, midcollicular transection did not effect the excitation of SND elicited by stimulation of medullary raphe nuclei. Taken together with the iontophoretic data, this study indicates that 5-HT neurons arising in the midline medullary raphe nuclei provide a tonic excitatory input to sympathetic neurons in the spinal cord.

91.8 NEUROTENSIN- AND ENKEPHALIN-IMMUNOREACTIVE NEURONS IN AUTONOMIC AREAS OF THORACO-LUMBAR CORD IN THE CAT. T.L. Krukoff, J. Ciriello and F.R. Calaresu. Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

The presence of neurotensin (NT) and enkephalin (ENK) immunoreactivity (ir) in sympathetic ganglia, preganglionic nerves, and adrenal medulla (Schultzberg, M. et al., Neurosci. 4 (1979) 249; Lundberg, J.M. et al., Acta Physiol. Scand. 114 (1982) 153) suggests that NT and ENK may be present in sympathetic preganglionic neurons of the thoracolumbar cord. Experiments were done in cats treated with colchicine to determine whether neurons in the intermediate gray region of the thoraco-lumbar cord were immunoreactive to NT and ENK. Colchicine (450 µg in 90 µl) was applied via an intrathecal polyethylene cannula to the spinal cord of cats anesthetized with ketamine (35 mg/kg i.p.). After a 24-48 hr survival period, cats were perfused with Zamboni's fixative and frozen transverse and longitudinal sections (50 µm) of different segments of spinal cord (C8 to L3) were cut. Series of sections were placed into antisera for each of NT, leucine-ENK, and methionine-ENK for 16-18 hr at 4°C. The biotin-avidin immunohistochemical procedure using a Vecta Stain Kit (Vector Labs, Burlingame, CA) was used to demonstrate NT-ir and ENK-ir.

NT-ir was found in neurons of the intermediolateral nucleus pars principalis (IML-p) at all levels studied, in the IML pars funicularis (IML-f) between T6 and L3, in the nucleus intercalatus (IC) at all levels except T7 and T8, and in small numbers in the central autonomic area (CA) between T1 and T3. Neurons with leu-ENK-ir were fewer in number than NT-ir neurons and were found in the IML-p and CA at all levels and in the IC between C8 and T5 and between T11 and L2. Neurons with met-ENK-ir had the same distribution as those with leu-ENK-ir.

The location, size, and shape of these NT-ir and ENK-ir neurons in the intermediate gray region of the thoracolumbar cord suggest that they are likely to be sympathetic preganglionic neurons. In addition, the uneven distribution of neurons immunoreactive to different peptides in the autonomic centres of the cord suggests that these peptides may be involved in peptide-specific pathways to different visceral organs.

(Supported by the Medical Research Council of Canada and the Ontario Heart Foundation).

91.9 VASOPRESSIN AND OPIOID PEPTIDE RELATIONSHIPS IN DISCRETE CARDIOVASCULAR NUCLEI OF RATS EXPOSED TO HYPOVOLEMIC HYPOTENSION. G. Feuerstein, C. J. Molineaux*¶, J. G. Rosenberger*¶, R. L. Zerbe#, B. M. Cox¶ and A. I. Faden. Neurobiology Research Unit and ¶Dept. of Pharmacology, USUHS, Bethesda, MD 20814; #Eli Lilly, Indianapolis, IN 46254.

The neuropeptides vasopressin (VP), enkephalins (Enk) and dynorphin (Dyn) were implicated in central cardiovascular control since they modulate cardiac, respiratory and hemodynamic functions. Yet, most of the previous evidence is based on pharmacological studies utilizing central or peripheral injections of vasopressin and opioid peptide agonists and antagonists. To study further the relationships between VP and the central opioid system, we examined the effect of acute hypovolemic hypotension (a known stimulus for vasopressin release and activation of the endogenous opioid system) on the distribution of VP (RIA), leu^5-Enk (LE [RIA]) and Dyn A (RIA, 1-13 or 1-8) in the pituitary (neurointermediate: NIL, or anterior: AL) and discrete cardiovascular brain nuclei (micropunch technique; Palkovits, *Brain Res.*, 59:449, 1970) of rats. Conscious rats were implanted with arterial lines (PE50, 24 hrs before the experiment) and bled: 8.5 ml/300 g over 5 min. Blood pressure and heart rate were also monitored. Rats were sacrificed 5 min, 2 hrs or 24 hrs (n = 8 - 15) after the hemorrhage, the AL, NIL and brain removed, frozen on dry ice, sliced by a cryostat, and brain nuclei punched out and kept at -80°C. Plasma VP was 5.3 ± 1.6, 1397 ± 275, 298 ± 94 and 7.5 ± 3 pg/ml at control, 5 min, 2 hrs and 24 hrs after the bleeding. VP in the NIL was reduced at 24 hrs by 33% ($p < 0.05$) but increased in the suprachiasmatic (SC): 66%; supraoptic (SO): 200%; and median eminence (ME): 44%. VP in paraventricular (PVN) was not changed. Dyn A decreased by 38% and 62% in the NIL and AL, respectively, at 24 hrs while Dyn A-(1-8) was reduced by 60% in the NIL only. Dyn A was also depleted (> 70%) in some AV3V nuclei; Dyn A was not changed in SO, SC, ME or PVN. LE in the NIL increased by 70% and 87% 2 and 24 hrs after the bleeding, 266% in the AL at 24 hrs, and 63% in the ME at 2 hrs after the bleeding. These data suggest that hemorrhage enhanced processing of the depressor opioid Dyn A to the pressor opioid LE; moreover, since Dyn A is an endogenous ligand for κ-opiate receptors, which inhibit VP release, the data suggest a facilitatory mechanism for VP release through enhanced conversion of Dyn A to other opioid peptides of different opiate receptor specificity.

FEEDING AND DRINKING: NEUROPHARMACOLOGY I

92.1 THE TRANSITION FROM EATING TO ANOREXIA: A CLUE TO THE MECHANISM OF CHOLECYSTOKININ INDUCED SATIETY? T. R. Maone*, J. M. Kaplan*, L. H. Schneider, S. M. Feldman. Department of Psychology, New York University, New York, NY 10003.

We used a stimulation-induced feeding paradigm to investigate the mechanism by which cholecystokinin octapeptide (CCK-8) may signal satiety. Research by Schneider et al. (1984) showed a dose-related suppression of feeding in rats following administration of CCK-8. Electrical stimulation of the lateral hypothalamus (ESLH; 30 sec trains followed by 2 min intertrial intervals (ITI)) was used to produce feeding behavior. It was found that the maximally suppressive effect of CCK-8 was never seen until at least one "transition" trial had occurred, during which ingestion took place. The present study investigated whether animals must eat before succumbing to CCK's anorectic effect, or whether transition trials reflect time for activation of appropriate receptors by CCK-8 following i.p. injection.

Daily sessions consisted of 32 feeding trials, during which 30 sec trains of ESLH (0.1 msec rectangular pulses, 60 pps) were followed by 2 min ITIs. Immediately after the 8th trial, rats were given CCK-8 (5 µg/kg, i.p.). By delaying the onset of the next (9th) feeding trial by 10 min following CCK-8 injection (delay condition), versus the 2-min ITI no-delay condition on control days, we were able to examine the significance of transition trials. The 10-min delay following CCK-8 injection meant that the next feeding trial occurred at a time when feeding would have been completely suppressed without the delay. In the no-delay condition, we replicated Schneider et al. (1984): 5 µg/kg of CCK-8 caused a complete suppression of food intake for as long as 24 post-injection trials (1 hour); and at least one transition trial preceeded the maximal anorectic effect of CCK-8. In the delay condition, the transition trials were also manifest; i.e., the initial post-CCK-8 trial showed only partial suppression. In addition, latencies to begin eating during the 1st transition trial, under delay and no-delay conditions, were not greater than pre-injection latencies. It was not until subsequent trials that the latencies increased substantially.

We conclude that the transition trials do not reflect the pharmacokinetics of CCK-8; rather, interaction of CCK-8 with orosensory, gastric and/or ESLH properties seem to be necessary for the anorectic effect.

92.2 EFFECTS OF CHOLECYSTOKININ AND ITS PUTATIVE BLOCKER, PROGLUMIDE, ON INGESTION INDUCED BY HYPOTHALAMIC STIMULATION. J. M. Kaplan*, L.H. Schneider, E. E. Coons*, R. B. Murphy, S. M. Feldman (Spon. D. I. Schuster). Depts. of Psychology and Chemistry, New York University, New York, NY 10003.

We investigated the effect of cholecystokinin octapeptide (CCK-8; 0.2 µg/kg, N=4; 1, 5 and 25 µg/kg, N=6, i.p.) and its putative blocker, proglumide (1, 10 and 50 mg/kg, N=5, i.p.), on eating elicited by electrical stimulation of the lateral hypothalamus (ESLH). Rats with chronic LH bipolar electrode implants were restrained in a harness (Kaplan & Feldman, 1983) and presented with wet mash while receiving 30 sec trains of ESLH (0.1 ms rectangular pulses, 60 pps). Both screening and experimental sessions consisted of 32 ESLH trials with 2 min intertrial intervals. For each rat, a single current was chosen which produced eating on all trials and 1.75±0.25 g consumption per trial for the first 12 trials in a session. Experimental sessions were run in sets of three over consecutive days with a single dose of CCK-8 (day 2) or saline (days 1,3) given immediately after the 8th trial. CCK-8 caused a highly significant reduction of food intake. (Desulfated CCK-8, 5 µg/kg, was without effect.) The magnitude of the suppression and its time course (onset and recovery) were dose-dependent. The recovery of ESLH-induced eating after CCK-8 contrasted with the monotonic decrease in eating seen in saline control sessions. At the highest dose (25 µg/kg), only 1 animal out of 6 showed any recovery. However, for every CCK-8 session regardless of dose level, there was only a partial suppression of eating on the trial following the injection. Post-CCK-8 saline sessions did not differ from pre-drug saline sessions; no sign of aversive conditioning was detected.

We next examined the ability of proglumide to reverse the suppression of ESLH-induced eating by CCK-8 (1.0 µg/kg, i.p.). Proglumide was injected prior to the 1st feeding trial, with CCK-8 administered after the 8th. Each dose tested significantly, but not completely, antagonized inhibition of ingestion. The 50 mg/kg dose caused a significant suppression of feeding over the trials preceding CCK-8 injection.

This feeding paradigm, which is not constrained by normal meal size or duration, provides a sensitive behavioral assay for the actions of both CCK-8 and proglumide.

92.3 INFLUENCE OF ADRENALECTOMY ON α_1- AND α_2-NORADRENERGIC RECEPTORS IN DISCRETE HYPOTHALAMIC AND EXTRA-HYPOTHALAMIC AREAS. Jhanwar-Uniyal, M.*, Roland, C.R.*, and Leibowitz, S.F. (SPON: N.E. Miller) Rockefeller Univ., New York NY 10021.

The adrenal glucocorticoid corticosterone (CORT) is known to influence peripheral and central catecholaminergic functions. The CRF cell bodies, which control the release of CORT, are densely packed within the paraventricular nucleus (PVN). This nucleus is also richly innervated by noradrenergic fibers which, in addition to modulating CORT release, are known to be involved in the control of ingestive behavior. PVN injections of norepinephrine (NE) and clonidine stimulate eating in satiated rats, and this response, mediated by α_2-noradrenergic receptors, is abolished by adrenalectomy (ADX) and restored, within short latency, by CORT. The present biochemical study was undertaken to investigate the effect of ADX on $\alpha 2$- and α_1-noradrenergic receptor binding sites in discrete brain areas.

Adult male albino rats were sacrificed by decapitation 7 days after ADX or sham-ADX. The brains were quickly removed and frozen on dry ice, and then 300 μ sections were cut to permit micropunching of discrete hypothalamic (8) and extra-hypothalamic (6) areas. Standard radioligand binding techniques were employed with the α_2-noradrenergic agonist [^{3}H]p-aminoclonidine ([^{3}H]PAC, 3.0 nM) and the α_1-noradrenergic antagonist [^{3}H]prazosin (0.5 nM). Nonspecific binding was determined in the presence of phentolamine (50 μM). To confirm ADX, serum CORT was measured by radioimmunoassay.

The results clearly indicate that the binding of [^{3}H]PAC to α_2-noradrenergic receptors of the hypothalamus was altered by ADX. Specifically, the [^{3}H]PAC binding sites were significantly down-regulated in ADX rats. This effect was highly site-specific, occurring significantly only in the PVN. In this nucleus, the number of α_2-receptor binding sites decreased by 53% ($p < 0.01$). In contrast, no change in radioligand binding was observed in 7 other hypothalamic and 6 other extra-hypothalamic areas analyzed. [^{3}H]Prazosin binding after ADX remained unchanged in all brain sites.

The results suggest that the removal of CORT by ADX may abolish the NE feeding response by down-regulating those PVN α_2 receptors upon which NE acts to elicit a meal. Consistent with other biochemical evidence (Leibowitz et al.) correlating circadian rhythms of PVN α_2-noradrenergic receptors and serum CORT levels, this study may explain the nature of CORT's permissive relationship with NE-induced feeding and may suggest a role for CORT and PVN NE in control of natural feeding. (Research was supported by grant MH-22879.)

92.4 REDUCED FOOD INTAKE AND DECREASED HYPOTHALAMIC NOREPINEPHRINE LEVELS IN GENETICALLY OBESE (obob) MICE FOLLOWING RAUWOLSCINE TREATMENT. G.A. Oltmans, M. Beales*, and M.F. Callahan. Dept. of Pharmacology, The Chicago Medical School, N. Chicago, IL 60064.

The genetically obese mouse (obob) exhibits hyperphagia and elevated hypothalamic (HT) norepinephrine (NE) levels. At low doses the opiate antagonist naloxone and the alpha-2 antagonist rauwolscine both produce a hypophagia in obob, but not lean, mice. Since alpha-2 blockade has been reported to potentiate central NE release (Anden, et al., J. Neural. Transm. 55:111, 1982) we have studied the effects of selectively hypophagic doses of rauwolscine and naloxone on HT NE levels in obob mice and their lean littermate controls. As previously reported, saline treated obob mice had significantly elevated HT NE levels compared to lean littermate controls ($\bar{X}$obob = 2.32 ± 0.23 ug/g; $\bar{X}$lean = 1.84 ± 0.07, $p < 0.01$). A low dose of rauwolscine (2 mg/kg), significantly reduced food intake in obob(-24%) but not lean(-7%) mice, and produced a significant decrease in HT NE levels in obob mice only (-13%, $p < .01$). A higher dose of rauwolscine (4 mg/kg), which reduced food intake in both obob(-20%) and lean(-15%) mice, also significantly reduced HT NE levels in both groups (obob=-14%; lean=-17%, $p < .05$ both comparisons). Naloxone treatment(5 mg/kg) did not affect HT NE levels in either obob or lean mice.

Decreases in HT NE as the result of drug-induced NE release might, in some cases, be masked by synthesis of new amine. To study this possibility the rauwolscine and naloxone treatments were studied in conjunction with alpha-methyl-p-tyrosine (ampt, a tyrosine hydroxylase inhibitor) pretreatment. This procedure did not produce any additional effect on HT NE levels in obob mice ($\bar{X}$rauw only=1.77 ±.14 ug/g; $\bar{X}$ampt only=1.84 ±.12; $\bar{X}$rauw+ampt=1.70 ±.26), but did produce a marginal effect in lean mice ($\bar{X}$rauw only=1.79 ±.31ug/g; $\bar{X}$ampt only=1.56 ±.11; $\bar{X}$rauw+ampt=1.33 ± 0.23; ampt only vs ampt + rauw, $.05 < p < .10$). The combination of naloxone plus ampt did not produce any effects different from those of ampt only. These results indicate that the obob mouse has an increased sensitivity to the anorectic effects of rauwolscine and that this is associated with an altered response to the neurochemical effects of rauwolscine. Naloxone does not produce an effect on HT NE levels in either obob or lean mice at the dose studied.

92.5 LOCALIZATION OF THE EFFECTS OF CORTICOTROPIN RELEASING FACTOR ON FEEDING. D.D. Krahn*, B.A. Gosnell, A.S. Levine and J.E. Morley, Neuroendocrine Research Laboratory, VA Medical Center, Minneapolis, MN, 55417.

Corticotropin releasing factor (CRF), a recently isolated 41 residue peptide, decreases food intake after deprivation when administered intracerebroventricularly (icv). CRF (icv) also is a potent suppressor of feeding induced by several pharmacological methods including administration of muscimol, norepinephrine, dynorphin and insulin.

Immunocytochemical studies of CRF distribution in the rat have revealed that CRF-containing cells are densely packed in the paraventricular nucleus. CRF containing cells are also scattered throughout the anterior hypothalamus. We hypothesized that so potent an inhibitor of feeding as CRF may have effects if administered locally to nuclei classically associated with control of feeding. Therefore, our initial experiment consisted of a survey of the effects of local injections of CRF into the lateral hypothalamus, ventromedial hypothalamus, striatum, globus pallidus and paraventricular nucleus.

Thirty-three male, Sprague-Dawley rats were stereotactically implanted with 23 gauge indwelling cannulae into one of these areas (striatum, n = 5; PVN, n = 9; VMH, n = 9; LH, n = 5; GP, n = 5). After 20 hours of food deprivation, the rats were injected with .5 µg of CRF in a volume of .5 µl or .5 µl of normal saline. All rats received both treatments. Food intake was measured at one and two hours.

CRF significantly decreased food intake at one hour (NaCl = 4.62 ± .45, CRF = 2.11 ± .51) and two hour intervals (NaCl = 5.28 ± .78, CRF = 3.22 ± .91) ($p < .05$) when injected in the PVN. CRF did not significantly alter food intake at the other sites.

Therefore, the maximal effect of CRF on food intake occurred in the PVN. This is consistent with the distribution of CRF immunoreactivity in the rat brain. Further work is necessary to determine whether CRF plays a role in the physiologic regulation of food intake.

92.6 ELECTROLYTIC PARAVENTRICULAR NUCLEUS (PVN) LESIONS AND FEEDING BEHAVIOR: RELATION TO FOOD RESTRICTION, DRUGS AND CORTICOSTERONE. G.Shor-Posner*, A.Azar* and S.F. Leibowitz (SPON: W.Wyrwicka). Rockefeller Univ. New York, NY 10021.

α-Noradrenergic stimulation of the PVN is known to enhance feeding, particularly of carbohydrate. Electrolytic lesions also produce hyperphagia and obesity. The present experiments assessed the ability of PVN-lesioned rats to react to drugs mediated by the noradrenergic system and to respond to food restriction. Adult male, albino rats were maintained ad libitum on three pure diets of protein, carbohydrate and fat. Sham surgery or bilateral electrolytic PVN lesions were performed under Pentobarbital anesthesia.

Following surgery, animals with electrolytic lesions directly focused on the PVN, demonstrated a dramatic increase in 24hr food intake ($p < .01$) relative to controls. This effect was attributed primarily to an increase in carbohydrate and in a few animals, to increased fat intake. Body weight was also enhanced. These effects appeared to be strongest during the first few days after surgery and seemed to become attenuated over the next two weeks. Circadian feeding patterns revealed a diurnal disruption with PVN-lesioned animals consuming more of their total calorie intake, specifically carbohydrate, during the day than control rats ($p < .01$).

Drug tests indicated that feeding response (carbohydrate intake) induced by clonidine was blocked by PVN lesions. Feeding induced by the α-2 antagonist yohimbine, and insulin was not attenuated, suggesting that yohimbine acts through different mechanisms than clonidine.

In response to food deprivation (5 or 24hr) rats with PVN lesions showed a remarkable deficit, particularly carbohydrate, in compensatory feeding. This suggests that the PVN may have neurochemical mechanisms, perhaps α-2 noradrenergic, essential for normal energy repletion after food restriction. This deficit may be related to the greatly attenuated plasma corticosterone levels observed in the PVN-lesioned animals. This is consistent with additional evidence suggesting that glucocorticoids may be closely linked with medial hypothalamic α-2 noradrenergic function in the process of controlling feeding behavior.

92.7 THE EFFECT OF INTRACEREBROVENTRICULARLY (I.C.V.) INFUSED SATIETIN (SAT) ON CONDITIONED TASTE AVERSION. L.L. Bellinger and V.E. Mendel. Dept. Physiol., Baylor Coll. Dentistry, Dallas, TX 75246; Dept. An. Physiol., An. Science and Food Intake Lab., Univ. CA, Davis, CA 95616.

SAT (Knoll, Physiol. Behav. 23:497, 1979) is a glycoprotein found in human serum (>2 µg/ml) that is reported to be a strong anorexigenic agent when infused I.C.V. into rats. In the present studies male S.D. rats (Exp. 1,376.1± 7.7g; Exp. 2, 269.6±6.0g body weight) were fitted with chronic third ventricle cannulas. The rats were trained for 7 days to drink their water in one hour a day, 1100-1200 h, (LD 12:12h, light out 12:15h). At the end of this period in Exp. 1 the rats were divided into two groups (Grp) and given either water flavored with (Grp) 0.5% banana (BFW) or (Grp 2) 0.5% almond (AFW) extract instead of tap water. Liquid and food intake (FI) were recorded (1100-1200h and 24h for FI). The next day the rats received tap water. On the third day the rats were infused I.C.V. with (10µl) of artificial cerebrospinal fluid (CSF) [Grp 1] or 100µg/rat of purified human SAT (Grp 2) 30 min. prior to fluid presentation (Grp 1, AFW; Grp 2, BFW). Again fluid and FI were recorded. Three days later Grp 1 and 2 were given BFW and AFW drinking bottles. Both one hour (1.0±0.4 vs. 6.3±1.0g) and 24h (8.3±1.2 vs. 18.0±1.1g) FI of Grp 2 after SAT was less (<0.01) than Grp 1. SAT also reduced (<0.01) the fluid intake of Grp 2 below preinjection levels (18.0±3.0 vs. 25.7±2.1 ml) while CSF treatment did not. After two bottle choice Grp 1 drank similar amounts of BFW and AFW (12.6±2.5 vs 14.3±3.4ml) while Grp 2 drank more (<0.01) AFW (22.6±1.6 vs. 3.9±1.5). Retesting the next day again revealed that Grp 2 preferred (<0.05) AFW. In Exp 2 the rats were infused with saline (Grp 1) or SAT (50 µg/rat) (Grp 2) 30 min. prior to presentation of a 0.125% saccharine water solution (SWS). SAT reduced (<0.02) one hour (1.5±0.5 vs 3.9±0.7g) and 24h (8.9±1.1 vs 18.7±1.8g) FI and (<0.01) one hour SWS (12.1±1.9 vs. 24.7±2.8 ml). Three days later the rats were given both SWS and tap water. Grp 1 preferred SWS (17.2±2.1 vs 11.1±2.2 ml) while Grp 2 preferred (<0.001) tap water (4.4±0.4 vs 20.8±1.7ml). Retesting again showed Grp 2 preferred (<0.01) tap water. The data suggest SAT may be aversive and thus a note of caution should be used in interpreting FI data until synthesized SAT is tested for aversion. It is also of interest to determine if SAT could be responsible for pathological anorexics. Supported by BCD research funds.

92.8 THE EFFECT OF INTRACEREBROVENTRICULAR (I.C.V.) INFUSED SATIETIN (SAT) ON FOOD INTAKE (FI), WATER INTAKE (WI) AND ACTIVITY (ACT). V. E. Mendel and L. L. Bellinger. Dept. An. Physiol., An. Science and Food Intake Lab., Univ. of Calif., Davis, CA 95616 and Dept. Physiol., Baylor College of Dentistry, Dallas, TX 75246.

SAT (Knoll, Physiol. Behav. 23:497, 1979) is a glycoprotein (~50,000 daltons) found in human serum (>2µg/ml) that suppresses FI of 96 h fasted rats when infused I.C.V. In the present study male S.D. rats (L:D 12:12 h, lights out 12:30 h) were fitted with chronic third ventricle cannulas (Exp 1, 412.5±5.0 g; Exp 2, 285.1±10.4 g body weight at surgery). After recovery, food was removed at 1130 h and the rats infused (10 µl) with saline (SAL) or human SAT at 12.5, 25.0 or 50.0 µg/rat. Food was returned one hour later and recorded for one hour and a 24 h total. The procedures were repeated 9 days later except the rats receiving SAL and 50 µg of SAT had their doses reversed as did the rats receiving 12.5 and 25.0 µg of SAT. SAT did not suppress one hour FI. Twenty-four hour FI was slightly suppressed (>0.05) by SAL (n=13) when compared to baseline (26.4±1.3 vs 30.2±1.2 g). SAT reduced 24 h FI with FI after all doses of SAT being less (<0.01) than SAL (12.5 µg (n=13), 14.1±2.0 g; 25 µg (n=11), 15.1±1.5 g; 50 µg (n=8), 9.1±1.1 g). The 50 µg dose suppressed FI more (<0.05) than the other two SAT doses. A follow up with 6.25 µg/rat SAT (n=6) showed no 24 h FI suppression. In Exp 2 rats were placed in activity wheels. After adjustment the rats were, on alternate days, infused (10 µl) with SAL (3 doses) or given mock injections. This procedure was followed giving 4 doses of SAT (25 µg/rat) instead of SAL. Daily FI, WI and ACT were recorded. Compared to noninfused days SAL did not suppress (>0.05) FI, WI or ACT. SAT suppressed (<0.05) FI (23.8±1.0 vs 9.7±2.2 g), WI (31.4±2.6 vs 17.0±3.8 ml) and ACT (1625±617 vs 228±98 revol.) compared to mean SAL values, but only after the first injection. ACT remained low following subsequent SAT injections. The data from Exp 1 revealed that SAT can suppress 24 h FI but the dose response curve is not linear. The second Exp indicated alternate day I.C.V. infusions of SAT are effective in reducing FI only on initial exposure. The selectivity of SAT is questioned since WI and ACT were also reduced. The results suggest caution be used in interpreting SAT FI data until synthesized SAT is available for testing. Supported in part by BCD research funds.

92.9 AMPHETAMINE FACILITATES OR INHIBITS INDEPENDENT FEEDING IN RAT PUPS DEPENDING ON DOSE, AMBIENT TEMPERATURE, AND METHOD OF MILK DELIVERY.
L.M. Terry, I.B. Johanson and D.L. Wolgin. Psychology Department, Florida Atlantic University, Boca Raton, FL 33431

Deprived rat pups tested in a warm environment show adult-like ingestive responses when milk is infused directly into their mouths (cannula method) or spread in a thin layer on the floor (self-feed method). Such independent ingestion is accompanied by an impressive behavioral activation (Hall, W.G., Science, 1979, 205, 206; Hall, W. G. & Bryan, T. E., JCPP, 1980, 94, 746). To determine the extent to which such feeding can be influenced by pharmacological manipulations of activation level, we gave 24-hr-deprived 9-day-old rat pups access to milk following injection of either 0, 1, 2, or 4 mg/kg of d-amphetamine sulfate. Tests were conducted in both warm (32° or 36° C) and cool (27° C) environments using both the cannula and self-feed methods of milk delivery. Behavioral activation was rated on a scale of 0 (no activity) to 6 (frenzied activity).

The effect of amphetamine on intake was found to vary with dose, ambient temperature, and method of milk presentation. When milk was delivered by cannula in a warm environment (32° C), amphetamine produced a dose-dependent suppression of intake, replicating earlier findings (Raskin, L. A. & Campbell, B. A., JCPP, 1981, 95, 425). In contrast, at 27° C, amphetamine facilitated intake at 1 and 2 mg/kg (to 135 and 140% of control levels, respectively), and suppressed intake at 4 mg/kg. A different pattern of results was found with the self-feed method. In the warmth (36° C), amphetamine greatly increased intake at 2 mg/kg (to 152% of control levels) and suppressed intake at 4 mg/kg. However, at 27° C, the drug had no effect at the lower doses, and suppressed intake at 4 mg/kg.

Amphetamine increased behavioral activation in a dose dependent fashion with both methods of milk delivery. However, although the effect of amphetamine on milk intake was differentially influenced by ambient temperature, the effect of the drug on behavioral activation was not. Thus, the effects of amphetamine on milk intake do not appear to be a direct result of drug-induced changes in activation level.

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92.10 **EFFECTS OF NEUROLEPTICS ON FOOD INTAKE.** W.B. Lawson, J. Byrd* and D. Reed*. Adult Psychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032

Neuroleptics frequently induce obesity in man. Studies in animals have reported both decreased and increased food intake. Both findings are presumed to be due to blockade of central dopamine receptors. Effects of several neuroleptics on food intake were studied, over a range of dosages to clarify this issue.

Experiment 1: Intake of wet mash (50% lab chow and 50% milk by weight) was measured in satiated albino rats during the five hours after receiving intraventricular injections of chloropromazine (CPZ) or vehicle. Rats receiving 50 ug of CPZ ate significantly more than animals that received vehicle or 100 ug of CPZ ($p < .05$). No differences were seen at 24 hours.

Experiment 2-4: Animals received I.P. injections of vehicle or CPZ (2, 4, 16 mg/kg), trifluoperazine (TFPZ: .05, .5, 5 mg/kg) or pimozide (PZ: .01, .05, .1, .5, 1 mg/kg). Wet mash intake was measured after three and 24 hours. Similar curves were seen for all neuroleptics, with increased intake at midrange doses followed by decreased intake at the highest dose. Significant increases (CPZ=2 mg/kg, TFPZ=.5 mg/kg, and PZ=.05 mg/kg vs. vehicle: $p < 0.05$) in eating after three hours were observed for all three neuroleptics, while TFPZ and PZ significantly decreased eating at the highest dosages. The dosage ranges which increased eating corresponded with the clinical potency of the neuroleptic. Only TFPZ produced a significant difference in 24 hours food intake which was a decrease in food intake at the highest dose ($p < .01$). Comparisons were made between the peak dose that elicited eating of the neuroleptics above plus three others from the literature and several measures of dopaminergic systems. Dosages which produced increased food intake were highly correlated with the average clinical dose and inhibition of (^{3}H) haloperidol binding (for both, $r_s = .94$, $p < .01$). Thus, at the appropriate dosage neuroleptics acutely increase food intake, probably through dopamine receptor blockade.

92.11 EFFECTS OF PERIPHERAL ANORECTIC AGENTS ON ZUCKER RATS: INHIBITORS OF CARBOHYDRATE ABSORPTION VERSUS INHIBITORS OF LIPID METABOLISM. J.A. Grinker, A. Drewnowski*, R. Gruen*, and A.C. Sullivan*. Univ. of Michigan, Ann Arbor, MI 48109 and Hoffmann-La Roche, Inc., Nutley, NJ 07110.

Peripherally active anorectic agents influencing lipid or carbohydrate absorption represent a new approach to the pharmacological management of obesity. In the present study, we compared two inhibitors of carbohydrate metabolism, a glucosidase inhibitor Acarbose (BAY g 5421) and an alpha-amylase inhibitor, Ro 12-2272, with two novel compounds affecting lipid metabolism, an inhibitor of human pancreatic lipase (Ro 20-0083) and an inhibitor of hepatic fatty acid synthesis (Ro 22-0654). Following a 4-day baseline period on powdered chow diet, 6 obese male Zucker rats and 6 lean littermates (age 3 mo.) received Acarbose or Ro 12-2272 as diet admixture (80 mg/100 g diet) over 4 consecutive days. In the second experiment, 5 obese and 5 lean Zucker rats (age 8 mo.) fed a semisynthetic diet containing 10% corn oil received a diet admixture of Ro 22-0654 (322 mg/100 g diet) or Ro 20-0083 (774 mg/100 g diet). Total food and water intakes, the temporal pattern of feeding and meal frequency and meal size were measured using computerized data collection procedures. The two inhibitors of carbohydrate absorption failed to suppress food or water intakes of either obese or lean rats and had no effect on the temporal profile or the microstructure of feeding behavior. In contrast, inhibitors of lipid metabolism reduced food intake by 56-77% through a reduction both in meal frequency and in meal size. Other investigators have reported significant decrements in the development of obesity in young Zucker rats and in rats exposed to obesity promoting diets following Acarbose treatment (Vasselli et al., Nutr Behav, 1982, Pharm Biochem Behav, 1984). It is possible that diet palatability and age of the animal are important modifiers of the effectiveness of the agent. The data reported here suggest that some peripherally acting compounds can have an acute effect on food intake in the mature obese rat and that a direct inhibition of lipid metabolism may be a viable mechanism for anti-obesity agents. NIH AM27980 and AM32944

92.12 TREATMENT WITH EITHER PARA-CHLOROPHENYLALANINE OR 5,7-DIHYDROXYTRYPTAMINE EXACERBATES THE DECREASED FOOD INTAKE OF RATS FED IMBALANCED AMINO ACID DIETS. D. W. Gietzen*, P. M. B. Leung*, W. J. Hartman* and Q. R. Rogers* (SPON: E. Sassenrath). Dept. of Physiol. Sci., Sch. of Vet. Med. and Food Intake Lab., Univ. of Calif., Davis, CA 95616.

Serotonin has been implicated as a major neurotransmitter involved in feeding behavior. It has previously been shown that food intake is markedly depressed in rats fed imbalanced amino acid diets (IMB). In order to determine whether serotonin may be involved in this response to IMB, male Sprague-Dawley rats (BW: 150-250 g) were treated with either p-chlorophenylalanine (PCPA, 300 mg/kg BW ip) or 5,7-dihydroxytryptamine (5,7-DHT, 200 µg/rat) intracerebroventricularly (icv) to reduce serotonin levels. The 5,7-DHT treated animals were pretreated with 25 mg/kg desmethylimipramine (DMI) ip 30 min before the icv injections to prevent the 5,7-DHT from being taken up by catecholaminergic neurons. Control animals were given saline injections either ip or icv, and controls for 5,7-DHT were also given DMI. The animals were maintained on a low-protein basal diet (BAS) and water ad libitum under a 12:12 lighting schedule with lights off at 12 noon. After daily food intake on BAS had returned to preinjection levels, groups of animals were given IMB, with either threonine or isoleucine as the limiting amino acid. Serotonin levels were determined in brain homogenates by fluorometric analysis. As expected, food intake was depressed in control animals given IMB, but the 24 hr food intake of IMB for the PCPA or 5,7-DHT treated animals was significantly less than that of the controls given IMB. Food intake at 2 hr was not different between PCPA treated and control animals given IMB, but at 6 hr there was a trend toward a greater decrease in the PCPA treated animals compared to controls, both given IMB. However, treatment of the animals with the serotonin precursor L-tryptophan (L-trp, 100 mg/kg ip at 11AM) did not alter the 2, 6 or 24 hr intake of either BAS or IMB by the animals. Therefore, depletion of serotonin by PCPA ip or 5,7-DHT icv exacerbates the 24 hr food intake response of rats to IMB, with differences beginning to be demonstrated at 6 hr. However, since L-trp did not alter the intake of IMB in these animals, the serotonergic system may play only a part in the complex mechanisms involved in the response to imbalanced amino acid diets. (Supported in part by NIH grants AM 13252 and AM 07355.)

92.13 NEWLY IDENTIFIED SUGAR ACIDS ACTING AS FEEDING MODULATORS. N. Shimizu*, Y. Oomura, K. P. Puthuraya*, S. Nemoto*, T. Sakata*, and N. Okukado*. (SPON: M. Tachibana) Dept. of Physiol. and Int. Med., Fac. of Med.; Dept. of Organic Chem., Fac. of Sci., Kyushu Univ. 60, Fukuoka 812, Japan.

The endogenous sugar acid 2-deoxytetronic acid (2-DTA) lactone was found to be increased in rats from a normal value of 38.4 µM to 1.5 mM and 2.5 mM, after food deprivation for 36 and 48 hrs respectively. But the serum concentration of another sugar acid, 3-deoxypentonic acid (3-DPA) lactone whose normal value was 73 µM, did not change so dramatically like 2-DTA lactone. Injection of the racemic type of 2-DTA into the third ventricle (2.5 µmol) reduced food intake for 24 hr even in 72 hr food deprived rats, whereas the racemic type of 3-DPA transiently elicited feeding as compared to the controls. Four kinds of 3-DPA optical isomers were further tested for the elicitation of feeding. Type 2S-4S of the isomer induced feeding with a latency of 8 to 12.5 min, while 2R-4S, 2S-4R or 2R-4R types did not. These results indicate that 2S-4S type of isomer is the active form in the living body, which is also confirmed by GC/MS analysis. Both S and R type of 2-DTA were effective. Simultaneous changes in single neuronal activity of the lateral hypothalamic area (LHA) and in feeding behavior occurred after injection of 2-DTA or 3-DPA into the third ventricle of the chronic rats.

Electrophoretically applied 2-DTA significantly and specifically suppressed the activity of glucose-sensitive neurons in the LHA, while 3-DPA facilitated the activity. Glucose-insensitive neurons were not affected much by these sugar acids. The inhibition caused by 2-DTA was blocked by the simultanious application of ouabain, which suggests that the inhibitory effect of 2-DTA might be mediated by the activation of Na-K pump. These sugar acids had the completely opposite effects on the glucoreceptor neurons in the ventromedial hypothalamus (VMH), i.e. excitatory effect for 2-DTA and inhibitory effect for 3-DPA. A good correlation was seen between the LHA glucose-sensitive and the VMH glucoreceptor neuronal activity and the feeding behavior change elicited by these sugar acids, indicating that 2-DTA act as an endogenous satiety substance and 3-DPA as a hunger substance. These effects are mediated through the modulation of the LHA and VMH neuronal activities.

93.1 EFFECTS OF NALOXONE ON THE ACQUISITION OF SCHEDULE-INDUCED POLYDIPSIA. A. Riley, D. Matallana* and J. Hahn*. Psychopharmacology Laboratory, The American University, Washington, D.C. 20016.

Naloxone hydrochloride, a narcotic antagonist, suppresses consumption of water under a wide range of conditions (Morley, J.E., Levine, A.S., Yim, G.K., & Lowy, M.T., Neurosci. Biobehav. Rev., 7: 281-305, 1983). In contrast to this general suppression, naloxone has no effect on schedule-induced polydipsia (SIP), i.e., drinking induced by the spaced delivery of food pellets (Brown, D.R., & Holtzman, S.G., Eur. J. Pharmac., 69: 331-340, 1981; Cooper, S.J., & Holtzman, S.G., Pharmac. Biochem. Behav., 19: 505-511, 1983).

A recent report by Sanger and his colleagues (Sanger, D.J., & McCarthy, P.S., Psychopharmacology, 77: 336-338, 1982) provides a possible explanation for this failure. Sanger and McCarthy noted that while naloxone suppresses food consumption in deprived rats, this suppression is substantially weakened if the rat is adapted to the feeding schedule. It is important to note that the drinking which occurs under schedules of spaced food delivery, i.e., SIP, is elicited by the consumption of the food pellet and typically develops only after the rat has extensive exposure to the schedule of food delivery. If naloxone fails to affect food consumption in rats adapted to the feeding schedule (see above), it would not be expected that naloxone would suppress SIP. If this is so, naloxone should suppress SIP if it is given at the outset of SIP training before the subjects adapt to the feeding schedule.

To test this prediction, in the present experiment different groups of food-deprived rats (n=4 per group) were given injections of naloxone (10 mg/kg) or the distilled water vehicle for 22 consecutive days 15 min prior to the start of the daily 75-min session during which time they received 45-mg Noyes pellets on a fixed time 75-min schedule. Water was available ad lib in the home cage and during the experimental session.

Naloxone dramatically retarded the acquisition of SIP. Although by the seventh session all control subjects were drinking approximately 30 ml, the naloxone-injected subjects were drinking only 5 ml. While these subjects eventually acquired SIP, their absolute level of consumption never reached that of control subjects. At the beginning of training, naloxone-injected subjects seldom drank following pellet delivery. Although the probability of post-pellet drinking did increase as training progressed, it remained low at the outset of each session. Control subjects were drinking following every pellet delivery by the seventh session.

These data suggest that SIP can be affected by naloxone. That this effect was evident only during the acquisition of SIP (vehicle-injected subjects showed only a minor supression of SIP when given naloxone after SIP had been acquired) suggests that the earlier failures to suppress SIP may have been due to the rat's adaptation to the feeding schedule.

93.2 FOOD INTAKE AND THE KAPPA OPIOID RECEPTOR. A.S. Levine and J.E. Morley. Neuroendocrine Research Laboratory, VA Medical Center, Minneapolis, MN, 55417.

A variety of studies have suggested a role for opioid receptors in the modulation of food intake. Several distinct classes of opioid receptors have been postulated. In an attempt to establish which opioid receptor(s) modulate feeding, we studied the effects of the kappa agonist, bremazocine on feeding and compared its effects to the preferential mu agonist, morphine, and the mixed kappa agonists/antagonists butorphanol tartrate and ethylketocyclazocine in male Sprague-Dawley rats. Bremazocine increased feeding to the same extent as morphine and was less potent than either butorphanol tartrate or ethlyketocyclazocine. The bremazocine effect demonstrated a bell-shaped dose response curve. Compared with a single exposure, a daily injection of bremazocine or morphine for 5 days enhanced the effect of these opioid agonists on the induction of food intake. If animals receiving daily injections of morphine were then injected with bremazocine, no enhancement in food intake occurred compared to a single exposure. The same was true for rats injected daily with bremazocine and then given a single exposure to morphine. The bremazocine effect is enhanced by the opioid antagonist, diprenorphine, and not inhibited by naloxone. Low doses of the dopamine antagonist, haloperidol, enhance the bremazocine effect and higher doses inhibit it. These studies provide further evidence for a role for the kappa opioid receptor in feeding. However, the lack of cross tolerance observed for bremazocine and morphine suggests that more than one subpopulation of opioid receptors is involved in feeding modulation.

In a separate series of studies we examined the role of kappa opioid receptors in humans by administering the mixed agonist/antagonist, butorphanol tartrate (1 µg/kg) to 10 humans. The subjects were injected with either saline or butorphanol and immediately offered a breakfast consisting of three types of sandwiches. Butorphanol increased caloric intake over a 6 hour period compared to saline controls. This effect was reversed by naloxone (6 µg/kg). These findings suggest that humans, like rats, have a kappa opioid receptor feeding system.

93.3 DOSE- and TRIAL-DEPENDENT ATTENUATION OF STIMULATION-INDUCED FEEDING BY NALOXONE AND PIMOZIDE. F. Jenck,* A. Gratton,* and R.A. Wise. Center for Studies in Behavioral Neurobiol. Dept. Psychol., Concordia Univ., Montreal, Canada H3G 1M8.

Latency to feed in response to lateral hypothalamic electrical stimulation was measured at a variety of stimulation frequencies at fixed intensity in each rat. Graded effects were seen; shorter latencies were associated with higher frequencies and higher intensities. Pimozide (0.062, 0.125, 0.25, 0.5 mg/kg) and naloxone (0.5, 1.0, 2.0, 4.0 mg/kg) increased latencies in a dose-dependent manner; different dose-sensitivity was seen in different subjects. Deficits were not evident on initial test trials in either case; rather, deficits developed with repeated trials. Thus simple motoric capacity for feeding was not critically disrupted. Rather, it appeared that feedback from food was not normally rewarding; it did not sustain responding at the usual latencies. Even when latency criterion was liberal, well within the demonstrated performance capability of the animals, higher than normal stimulation frequency was required for normal performance.

Thus the drugs impaired the motivational impact of either the food or the stimulation. This suggestion is consistent with the observation that pimozide attenuates both the rewarding effects of lateral hypothalamic stimulation and the rewarding effects of food, and with the suggestion that naloxone attenuates either the motivating effects of stimulation or the rewarding effects of food (Carr, 1983). The similarity between the effects of opiate receptor blockade and dopamine receptor blockade suggests synergistic actions in a common feeding or food-reward mechanism; it may be that dopaminergic and opioid peptide circuit elements are connected in series in motivational circuitry of feeding.

93.4 NALOXONE AND FLUID CONSUMPTION IN RATS: DOSE-RESPONSE RELATIONSHIPS FOR 15 DAYS. G. A. Olson, S. W. Delatte*, A. J. Kastin, T. A. Riedl*, J. H. McLean, D. F. Phillpott*, and R. D. Olson. Dept. of Psychology, Univ. of New Orleans, New Orleans, LA 70148

Rats were given daily intraperitoneal injections of 10.0, 1.0, 0.1, 0.01, 0.001, or 0.0 mg/kg of naloxone for 15 days. Daily, after injections, animals were allowed access to a 20% sucrose solution for two hours and to tap water for the subsequent 10 hours. Consumption of the sucrose solution by the group that received 10.0 mg/kg, although reliably less than that of the control in each of the 15 sessions, showed a trend toward increasing over the experiment. Consumption by the group given 1.0 mg/kg of naloxone was significantly supressed only on days 1 and 2. Drinking patterns of the other groups did not differ statistically from the control although the group treated with 0.001 mg/kg did have a higher mean consumption than the control group. Intake of tap water was highest by the groups which drank the least sucrose solution and decreased over days as their sucrose consumption increased. Total daily fluid intake, therefore, was comparable among the groups.

A second study compared rats injected with 1.0 or 0.0 mg/kg of naloxone using the same design except injections were administered only on days 1 and 15. The 1.0 mg/kg group exhibited reliable supression on day 1 but not on day 15. These findings suggest that rats become less susceptible to the supressant effects of naloxone with repeated injections.

After obtaining the behavioral data, the 1.0 mg/kg dose of naloxone in diluent solution was analyzed by high performance liquid chromatography. This sample of naloxone was applied to a column of C-18 ODS in a solution of 0.1% trifluoroacetic acid (TFA) in 3% methanol. After two minutes under these initial conditions, the concentration of methanol was raised to 16% over 11 minutes followed by a further increase to 40% over 10 minutes, and finally to 100% methanol over one minute. The flow rate was 1.5 ml per minute throughout the procedure. The retention time of the naloxone used in the original experiment was 17.62 minutes as compared to the retention time of 17.74 minutes for the freshly prepared standard. Only one peak was observed, the area of which was 98.3% of the standard.

The HPLC results indicate that the behavioral data were not due to naloxone degradation. An endogenous process such as the development of tolerance provides the best explanation for these data.

93.5 THE EFFECTS OF MORPHINE AND KETOCYCLAZOCINE ON DAY-NIGHT FOOD INTAKE OF YOUNG AND OLD MICE. M. Hirst* and M. Kavaliers. Depts. of Pharmacology-Toxicology and Zoology, University of Western Ontario, London, Ontario, Canada N6A 5B7

Nocturnally active animals such as mice consume most of their food at night. Both mu and kappa-opiate agonists stimulate feeding behavior in mice. However, the majority of investigations of opioid influences on feeding have been conducted in young animals during the daytime. The present study was undertaken to examine the day and night-time feeding responses of young and old mice following administration of the opiate agonists morphine and ketocyclazocine.

Young (1-2 months) and old (24-30 months), male CF-1 mice were housed individually at 23°C under LD 12:12 with food (Purina mouse chow 5015) and water available ad libitum. In the first study mice ate powdered chow ad libitum with the quantity consumed being measured at 30 min intervals over a 24 h period. Following this animals were injected with saline, morphine sulfate (1,10 mg/Kg), or ketocyclazocine hydrochloride (1,10 mg/Kg) in saline i.p. (10 ml/Kg) at 1300 h (light period) and 2100 h (dark period) and their food intake monitored for the next 3 hours.

Young and old mice display differences in their day-night patterns of food acquisition. Young mice show acute changes of feeding behavior with the onset and offset of light, whereas old mice consume a much greater quantity of food during the day. Further, in old mice there is a less prominent influence of altering the light condition, there being a more gradual increase and decrease in nocturnal feeding. Young mice showed a greater sensitivity to the opiate agonists. A dose and time dependency was observed in young mice injected with morphine of ketocyclazocine during the day whereas the old animals were little influenced by the drug treatments. Both morphine and ketocyclazocine had more potent stimulatory effects in the young mice during the night-time but again the old mice showed little sensitivity to the drug treatments.

Accordingly, advancing age in mice disrupts the day-night patterns of feeding and sensitivity to opiate compounds that stimulate feeding behavior.

93.6 NALOXONAZINE AND FOOD INTAKE: SELECTIVE ROLE FOR HIGH-AFFINITY OPIATE RECEPTORS. D. A. Simone*, R. J. Bodnar, E. Goldman* and G. W. Pasternak. (SPON: J. Haldar) Dept. of Psychology, Queens College, CUNY, Flushing, NY and George C. Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Dept. of Neurology and Pharmacology, Cornell University Medical Center, New York, NY.

Studies over the past decade indicate a role for opioids in the mediation of ingestive behavior. The opiate antagonist naloxone (NAL) decreases food intake in free feeding, deprived and glucoprivic rats. Its short duration of action and selective affinity for opiate receptor sub-types limit interpretation of some of NAL's effects however. Naloxonazine (NAZ) is a long-lasting opiate antagonist which selectively blocks the high-affinity binding site of all opiate receptor sub-types. The present study examined NAZ's effects upon intake in free feeding, deprived and glucoprivic rats. Two groups of sixteen male albino Sprague-Dawley rats, matched for baseline food intake, received either NAZ (10 mg/kg, iv) or vehicle and food intake was assessed 24 h later. Pretreatment with NAZ (68% of baseline) significantly reduced free feeding relative to vehicle treatment (90% of baseline). In a second experiment, six groups of rats were food deprived for 24 h. Four groups received NAZ (10 or 20 mg/kg, iv), NAL (10 mg/kg, iv) or vehicle respectively just before deprivation. Two groups received either NAL (10 mg/kg, iv) 15 min prior to or NAL (10 mg/kg, sc) immediately before reintroduction of food. Intake was determined 2 h thereafter. Pretreatment with NAZ doses of 10 (67% of vehicle) and 20 (66% of vehicle) mg/kg, but not NAL (109% of vehicle) prior to deprivation, significantly reduced deprivation-induced intake. Naloxone was effective at reducing deprivation-induced intake only when administered iv (59% of vehicle) or sc (61% of vehicle) shortly before food reintroduction. In a third experiment, 2-deoxy-D-glucose (2-DG) (400 mg/kg, ip) was administered 24 h after either NAZ (10 mg/kg, iv) or vehicle, or immediately after NAL (10 mg/kg, sc). Food intake was determined at 2 and 4 h following 2-DG administration. While NAL significantly reduced 2-DG hyperphagia at 2 and 4 h, NAZ potentiated 2-DG hyperphagia at 4 h following 2-DG administration. These data indicate that the reductions in free feeding and deprivation-induced feeding induced by NAZ and NAL suggest a role for high-affinity binding sites in these responses. The respective ability and inability of NAL and NAZ to reduce 2-DG hyperphagia suggest a different mechanism of opioid action. (Supported by PSC/CUNY Grant 6-63210).

93.7 LOCALIZATION OF NALOXONE-SENSITIVE BRAIN AREAS IN RELATION TO FOOD INTAKE. B.A. Gosnell, J.E. Morley & A.S. Levine, Neuroendocrine Res Lab, VA Medical Center, Minneapolis, MN, 55417.

Several studies have suggested that the paraventricular nucleus of the hypothalamus (PVN) is part of a noradrenergic feeding system. This area is also thought to play a role in opioid-induced feeding. To further localize the neural sites mediating opioid effects on feeding, 23-gauge guide cannulae were stereotaxically implanted into the striatum, globus pallidus (GP), PVN, lateral hypothalamus (LH), and ventromedial hypothalamus (VMH) of male rats. After food deprivation (19-20 hours), rats were injected with saline or naloxone hydrochloride (50 µg) in a 0.5 µl volume. All rats were tested under both conditions. Naloxone significantly reduced 1 hour intake when injected into the PVN or VMH (median reductions of 68% and 29.5%, respectively). Two hour intake was reduced by naloxone injected into the PVN and the GP (median reductions of 55% and 34.5%, respectively). In earlier trials, it was found that the injection of norepinephrine (50 nmol) into the PVN and VMH induced feeding (mean intakes of 4.2 and 5.9 g, respectively). Norepinephrine had no effect on feeding when injected into the other areas. The globus pallidus, therefore, may represent part of an opioid feeding system that is largely independent of noradrenergic influences. This is consistent with our finding that electrolytic lesions of the GP attenuate the feeding response to subcutaneous injections of the opiate agonist, ketocyclazocine.

In a separate group of rats, knife-cuts (or a sham procedure) were made in the medial hypothalamus. Rats were maintained on a high-fat diet (33%) beginning 1 day after surgery. Knife-cut rats became hyperphagic and gained more weight than shams. While still in the dynamic stages of weight gain, nocturnal food intake was measured following subcutaneous injections of naloxone (0-10 mg/kg) in a repeated measures design. In sham and obese knife-cut animals, naloxone (1 and 10 mg/kg) significantly reduced 2 hour intake. This experiment demonstrates a functional opioid feeding system is present in knife-cut obese rats; the hyperphagia and obesity may be due in part to a removal of inhibition of this opioid system. These studies support the suggestion that the PVN and VMH are parts of opioid and noradrenergic feeding systems within the hypothalamus. That naloxone also reduces intake when injected into the globus pallidus suggests that the opioid feeding system also includes areas outside the hypothalamus.

93.8 A TEMPORAL PATTERN ANALYSIS OF DRINKING IN NALOXONE-TREATED RATS DURING LIGHT/DARK PERIODS. D.A. Czech and K.J. Gill* Dept. of Psychology, Marquette University, Milwaukee, WI 53233.

It is well known that both peripheral and central administration of opioid antagonists will reliably attenuate drinking, as well as feeding, in a number of animal species and in variety of situations. These observations have resulted in suggestions that one or more of the endogenous opioid peptides plays an important modulatory role in mechanisms regulating consummatory behaviors. More recent studies have begun to probe issues such as latency to drink and patterns of drinking behavior under different conditions. We report here the results of a study examining effects of several doses of naloxone hydrochloride on temporal patterning of water intake in rats during both the light and dark phases of their L/D cycle.

Male Sprague-Dawley rats were placed on a 12/12 L/D cycle and adapted to a 23-hr water deprivation schedule. Following stable intakes, all rats were injected SC with three doses of naloxone (0.3, 1.0 & 3.0 mg/kg) and a normal saline vehicle during both the light and dark periods. All rats received all treatment conditions, and tests were separated by 3-4 days. Fifteen min after injection, they were given access to distilled water during a one hour test session in the home cage. Food was removed during testing. Water intake was recorded automatically at one minute intervals throughout the test session. Intakes were analyzed with repeated measures ANOVA procedures with significance level set at $p < 0.05$.

No latency to drink was observed in any of the animals, which is in general agreement with the literature. Significant effects were found for light-dark period, dosage and time over the primary drinking period. Several interactions were also significant. Drinking was most vigorous during approximately the first 4-8 min. Drinking declined more sharply during the dark period, and total fluid consumed was significantly lower during this period. These data clearly suggest that circadian rhythmicity and temporal patterning of drinking behavior need to be considered. Results are further discussed.

94.1 **AUTOTITRATION OF SELF-STIMULATION: REWARD THRESHOLD OR COULOMB OPTIMIZATION?** **J.B.Richards and D.B.Neill (SPON: K.Wallen). Dept. of Psychology, Emory University, Atlanta, GA 30322.**

Autotitration is a procedure in which rats are allowed to self-regulate brain stimulation current intensity. The rats are placed in an operant chamber with two levers. Pressing one of the levers delivers a train of electrical brain stimulation to the rat. The intensity of the current gradually diminishes as a function of the number of presses on the stimulation lever. Pressing the other lever resets the current to maximum intensity. Rats trained on this procedure reset at the same current intensity from day to day. This current level has been interpreted as indicating the rewarding threshold of the stimulation current.

However, a recent experiment (Fouriezos, G. & Wawiesniak, E., Soc. Neurosci. Abstr., 8:624, 1982) has produced data inconsistent with a simple reward threshold interpretation of autotitration. We have developed another interpretation which says that autotitrating rats are optimizing the total charge consumed (measured in coulombs) during short experimental sessions.

In order to test the optimization model we challenged autotitrating rats in three different ways. First, we tested rats with three different peak intensities. We found that the rats did not maintain the same reset intensity across all the peak intensity values. In general, the rats reset at lower current intensities when the peak intensity was low and reset at higher current intensities when the peak intensity was high. In a second group, a delay period was imposed after each reset so that brain stimulation was not available at the stimulation lever for a specified amount of time after each reset. Reset delays of 0, 5 & 15 seconds were used. Reset intensity decreased as the length of the reset delay increased. Third, a minimum inter-stimulation time of 0, 150 & 300 milliseconds was imposed between the end of one train of stimulation and the beginning of the next train. In effect, this manipulation slowed the rate at which the rats received trains of stimulation for pressing the lever. Increasing the minimum inter-stimulation time caused the rats to increase reset intensity.

The above results are consistent with the notion that rats are conserving the amount of coulombs consumed during an experimental session as predicted by the optimization model. The results are not consistent with a simple reward threshold interpretation of autotitration.

94.2 AMPHETAMINE EFFECTS ON INTRACRANIAL SELF-STIMULATION AS ASSESSED BY THE QUANTITATIVE 2-DEOXYGLUCOSE METHOD T.F. Seeger*,L.R. Porrino,R.U. Esposito,A.M. Crane, T.L. Sullivan*,and A. Pert (SPON: C.T. Bennett) NIMH, Bethesda, MD 20205.

Amphetamine pretreatment induces an increase in the rate of responding for intracranial self-stimulation (ICSS). We used the quantitative 2-(14C)deoxyglucose method (Sokoloff, J.Neurochem.28:897,1977) to study this effect of amphetamine on ICSS. Local cerebral glucose utilization (LCGU) was measured in select brain areas which have been implicated in ICSS of the ventral tegmental area (VTA) in freely moving rats (Porrino et al,Science 224:306,1984, Esposito et al, PNAS,81:635,1984). The following four groups were compared: 1)HIGH CURRENT: rats self-stimulating at an amplitude of 250-300uamps (response range: 65-90 stims/min. N=8) 2)LOW CURRENT +AMP: rats self-stimulating at 100uamps, given 0.5mg/kg D-amphetamine 15 min prior to test (response range:70-120 stims /min. N=6) 3)AMP ALONE: rats given 0.5mg/kg D-amphetamine but not allowed to self-stimulate (N=5) 4)CONTROL: rats which did not receive amphetamine or stimulation (N=6). Groups 1 + 2 thus responded for equivalent rates of ICSS and were intended as the primary comparison groups.

All rats were trained for ICSS of the VTA, after which the CONTROL and AMP ALONE groups were extinguished. 2-DG was injected 10 min after the start of ICSS/placement in chamber, followed by the standard protocol for determination of LCGU. The distribution of alterations in metabolic activity in the HIGH CURRENT and LOW CURRENT+AMP groups was largely as previously described (Esposito et al,1984). Rates of LCGU at the stimulation site and pathway were much lower in the LOW CURRENT+AMP group than in the HIGH CURRENT group. In contrast, rates of LCGU in a number of the projection areas of the VTA were equivalent in the two groups, including the nucleus accumbens, the medial prefrontal cortex, the basolateral amygdala, and the locus coeruleus. In the olfactory tubercle and the sulcal cortex, LCGU was higher in the LOW CURRENT+AMP group than in the HIGH CURRENT group. Rates of LCGU in the AMP ALONE group indicated that these effects were in no case due to the amphetamine administration alone.

These findings indicate that equivalent response rates for reinforcing stimulation, whether arising from pharmacological manipulation or varying current amplitude, yield strikingly similar paterns of local cerebral glucose utilization.

94.3 A COMPARISON OF SELF-STIMULATION TO THE VENTRAL TEGMENTAL AREA AND SUBSTANTIA NIGRA IN THE RAT BY MEANS OF 2-[^{14}C]DEOXYGLUCOSE AUTORADIOGRAPHY. L.J. Porrino, R.U. Esposito, T.F. Seeger*, A.M. Crane*, J.W. Jehle*, T. Sullivan*, A. Pert and L. Sokoloff. National Institute of Mental Health, Bethesda, MD 20205.

Rats will self-stimulate to both the ventral tegmental (VTA) and substantia nigra pars compacta (SNC) portions of the ventral midbrain. These anatomically continuous regions have, however, different patterns of afferent and efferent connections. In the present study we used the quantitative 2-[^{14}C]deoxyglucose autoradiopgraphic method (J. Neurochem. 28:897-916, 1977) to compare and contrast the patterns of local cerebral metabolic activity that result from self-stimulation to these two areas. Male Sprague-Dawley rats were implanted with bipolar platinum electrodes in either the VTA (N=8) or SNC (N=5) and trained to lever press for electrical brain stimulation at identical parameters (biphasic rectangular wavepulses; 100 Hz; 250-300 μA; 400 msec trains). Local cerebral glucose utilization (LCGU) was determined following the standard protocol. Rats in the VTA group self-stimulated at rates of 65-90 responses/min, while rats in the SNC group at rates of 40-60 responses/min. VTA rats were also more active and behaviorally aroused than SNC rats during the experimental procedure. LCGU was measured in the VTA and SNC, as well as in the terminal fields of each system, both ipsilateral and contralateral to the electrode site. Despite differences in response rates and behavior, similar increases in LCGU at the sites of stimulation and in the ascending and descending fiber pathways were found in both groups with some evidence of topographic organization. Metabolic activation in the SNC rats extended rostrally in the medial forebrain bundle in an area located dorsolateral to the corresponding area in VTA rats. Divergent patterns of LCGU alteration were evident in the terminal fields of the SNC and VTA. For example, in the caudate, a region more heavily innervated by the SNC than the VTA, extensive changes were seen in the SNC rats, but not in the VTA group, whereas in the septum which receives projections from the VTA, but not the SNC, changes were found in VTA rats, but not in SNC rats. In contrast there were several regions including the nucleus accumbens and prefrontal cortex in which similar changes in LCGU were found in both groups. This convergence of metabolic activation despite differences of anatomical innervation suggests a significant role for these regions in mediation of goal-oriented self-stimulation behavior.

94.4 CHANGES IN LOCAL CEREBRAL GLUCOSE UTILIZATION DURING REWARDING BRAIN STIMULATION TO THE SUBSTANTIA NIGRA. R.U. Esposito, L.J. Porrino, T.F. Seeger*, A.M. Crane*, J. Jehle*, T. Sullivan*, A. Pert and L. Sokoloff, National Institute of Mental Health, Bethesda, MD 20205.

The quantitative 2-[^{14}C]deoxyglucose (2-DG) method (J. Neurochem. 28, 897-916, 1977) was used to determine alterations in central metabolic activity during self-stimulation to the zona compacta of the substantia nigra (SNC). Male Sprague-Dawley rats were unilaterally implanted with bipolar platinum electrodes and screened for self-stimulation to this area (current parameters: biphasic rectangular wave pulses; 250-300 μA; and 400 msec). All subjects positive for self-stimulation were trained to stable response rates (40-60/min) and then divided randomly into 3 groups: ICSS- self-stimulators; EAS- received experimenter administered stimulation at the subject's preferred rates and; NS- animals that received no stimulation. Lever pressing was extinguished in rats in the EAS and NS groups prior to the experiment. The standard protocol for determination of local cerebral glucose utilization (LCGU) was followed.

Both the ICSS and EAS groups showed a similar pattern of metabolic activation, as assessed by changes in LCGU), at the stimulation site and in the direct rostral and caudal projections. There was an intense increase in LCGU at the stimulation site in the SNC which continued rostrally within the dorsolateral aspect of the medial forebrain bundle, extending to the lateral hypothalamic preoptic area. Caudal to the stimulation site there were bilateral LCGU increases in the projection fibers extending through the pontine gray. In thalamic sensory-motor nuclei, as well as in sensory motor neocortex and cerebellum, LCGU was bilaterally increased in both the ICSS and EAS relative to the NS group, reflecting the general behavioral activation of stimulated rats.

The pattern of LCGU changes within cortical and striatal terminal fields and major striatal efferent pathways was strikingly different in the ICSS and EAS groups. Particularly noteworthy were bilateral increases in LCGU in the medial prefrontal cortex, entorhinal cortex, caudate nucleus, nucleus accumbens and the ventral pallidus in the ICSS group, which were not activated in the EAS group. These data indicate that the distribution of changes in LCGU found in ICSS rats is the result of the goal-oriented nature of their behavior and not simply the consequence of electrical stimulation to the substantia nigra.

94.5 STRESSOR-INDUCED ALTERATIONS IN ICSS FROM THE NUCLEUS ACCUMBENS OR SUBSTANTIA NIGRA. W.J. Bowers, R.M. Zacharko and H. Anisman, Carleton University, Ottawa, Ontario K1S 5B6

Inescapable footshock reduces ICSS from the nucleus accumbens (Nas), without affecting responding from the substantia nigra (SN). Since inescapable shock affects mesolimbic and not nigrostriatal DA activity, it was suggested that stressor-elicited alteration of motivation is region specific. In the present investigation the motivational alterations induced by a stressor were further evaluated using a current titration procedure. A decreasing current intensity function was established for ICSS responding from the SN or Nas. Following training SN animals were exposed to one session of inescapable shock and tested immediately, 24-, 48- and 168-hr later. Response changes after stressor application were evident only among animals tested using current intensities which appreciably exceeded the optimal value. When optimal current intensities were employed the stressor treatment did not affect performance. In contrast, responding for ICSS from the Nas was reduced using optimal current intensities. The response reductions were evident immediately and 24-hrs following inescapable footshock, while 48 or 168 hr following exposure to the stressor elevated response rates were observed. These data are consistent with the suggestion that stressor induced ICSS alterations in the mesolimbic and nigrostriatal systems are fundamentally different from one another. While the reduction in ICSS from the SN was evident at all currents employed, the reduction in ICSS from the Nas was evident only at the higher currents. Moreover, at lengthy intervals following stressor application elevated response rates were observed using the same current intensities. The time-dependent biphasic alterations of responding for ICSS from the Nas may reflect an initial decline of motivation, coupled with the sensitization of neural substrates mediating ICSS (e.g., mesolimbic system).

94.6 NALTREXONE AND SELF-STIMULATION: EXTINCTION-LIKE RESPONSE PATTERN SUGGESTS SELECTIVE REWARD DEFICIT. K.A. Trujillo, J.D. Belluzzi and L. Stein. Department of Pharmacology, University of California, Irvine, CA 92717.

The effects of opiate antagonists on self-stimulation behavior have been widely studied, yielding different results from different laboratories. While some groups have reported that naloxone or naltrexone strongly suppresses responding for rewarding brain stimulation, others have reported no significant effects. One factor suggested to be involved in these discrepancies is the length of the test session (West et al, 1983). In addition, there is disagreement over interpretations of the suppressant effects of these compounds. While some investigators suggest that the suppression is due to a selective effect on reward processes (Belluzzi and Stein, 1977), others attribute this effect to a non-specific performance deficit (Franklin and Robertson, 1982). One method of distinguishing between reward and performance is to examine the pattern of responding during drug treatment. An extinction pattern showing normal initial response rates followed by suppression later in the session would indicate a selective reward deficit, whereas suppression throughout the session would be more consistent with a performance deficit (Fouriezos and Wise, 1976; Wise, 1982). We have preliminary evidence suggesting that naltrexone (NTX), a long-acting opiate antagonist, produces an extinction-like suppression of nucleus accumbens self-stimulation in rats. Animals were trained to stable self-stimulation response rates in daily 1-hr sessions. NTX (10 mg/kg s.c.) or saline (1 ml/kg) was injected immediately after the control session—approximately 23 hours before the test session. This delay allowed NTX to reach the slow decay phase where brain concentrations are most stable (Misra et al, 1976). NTX response rates for the total session expressed as percent of the preceeding control day were significantly different when compared to saline (79.9 ± 7.32 vs 100.3 ± 3.13, $p < .05$). The first 30 minutes showed a nonsignificant suppression (86.1 ± 6.16 vs 98.7 ± 1.22, n.s.) whereas the second 30 minutes showed significant suppression of responding (69.9 ± 10.46 vs 103.0 ± 6.81, $p < .05$). This pattern suggests an extinction-like effect which is consistent with a reward deficit in the naltrexone-treated rats, and may explain why long test sessions are necessary to observe the suppressant effects of opiate antagonists on self-stimulation. (Supported by AFOSR F49620-81-K-0015).

94.7 DORSAL TEGMENTAL AND LATERAL HYPOTHALAMIC SELF-STIMULATION ARE BLOCKED BY ATROPINE INJECTED INTO VENTRAL TEGMENTUM. O. Kofman and J.S. Yeomans, Dept. Psychology, Univ. Toronto, Canada, M5S 1A1.

Stimulating electrodes were placed in dorsal tegmentum (DT) just rostral and ventral to the lateral dorsal tegmental nucleus, and in lateral hypothalamus (LH), and an injection cannula placed in ventral tegmentum near dopamine cell groups. At a frequency of 40 Hz, the current was adjusted on both electrodes until half-maximal bar-pressing rates were obtained.

Summation between DT and LH electrodes was then tested by using paired pulses, stimulating at the obtained currents via alternate electrodes at various intrapair (C-I) intervals. At long C-T intervals, the frequency threshold shifted to a mean of 26 Hz, suggesting that summation between the two electrodes was near 70%. At C-T intervals of 0.2 and 0.4 msec, the frequency threshold was also near 26 Hz, suggesting no collision.

Injections of atropine into ventral tegmentum have been previously shown to increase thresholds for LH self-stimulation (Yeomans, Kofman and McFarlane, Brain Res., in press). In this study, an injection of 30 ug atropine sulphate in 0.5 ul artificial CSF increased thresholds on the LH electrode, but produced greater and more prolonged changes on the DT electrode. Ten ug atropine increased thresholds less on the DT electrodes, but had no effect on the LH electrode.

It is not clear why LH self-stimulation can be blocked since descending cholinergic projections from basal forebrain nuclei to ventral tegmental area have not been found (Grove, Haber, Domesick and Nauta, Neurosci. Abstr. 9, 16, 1983). DT self-stimulation sites mapped in previous studies are anatomically near cholinergic cell groups in the lateral dorsal tegmental nucleus. The blockade of DT self-stimulation by atropine in ventral tegmentum raises the possibility that these electrodes may be stimulating cholinergic fibers projecting rostrally to ventral tegmentum (Groenwegen, Kowall, and Nauta, Neurosci. Abstr. 9, 516, 1983).

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94.8 HYPOTHALAMIC SELF-STIMULATION IS SEVERELY WEAKENED IF REINFORCEMENT IS DELAYED FOR ONE SECOND. J. Black, J.D. Belluzzi, and L. Stein. Dept. of Pharmacology, University of California, Irvine, CA 92717.

The effect of delayed reinforcement on the acquisition of lateral hypothalamic self-stimulation was investigated. Brain stimulation reinforcement minimizes cues associated with reinforcement delivery (secondary reinforcement) and, by eliminating consummatory responses, permits precise temporal control of the interval between the operant response and reinforcement. Different groups were trained in daily 1-hr sessions for brain stimulation reinforcement at one of four delay intervals (1, 2, 3, or 6 sec). Responses made during the delay interval were not reinforced and reset the delay timer. Control groups were reinforced immediately, but were required to space responses—according to a DRL schedule—for an interval corresponding to one of the delay of reinforcement intervals. The DRL schedule equalized opportunities for reinforcement and non-reinforcement. At all intervals, rats trained with delayed reinforcement had significantly lower bar-press rates than controls trained under DRL (see FIGURE for 1-sec interval data). When reinforcement schedules were switched (DELAY groups now get DRL and vice versa), response rates rapidly shifted to levels appropriate to the new schedule. The pre-switch results indicate that delays even as short as 1 second markedly impede the acquisition of self-stimulation behavior. The post-switch results suggest that delay of reinforcement, like stimulation intensity, may determine the strength of hypthalamic reinforcement and hence final levels of performance.

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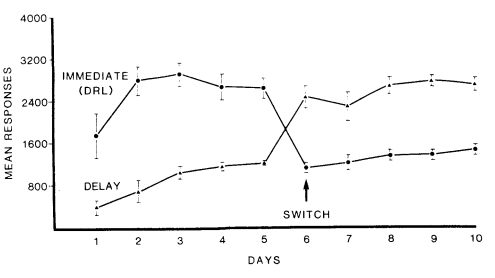

94.9 DRUG SPECIFICITY ON BRAIN STIMULATION REWARD

E. Miliaressis, P.P. Rompré, L. Philippe* and P. Laviolette*. School of Psychology, University of Ottawa, Ottawa, K1N 6N5, Canada.

The specificity of drug action on brain stimulation reward was examined using psychophysical methods.

Adult male rats were chronically prepared with a monopolar moveable electrode implanted slightly above the lateral hypothalamus (LH). The stimulation consisted of trains (.3sec) of cathodal rectangular pulses of fixed duration (.1msec) and intensity (100 to 400µA depending on the subject) and variable frequency. Following post-surgical recovery, the electrode was driven ventrally by steps of .16mm until threshold selfstimulation was obtained using pulse frequencies and intensities not greater than 50Hz and 400µA. During subsequent training, the pulse frequency was varied in ascending and descending order to cover the entire performance range of the animal. Training was continued until the pulse frequency/response (or F/R) functions showed good inter-day stability. The last phase consisted in comparing the F/R functions obtained under saline and drug treatment. Methocarbamol (200 and 300mg/Kg), a depressor of polysynaptic reflexes, was used in order to examine the alterations of the F/R functions due to motoric debilitation. This drug was therefore used as a control for Pimozide (.25 and .35mg/Kg), an experimental neuroleptic suspected to inhibit selfstimulation through its motoric effects. The following observations were made: When the pulse frequency is plotted on log scale, Methocarbamol produces an angular as opposed to Pimozide which produces a parallel shift of the F/R functions. In addition, Methocarbamol produced a severe depression of the assymptotic performance, while with Pimozide, significant depression was noted only at the higher dose. The degree of specificity of the two drugs, related to brain reward, was inferred from the increase in the frequencies corresponding to a) threshold (Θo) and b) half-maximal (M50) performance. M50 predicted a reward-specific effect for both drugs while such an effect was predicted by Θo only for Pimozide. The above discrepency lies in the fact that M50 provides accurate reward measurements only when the performance maxima of control and drug conditions are reached at the same frequency.

Two basic conclusions were drawn: a) Pimozide reduces the rewarding value of hypothalamic stimulation. b) When Θo and M50 procedures are compared in a bar-ressing paradigm, the former operates more reliable dissociation between the rewarding and motoric effects of drug treatments.

94.10 EFFECTS OF INTRASTRIATAL MICROINJECTIONS OF β-PHENYLETHYLAMINE ON RATE AND SELF-REGULATED DURATION OF LATERAL HYPOTHALAMIC STIMULATION IN RATS.

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β-Phenylethylamine (PE) is an endogenous amine in mammalian brain which is structurally similar to amphetamine. There is increasing evidence that PE may play a role in neural regulation, particularly in relation to neural substrates of reinforcement (Shannon & Thompson, 1984, J. Pharmac. Exp. Therap. 228, 691-). The present study investigated effects of intrastriatal microinjections of this amine on self-stimulation behaviour. Both response rate and a rate-independent correlate of reinforcement magnitude were employed to assess PE effects on reinforcement processes. Male Wistar rats were unilaterally implanted with twisted bipolar electrodes in the medial forebrain bundle at the level of the lateral hypothalamus, and with guide cannulae in the anterior ventral striatum ipsilateral to the electrode. Each animal was trained on a fixed-interval 6 sec schedule of self-regulated electrical hypothalamic stimulation (as described previously, Greenshaw et al., 1983, Psychopharmacology 81, 231-). Under these conditions, when stimulation was available, depression of the response lever initiated stimulation which remained on until the rat released the lever or until a maximum of 6 sec of stimulation had elapsed. Test sessions were of 30 min duration. Baseline current (sinusoidal 60 Hz) was 70 µA (rms). Response rate, the duration of self-regulated electrical stimulation and the duration of non-reinforced responses (i.e. during the fixed interval), served as dependent variables. The effects of several doses of PE (6.34-634 nmol) were assessed in animals pretreated with the monoamine oxidase inhibitor pargyline (50 mg/kg s.c. 24 h). Each animal received a single microinjection (1 µl delivered over a 3 min period) of PE or the vehicle. PE significantly increased response rate. The duration of self-regulated hypothalamic stimulation was decreased by PE:at 63.4 nmol this effect was associated with a decrease in response duration whereas at 190.2 and 634 nmol there was no consistent effect of PE on this measure. Increasing current to 106 µA resulted in a decrease in the duration of hypothalamic stimulation but the duration of non-reinforced responses and response rate were variably affected. These data suggest that PE may influence both motor and reinforcement systems in the rat forebrain.

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94.11 TEMPORAL SUMMATION CHARACTERISTICS OF PROLONGED BRAIN STIMULATION AS A FUNCTION OF INTERPULSE INTERVAL.

P.A. Mason and P.M. Milner*. Dept. of Psychology, McGill University, Montreal, Quebec, H3A 1B1, Canada.

This study investigated the temporal summation characteristics of prolonged brain stimulation. Seven rats with mid-posterior lateral hypothalamic implanted electrodes were tested in a Y-maze. They were required to choose between a standard reinforcement, which was either 3.0 or 5.0 sec in duration, and a test reinforcement. The duration of the test reinforcement was always equal to the duration of the standard reinforcement. The first 2.50 sec of the test reinforcement (part A) always had the same parameters as the standard reinforcement. The interpulse interval was sometimes changed during the remaining 0.50 or 2.50 sec of the test reinforcement (part B). Throughout the experiment, the current intensity remained constant for each rat.

In the first phase of the experiment, the interpulse interval of the standard reinforcement and part A of the test reinforcement was 10.0 msec. The interpulse interval of part B was 5.0, 10.0, or 20.0 msec. The results indicated that decreasing the interpulse interval of part B produced a strong preference for the test reinforcement. Augmenting the duration of part B failed to alter the preference produced by decreasing the interpulse interval.

In the second phase, the interpulse interval of the standard reinforcement and part A of the test reinforcement was 4.0 msec. The interpulse interval of part B was 2.0, 4.0, or 8.0 msec. The results showed that varying either the interpulse interval or duration of part B failed to produce a preference for the test reinforcement.

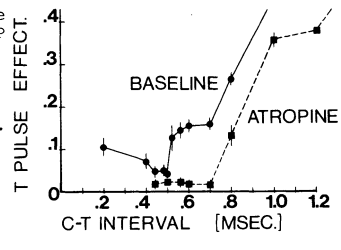
The first phase of the experiment demonstrated that when prolonged brain stimulation is delivered with a long interpulse interval (10.0 msec), a decrease in the interpulse interval is preferred over either increasing or not changing the interpulse interval. However, the second phase showed that when prolonged brain stimulation is delivered with a short interpulse interval (4.0 msec), a decrease in the interpulse interval is not preferred. These results elaborate on those of Atrens, Sinden, and Hunt, Physiol. Behav., 31: 787-799, (1983); and Deutsch and Hawkins, Behav. Biol., 7: 285-290, (1972). The present data are inconsistent with an adaptation model of temporal summation. Instead, the data support a model of temporal summation which involves neuronal and synaptic fatigue.

(Supported by NSERC Canada grant A66 to P.M. Milner).

94.12 THE SUBSTRATE FOR MEDIAL FOREBRAIN BUNDLE SELF-STIMULATION CONTAINS A SUB-POPULATION OF FAST CHOLINERGIC FIBERS.

A. Gratton* and R.A. Wise (SPON: B. Jones). Center for Studies in Behavioral Neurobiol., Concordia U., Montreal H3G 1M8.

The first stage, reward-relevant fibers of medial forebrain bundle (MFB) self-stimulation have post stimulation excitability cycles ranging from 0.5 to 2.0 msec (Gallistel et al. 1981). The refractory periods (RPs) of the reward substrate are inferred behaviorally using a double pulse technique, where the animal's response to a train of paired pulses is compared to its response to a train of single pulses. By varying the delay (C-T interval) between the two constituent pulses (C and T pulses) of each pair of the paired pulse condition, a distribution of RPs of the reward-relevant fibers is obtained. The fact that the reported function relating T pulse effectiveness to the C-T interval is continuously graded has suggested either a single fiber population or several populations with overlapping ranges of RPs. However, when C-T intervals are varied in smaller increments (0.02-0.04 msec) we find a flat portion of the function; between 0.6 and 0.7 msec no increase in T pulse effectiveness is seen. This plateau suggests that at least two distinct populations of reward-relevant fibers are activated by the stimulation; a fast set of fibers with RPs ranging from 0.44 to 0.60 msec and a slow set with RPs all longer than 0.7 msec. In a second experiment we attempted to selectively block the contribution of one subset of fibers pharmacologically. Atropine sulphate, a cholinergic muscarinic blocker, was found to eliminate the contribution of the fast fiber population (Fig); only fibers with refractory periods of 0.7 msec or greater contributed to T-pulse effectiveness in this condition. On the other hand, methyl atropine (which does not cross the blood-brain barrier) and the dopaminergic antagonist pimozide had no effect on the RP curves. These data extend recent reports of a cholinergic involvement in MFB self-stimulation (Yeomans et al. 1983) and suggest that 15% to 30% of the fibers directly activated by rewarding brain stimulation utilize this transmitter.



94.13 ELECTROPHYSIOLOGICAL CHARACTERISTICS OF FOREBRAIN CELLS DIRECTLY DRIVEN BY MEDIAL FOREBRAIN BUNDLE STIMULATION IN THE RAT. Peter Shizgal and Pierre-Paul Rompré. Center for Studies in Behavioural Neurobiology, Concordia University, Montreal, Quebec H3G 1M8.

Psychophysical experiments have suggested that descending projections are at least in part responsible for the rewarding effects of medial forebrain bundle (MFB) stimulation, and that the refractory periods of these fibers range principally from 0.4 to 1.2 msec. The purpose of the present study was to record from forebrain somata directly activated by MFB stimulation and to compare the refractory periods of these cells to the psychophysically derived estimates.

Under urethane anesthesia, single unit action potentials were recorded extracellularly using tungsten microelectrodes. Stimulation was delivered through steel macroelectrodes aimed at the MFB. A response was considered to be antidromic if it showed collision; responses that were not spontaneous but showed good latency stability and frequency following were categorized as "probably antidromic". Two procedures were used to estimate refractory periods. Cells from both categories were tested with the "standard" procedure which consists of determining the least interpulse interval that triggers two spikes. Cells that showed collision were subjected to a further test in which a pair of pulses was delivered at a given interval after a spontaneous spike. This "Swadlow test", like the psychophysical test, is believed to estimate recovery at or near the site of stimulation while the standard test may reflect the characteristics of the soma and initial segment (Swadlow, Exp. Neurol., 1982, 65, 514).

In the 15 hooded rats studied to date, 58 cells met the abovementioned criteria. Refractory period estimates obtained in the standard manner ranged from 0.6 msec to 5.8 msec. The standard estimates for the 11 spontaneously active cells ranged from 1.0 - 3.7 msec, while the values from the Swadlow test were always shorter: 0.4 - 2.2 msec. Histological analysis showed that almost 60% of the cells were located in the septum while the rest were scattered among several basal forebrain nuclei.

The results show that MFB stimulation activates forebrain cells with refractory periods within the range of the psychophysical estimates. The overlap is increased when the appropriate electrophysiological test is used.

94.14 DOPAMINE TERMINAL ABLATIONS ATTENUATE IPSI- AND FACILITATE CONTRA-LATERAL MEDIAL FOREBRAIN BUNDLE (MFB) BRAIN STIMULATION REWARD. L. Colle* and R. A. Wise . (Spon: R. Malmo) Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia Univ., Montreal, Canada H3G 1M8.

Pharmacological studies implicate dopamine in MFB brain stimulation reward. Animals can still learn primitive responses for MFB reward after major bilateral forebrain ablations,however,despite loss of most forebrain dopamine terminals (Huston and Borbély, 1973). Moreover, lever-pressing for MFB reward seems quantitatively normal after unilateral forebrain ablations (Stellar, Illes and Mills, 1982). The present study was designed to examine the effects of unilateral dopamine terminal field ablations on MFB reward in a within-subjects paradigm. Frequency-response rate functions at each of six stimulation intensities (in 8 rats) and refractory period distributions (in 2 rats) were determined bilaterally before and after unilateral suction ablations of the forebrain rostral to the anterior commisure. Frequency required for maintenance of criterion performance increased in some animals, but all animals continued to show robust self-stimulation despite loss of all of the dopamine terminals of nucleus accumbens and most of those in the rostral caudate and frontal cortex, as confirmed by flourescence histochemistry. No change in refractory period was seen; thus no subpopulation of contributing fibers seemed lost after the lesions. Contralateral self-stimulation dramatically improved after lesions in every animal; permanent (12 weeks) 25-40% decreases in frequency thresholds were seen. These data are difficult to reconcile with current notions of the involvement of dopamine in reward. They indicate that while forebrain dopamine may contribute to reward function, ipsilateral accumbens and frontal cortex are not necessary for MFB reward. If ipsilateral caudate is important then only a small caudal portion is essential. The surprising finding that contralateral self-stimulation was facilitated by these lesions suggests that portions of one forebrain may normally act to inhibit reward mechanisms in the contralateral hemisphere. Since less pronounced facilitations were seen after lesions restricted to the frontal cortex, the mechanism of this effect would seem difuse.

94.15 DISSOCIATION OF MEDIODORSAL AND SULCAL DIVISIONS IN THE PRODUCTION OF ELECTRICAL SELF-STIMULATION OF PREFRONTAL CORTEX A. Robertson, A. Laferrière* and P.M. Milner*. Dept. Psychology, McGill University, Montreal, PQ H3A 1B1, Canada

Lever pressing behavior rewarded with electrical stimulation of either the mediodorsal or sulcal divisions of the rodent prefrontal cortex (PFC) requires several daily training sessions to become manifest, but can procede quickly if animals are repeatedly stimulated before self-stimulation (SS) training (Corbett et al, 1982). Acquisition of mediodorsal PFC SS is equally facilitated by pre-training stimulation of the sulcal PFC (Robertson et al, 1982). Moreover, bilateral parasagittal knife cuts of the projections directly linking the mediodorsal and sulcal PFC result in the elimination of mediodorsal PFC SS which does not recover when sampled 5 or 6 times over a 3-week period (Corbett et al, 1982). These observations suggest that the cortico-cortical connections between the two PFC areas are critical for the production of the reinforcing effects of mediodorsal PFC stimulation. In the first experiment testing this possibility rats with bilateral knife cuts subjected to daily acquisition sessions displayed mediodorsal PFC SS within the same time period as control rats. Furthermore, rats with similar cuts performed after mediodorsal PFC SS had been established recovered completely within a week if postlesion testing was carried out on a daily basis, but not if testing was done 6 times over a 3-week period. Other experiments showed that pre-training stimulation of the mediodorsal PFC in rats with knife cuts was no less effective than normal in facilitating later acquisition of SS of this site, nor was the effectiveness of pre-training stimulation of the sulcal PFC in facilitating onset of sulcal PFC SS altered in similarly treated rats. However, pretraining stimulation of the sulcal PFC in rats with knife cuts was no longer effective in facilitating onset of mediodorsal PFC SS. These results suggest that (1) mediodorsal PFC SS can occur in the absence of a direct cortical link to the sulcal area and (2) the recovery of mediodorsal PFC SS following destruction of these connections seems to require a minimum exposure to the repeated effects of stimulation, akin to what is needed to observe normal mediodorsal PFC SS. The data further suggest that (3) the substrates for this process in the mediodorsal and sulcal PFC can be dissociated and that the role of the corticocortical path between these two PFC areas could perhaps be to provide an associative and/or subordinating link in the processing of reward-related signals.

95.1 THE DEVELOPMENT OF THE PREFERENCE FOR SALT IN NEONATAL RATS. Karen E. Moe. Dept. of Biology, Univ. of Pennsylvania, Philadelphia, PA 19104.

Many mammalian species demonstrate a preference for the taste of salt when it is offered in low concentrations but they show an aversion to higher concentrations. The means by which animals develop this need-free preference for salt has been virtually ignored. The experiments described here show that neonatal rats are precociously capable of demonstrating the concentration-dependent preference-aversion response to sodium chloride.

Rat pups were offered one of several concentrations of NaCl (0.3% - 18%), sucrose (5% - 15%), quinine (0.005% - 0.4%)or NH_4Cl (0.25% - 15%) by pulsatile infusion through an anterior mouth catheter for 30 minutes. At 6, 12 and 18 days of age, pups show the inverted U-shaped preference-aversion curve characteristic of adult rats. However, the curves are shifted to the right along the concentration axis in an age-related fashion. This may reflect the postnatal timing of much of the development of the rat's gustatory system.

Because of this finding and the postnatal gustatory development of the rat, it was predicted that 3 day old pups would either be completely indifferent to NaCl or would show an adult-like preference-aversion function shifted even farther along the concentration axis. However, the response of the 3 day old pups was very different from the response of the older pups. In general, they seemed more sensitive that the older pups. They showed no preference for any saline solution offered and rejected concentrations which older pups either preferred or found neutral. They also rejected a quinine solution to which the older pups were indifferent, and ammonium chloride (preferred or neutral to older pups). These responses of 3 day olds may be trigeminally mediated.

Supported by NSO3469 and NRSA NSO7304.

95.2 TASTES GET ASSOCIATED WITH THE AVERSIVE ASPECTS OF MORPHINE IN THE VISCERAL CORTEX. W.B. Mackey*, J. Keller* and D. van der Kooy. (Spon: T. Hattori). Dept. of Anatomy, Univ. of Toronto, Toronto CANADA M5S 1A8

Little information exists with regard to the brain systems involved in morphine's aversive aspects. Using the conditioned taste aversion paradigm, we attempted to discover the brain area(s) involved in associating the aversive aspects of morphine with taste. We paired the presentation of 0.1% saccharine with morphine (15 mg/kg i.p.) in rats bilaterally lesioned with 0.1 l of 4% ibotenic acid into the visceral cortex. The area of lesion was chosen because it is a cortical projection area for neurons involved in taste, it contains a large population of dopamine fibers (dopamine is implicated in the psychoactive effects of numerous drugs) and cells from this area project to the nucleus of the solitary tract, a visceral and taste center in the medulla. Three groups of rats were used (N's=8). Group's 1 and 2 received bilateral ibotenic acid lesions and had saccharine consumption paired with morphine and saline injections respectively. Group 3 received sham saline lesions and had saccharine consumption paired with morphine injections. On the test day each rat's consumption of saccharine and tap water over a 20 min period was recorded. Saccharine consumption represented 86%,93% and 19% of the total liquid consumed for groups 1,2 and 3 respectively. These results showed that the lesion disrupted the abilities of the rats to associate saccharine with the aversive properties of morphine (Group 1), taste discrimination in general was not affected (Group 2) and that taste aversions do develop to morphine in normal rats (Group 3). To test if the lesion was specific for disruption of the associability of tastes to morphine's aversive properties, we repeated the experiment with LiCl (75 mg/kg i.p.) and used two novel flavours as tastes. Consumption of the flavour not paired with morphine represented 90%,57% and 98% of the total liquid consumed over the 20 min test period in groups 1,2 and 3 respectively. These results showed that the lesion had no effect on LiCl taste aversions and demonstrated that the two flavours used were equally preferable to rats (Group 2). Place preference conditioning was used to test if our lesion had any effect on the association of morphine's positive reinforcing properties to other sensory stimuli. No differences were seen between each of the groups. We conclude that the area responsible for associating tastes with morphine's aversive aspects is the visceral cortex. This site plays little role in the associability of LiCl or in the association of morphine's rewarding aspects with other sensory stimuli.

95.3 KAINATE AND ELECTROLYTIC LESIONS OF THE LATERAL HABENULA: EFFECT ON AVOIDANCE RESPONSES. K. S. Wilcox,* G. R. Christoph,* B. A. Double* and R. J. Leonzio.* (Spon: George F. Steinfels)
Central Res. & Dev. Dept., E.I. duPont de Nemours & Co., Glenolden, PA 19036

The habenula of the rat is an epithalamic structure whose efferents project to midbrain structures containing monoaminergic cell bodies. Electrolytic lesions of the habenula are reported to impair performance on a conditioned avoidance response (CAR) task, a paradigm which has been shown to be sensitive to dopaminergic manipulations. The present study compares the CAR behavioral effects of kainate and electrolytic lesions of the LHb in order to determine whether neuronal perikarya or fibers of passage within the LHb are responsible for the observed deficits in CAR paradigms. Male, Sprague-Dawley rats (N=24) received either bilateral electrolytic lesions, kainic acid lesions or sham lesions in the LHb and were tested for one-way CAR acquisition. All rats with electrolytic lesions failed to learn the avoidance response within 15 trials and had significantly longer response latencies than sham controls, $F(1,9)=22.6$, $p<.01$. In contrast, 80% of the rats with kainate lesions learned the avoidance task (mean=5.5 trials) and the response latencies were not significantly different from controls. The results suggest the destruction of fibers of passage or neighboring structures, rather than LHb perikarya, are responsible for CAR deficits in electrolytically lesioned rats.

95.4 CHEMICAL BLOCKADE OF THE NUCLEUS ACCUMBENS: EFFECTS ON EXCITATORY AND INHIBITORY CLASSICALLY CONDITIONED RESPONSES IN THE CAT. W. J. Wilson, N. K. Dess*, and S. S. Soltysik. Mental Retardation Research Center, Neuropsychiatric Institute Rm 58-258, University of California, Los Angeles, CA 90024.

We have previously shown that both the infusion of dopamine into, and the electrical stimulation of the nucleus accumbens attenuate motor conditioned responses without affecting preparatory conditioned responses. Electrolytic lesions enhance the motor conditioned responses without affecting the preparatory conditioned responses. Our sensitive method of measuring conditioned inhibition provided a means of examining the functional role of the nucleus accumbens in behavioral inhibition.

Adult cats with chronically implanted cannulae aimed bilaterally at the nucleus accumbens were used. Five sec visual or auditory CSs were paired with a 300 msec, 3 mA foot shock US. On inhibitory trials, the US was omitted and its nonoccurrence was signalled by a 3 sec conditioned inhibitor (CI) which was presented 2 sec after CS onset. Each daily session consisted of 6 CS-US and 6 CS-CI trials, ordered randomly, with 2 or 3 min intertrial intervals.

Five types of responses were observed: leg flexion, vocalization, heart rate, respiration rate, and respiration amplitude.

Reversible blockade of activity within the accumbens was achieved by the bilateral infusion of 2% (20 mg/ml) lidocaine at a rate of 0.2 ul/min throughout the course of four 30 min sessions. Four sessions in which isotonic saline was infused at the same rate served as controls. Data analyzed thus far confirm our previous findings, i.e., that only motor conditioned responses are affected by manipulations of the nucleus accumbens.

95.5 ENDOGENOUS OPIOIDS: OPPOSITE MOTIVATIONAL EFFECTS IN BRAIN AND PERIPHERY. A. Bechara• and D. van der Kooy. Neurobiology Research Group. Dept. of Anatomy, Univ. of Toronto, Toronto Canada

Opiates have paradoxical reinforcing effects. When paired with visual and textural environmental stimuli, opiates produce positive reinforcing effects and yet at similar doses over the same routes of administration, opiates appear to produce aversive effects when paired with taste stimuli. We now report tests of the hypothesis that endogenous and exogenous opioids produce positive reinforcing effects in the brain and aversive effects in the periphery. Employing a place preference paradigm, separate groups of drug naive rats were administered various doses (.001-100.0 mg/kg)s.c. or i.p. of naltrexone or methylnaltrexone which does not cross the blood-brain-barrier effectively. Regardless of the route of administration, increasing doses of naltrexone were aversive whereas increasing doses of methylnaltrexone were positively reinforcing. Since naltrexone has almost no agonist activity on the opiate receptor, we suggest that the place aversions observed were due to an antangonism by the antagonists of endogenous opioid peptides acting on central opiate receptors. The preferences produced by methylnaltrexone must be due primarily to its blockade of peripheral endogenous opioid actions on peripheral opiate receptors. Interestingly, one anomalous result occurred at 0.1 mg/kg of naltrexone (i.p.), but not (s.c.), which produced a place preference. We hypothesized that this was due to a local block of aversive opiate effects in the gut, without significant central effects at this low dose. Based on this hypothesis, we predicted that a low dose of morphine (i.p.) should produce aversions. A dose of .05 mg/kg of morphine (i.p.) produced significant place aversions. The same dose administered s.c. had no effect; higher doses of morphine (s.c. and i.p.) were positively reinforcing in the place preference paradigm. As a further confirmation of the hypothesis that opiate aversive effects are mediated through an action in the gut, we found that rats with subdiaphragmatic vagotomies did not show the aversion to saccharin seen in normal control animals when tested in the conditioned taste aversion paradigm after pairings of a saccharin taste with 15.0 mg/kg morphine (i.p.). We conclude that endogenous and exogenous opioids acting on peripheral receptors (primarily in the gut) produce aversive effects whereas opioids acting on central brain receptors produce positive reinforcing effects.

95.6 EFFECTS OF CEREBELLAR VERMIS LESIONS ON CAT AND SHOCK-ELICITED FEAR BEHAVIOR IN RATS. W. F. Supple*, M.S. Fanselow* and R. N. Leaton. Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Reports of cerebellar influences on affective behavior have been scattered through the literature. Berman, et. al. and Peters & Monjan report that vermis lesions "tame" previously intractable feral rhesus monkeys. Chambers & Sprague note increased "pleasure reactions" in cats following vermis ablation. The present experiments were initiated to determine more accurately the form of the alteration in affective behavior following vermis lesions in rats. One of a rat's species specific fear related behaviors is the immobility or freezing response. Therefore we examined the effect of vermis lesions on the rat's freezing response to various stimuli.

Vermis lesioned (N=20) and sham control rats (N=19) were placed in an observation chamber. A 4.5 kg active cat was placed in a larger enclosure that surrounded the observation chamber. Each rat's behavior in the presense of the cat was rated every 4 sec. for a total of 8 min. The vermis rats froze significantly less, spent more time near the cat, and defecated less than controls.

To assess fear to another stimulus the rats were placed in an operant chamber and given a single 1 mA shock of .75 sec. duration. Behavior was time-sampled every 4 sec. for 8 min. Time spent freezing was not different for vermis and controls. Two days later the rats were reintroduced to the chamber with no shock presented; substantial freezing did occur, however there were no group differences .

The lack of freezing and defecation in the vermis group is interpreted as indicating less fear to a predatory stimulus; the cat. That the animals could make the response (freeze) is indicated in the shock condition. Vermis lesions result in a less fearful animal when fear is indexed by a rat's freezing to a cat. The data add to the literature implicating the cerebellum in the expression of emotional behavior.

95.7 EFFECTS OF REWARD-RELATED HYPOTHALAMIC STIMULATION ON NEURON ACTIVITY OF THE MOTOR CORTEX IN THE MONKEY. S. Aou*, C. D. Woody, Y. Oomura and H. Nishino* (SPON: K. Uchizono) Dept. of Biological Control System, National Inst. Physiol. Sci., Okazaki 444, Japan and Dept. of Anatomy and Psychiatry, UCLA Med. Ctr., Los Angeles, CA 90024.

Connection between the motor cortex and the lateral hypothalamic area in the monkey have been revealed anatomically (Kievit, J. and Kypers, H. G. J. M., Brain Res., 85:261, 1975) and electrophysiologically (Oomura, Y. et al. Brain Res Bull., 5(suppl.4):151, 1980). But its physiological significance is poorly understood. The present study investigated intracellular responses of the motor cortical neurons to hypothalamic stimulation that was effective in producing intracranial self-stimulation (ICSS) in awake monkeys.

One hundred and twenty five neurons which showed action potentials of more than 20 mV (mean 43 mV, max. 78 mV) and clear shift of base-line potential (mean 45 mV) were analyzed. Lateral hypothalamic stimulation, which was effective for ICSS, evoked action potentials with short latency (less than 20 ms) in 52 of 98 motor cortical neurons tested. Hypothalamic stimulation, which was not effective for ICSS, evoked short latency excitation in significantly smaller number of cells (4 out of 27 cells) tested ($p<0.01$). Excitation with long latency (more than 20 ms) was elicited in about 10% of neurons tested by both types of stimulation (9 of 98 cells in ICSS-related hypothalamic stimulation and 3 of 27 in non-ICSS-related hypothalamic stimulation). Spontaneous firing of 13 of 98 and 2 of 27 neurons was suppressed by ICSS-related and non-ICSS-related hypothalamic stimulation, respectively. The incidences of long latency excitation or inhibition were not significantly different between the case of ICSS-related hypothalamic stimulation and that of non-ICSS.

Short latency (less than 20 ms) activation of neurons of the motor cortex by hypothalamic stimulation is predictive of loci of stimulation within the hypothalamus that accelerates the rate of acquisition of a classically conditioned eye blink reflex in the cat (Woody, C. D. et al., J. Neurophysiol., 49:780, 1983). The present results suggest that neurons of the motor cortex receive hypothalamic information related to ICSS through short latency activation in the monkey. Thus the functional significance of hypothalamic activation of motor cortical neuron with short latency may be extended to the case of operant conditioning in the monkey.

96.1 INTERHEMISPHERIC TRANSFER OF VISUAL DISCRIMINATION LEARNING IN PIGEONS AND DUCKLINGS. S.Watanabe and F.Murakami*. Dept.of Psychology, Keio Univ.,Mita,Minato-Ku,Tokyo,Japan.

If memory trace of monocularly trained discrimination is formed only in the contralateral hemisphere to the training eye in birds,DSO section after the monocular training willcause a failure of interhemispheric transfer of the discrimination. Whereas,if memory trace is formed in both hemispheres,DSO section after the monocular training will not disrupt the transfer.

Exp.1. Two groups of pigeons were monocularly trained on horizontal vs vertical line discrimination in an operant chamber with a single key. One group received DSO lesion before the monocular training and the other group receivd DSO lesion after accomplishment of the monocular learning. Then,all subjects were retrained with the previously untrained eye.

Both groups showed a failure of interhemispheric transfer. However,successful transfer was observed when the lesion was restricted to the dorsal part of DSO.

Exp.2. Two groups of newly hatched peking ducklings were monocularly exposed to a blue ball or a yellow cube for 48hr. One group received DSO lesion just after hatching and the other group received the lesion after 48hr monocular exposure. Then a choice test between two objects,one was imprinted object and the other was novel,was carried out.

Both groups showed preference of the imprinted object when tested with the trained eye,whereas they did not show such preference when tested with the untrained eye.

Conclusion. Results of the two experiments support the idea of unilateral memory formation with monocular learning in birds and suggest that successful interhemispheric transfer in intact animals is due to bilateral read-out of the lateralised memory through DSO.

96.2 EARLY EXPERIENCE, SEX, AND HIPPOCAMPAL ASYMMETRY IN THE ALBINO RAT. G.F. Sherman, M.E. Hasselmo*, and A.M. Galaburda. Department of Neurology, Beth Israel Hospital and Harvard Medical School, Boston, Ma.

The hippocampus is thicker on the right side in both infant and adult male rats, and is thicker on the left side in 90-day old females (Diamond et al., Exp. Neurol., 76: 553, 1982). Another report, however, has found that the left hippocampus is heavier in male rats (Valdes et al., Physiol. Behav., 27: 381, 1981). We, therefore, measured the total volume of the hippocampus in order to further explore the hippocampal asymmetry in regard to sex and to examine the influence of early experience.

Twenty-nine brains were obtained from adult male and female Purdue-Wistar rats that were either handled in infancy and raised in enriched environments (HEE), or not handled and raised in standard lab cages (NHLC). The volume of the hippocampus was calculated from measurements in whole brain serial sections. A measure of asymmetry was calculated using the formula (R-L)/ .5x (R+L) x 100. A positive score indicated a larger right side and a negative score a larger left. Symmetry was judged to be present when the score was less than 0.5.

The right hippocampus was larger in volume than the left in HEE rats. Thirteen animals had a larger right hippocampus, 1 had a larger left, and 3 were symmetrical. The mean asymmetry quotient was 1.1 and was significantly different from zero (t=3.56, df=16, $p< .005$). There was no difference between the HEE males and females (t=1.10, df=15) and there was a significant difference between the early experience groups ($F(1,25)$=7.59, $p< 0.01$). NHLC animals in this sample showed no significant asymmetries, although there was a suggestion of a left bias in NHLC females (mean= -2.10, t=2.03, df=4, $p< .11$).

Another sample of nonhandled animals (10 males and 10 females) was measured and a similar distribution was seen. In NHLC males the asymmetry quotient was -0.6 and was not significantly different from zero (t=0.63, df=9), whereas the quotient of -1.4 in females was significantly different from zero (t=2.23, df=9, $p< 0.051$). The left hippocampus also was larger when the nonhandled females were combined over the 2 samples (mean= -1.6, t=3.09, df=14, $p< 0.01$). When the NHLC asymmetry is compared to the HEE asymmetry it is apparent that handling plus enrichment shifts hippocampal asymmetry towards the right side.

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96.3 SEX DEPENDENT BEHAVIORAL RESPONSE TO FRONTAL CORTICAL SUCTION LESIONS IN THE RAT. J. R. Lipsey* and R. G. Robinson (SPON: J.R. DePaulo). Dept. of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD, 21205.

It has previously been reported (Pearlson GD, Robinson RG, Brain Res., 218:233, 1981) that suction lesions of the right frontal cerebral cortex in the male rat produce spontaneous hyperactivity and bilateral hemispheric decreases in norepinephrine concentrations, whereas left frontal cortical suction lesions produce neither of these effects. In the present experiment, a search for similar asymmetrical responses was made in female rats.

26 female and 15 male Sprague-Dawley rats (225-250g and 250-275g, respectively) were housed for 8 weeks acclimatization in individual mesh cages with running wheels. After i.p. chloropent anesthesia, female animals were given 1.5mm right frontal cortical suction lesions (N=8), left frontal cortical suction lesions (N=9), or sham surgery (N=9). Males were given the same right lesions (N=7) or sham surgery (N=8). Over a 36 day post-operative period, 4 day mean activities were measured as percent of 8 day mean pre-op activities. (Four day means were used because of the 4 day female estrus related activity cycle.) Postoperatively, right or left operated female animals had no significantly different running wheel activity from female sham controls ($F_{14,161}$=1.01,NS). Right operated male animals were significantly hyperactive compared to male sham controls ($F_{8,104}$=2.43,$p<.025$). When male and female right operated animal activity was compared (as percent of control), males were significantly more active than females ($F_{7,91}$=3.26, $p<.01$). Thus, the previously documented asymmetrical response to frontal cortical injury in the rat appears to depend on sex. (Data concerning brain catecholamine changes following the above surgery will be presented at the annual meeting.) (Supported by NIH Grants NS15178, NS18622, and RSDA MH00163.)

96.4 AUDITORY LATERALIZATION IN JAPANESE MACAQUES AND ITS RELATIONSHIP TO CORTICAL AND SUBCORTICAL ASYMMETRIES. G.D. Rosen, M.R. Petersen*, F. Aboitiz* and A.M. Galaburda. Neurological Unit, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

The two hemispheres of the human brain are functionally and anatomically asymmetrical -- the left hemisphere of most individuals is specialized for language, as determined by, among other means, dichotic listening studies. Anatomically, the left planum temporale is larger than the right in approximately 65% of studied brains. Cytoarchitectonic area Tpt can be as much as 620% larger on the left and its size correlates with that of the planum temporale (Geschwind and Levitsky, Science, 161: 186, 1968; Galaburda et al., Arch. Neurol., 35: 812, 1978). It has been speculated that these neural asymmetries may underlie the language lateralization.

In Japanese macaques, Petersen et al., (Science, 202: 324, 1978) reported auditory asymmetries of species-specific vocalizations. These animals, as well as old world monkeys, were trained to discriminate between acoustic features of field-recorded Japanese macaque vocalizations. All macaques exhibited a right ear advantage in the processing of the stimuli while only one of the five old world monkeys showed any ear advantage. These data were interpreted to indicate that processing of species-specific vocalizations by Japanese macaques preferentially takes place in the left hemisphere.

The present experiment was designed to determine whether there were any subcortical or cortical asymmetries which might underlie this auditory asymmetry among Japanese macaques. The brains of five Japanese macaques previously tested for auditory asymmetries were embedded in celloidin and sectioned at 35μ with every 20th section (for cortical architecture) and every 5th section (for subcortical structures) stained for Nissl substance and the adjacent sections stained for myelin. In the auditory cortex the following areas were parcellated after Galaburda and Pandya (J. Comp. Neurol., 221: 169, 1983) and their volumes determined: reIt, paI, proA, paAlt, paAc, paAr, KA, Tpt, and Ts3. In addition, area 17 was measured as a control. Subcortically, the medial geniculate nucleus and the inferior colliculus were measured.

Preliminary results indicate no relationship between the auditory asymmetries and the cortical asymmetries. These results and those of the subcortical asymmetries will be discussed.

(Supported by NIH grants NS14018 and NS07211).

96.5 LATERALITY IN MONKEYS WATCHING AND REACTING TO TELEVISION. B. A. Vermeire, C. K. Ifune* and C. R. Hamilton. Division of Biology, Caltech, Pasadena, CA 91125.

Sixteen split-brain rhesus monkeys, eight males and eight females, viewed color videotape recordings of interesting stimuli with their left eyes, right eyes, and binocularly. Two similar stimulus tapes were used, each composed of 10 tape clips 1.5 min in length and containing subject matter such as monkeys, people, zoo animals, and scenery. Half of the monkeys viewed tape A monocularly and tape B binocularly, while the rest of the monkeys saw the converse. Vision was restricted to one hemisphere by temporarily suturing closed the lids of the opposite eye. While the monkeys watched television their behavior was recorded on a second videorecorder; the amount of time spent watching each tape clip and any facial expressions made were noted. Scores from the left and right hemispheres were compared by means of a dominance index, DI=100 (L-R)/L+R).

Across monkeys the two hemispheres differed slightly, but not quite significantly, in the amount of time watched (DI= -13.73, t_{15}=-1.92, $p<0.1$) in favor of the right hemisphere watching longer. Female monkeys watched significantly longer with their right hemispheres (DI=-18.84, t_7=-2.71, $p<0.05$), while males did *not* (DI=-8.63, t_7=-0.67, ns). Similarly, monkeys made more facial expressions (lipsmacks, yawns, threats, fear grimaces) while viewing with their right hemispheres than with their left; this difference almost attained significance for female monkeys (DI=-36.35, t_7=-2.18, $p<0.1$), but not for males (DI=-1.53, t_7=-0.08, ns).

The profiles of the two hemispheres in terms of relative time spent watching individual tape clips were quite similar, indicating that the two hemispheres had similar preferences. Furthermore, the amount of time the monkeys watched binocularly was more similar in magnitude to the watching time of the right hemisphere than to that of the left, suggesting that the behavior of the whole monkey is more like that of the right hemisphere.

The monkeys' overall greater right hemisphere tendency to watch and react to color videotaped stimuli may reflect the right hemisphere advantage in processing emotional stimuli found with human subjects. In addition, the significant right hemisphere advantage found with female, but not male monkeys mirrors our previous result that female, but not male monkeys display a right hemisphere advantage in discriminating photographs of monkey facial expressions. Supported by USPH grant MH-34770.

96.6 INTERHEMISPHERIC INTEGRATION VIA BRAINSTEM CHANNELS AFTER COMPLETE FOREBRAIN COMMISSUROTOMY. J.J. Myers* (SPON: R.W. Sperry) Division of Biology 156-29, California Institute of Technology, Pasadena, CA 91125

In patients with complete section of the forebrain commissures, the contribution of brainstem channels to the integration of mental processing between left and right hemispheres can be segregated for separate study. Given the absence of right hemisphere speech in these patients, evidence of interhemispheric transfer can be obtained from spoken responses when input is projected exclusively to the right hemisphere.

The present study employed a newly developed technique for prolonging the exposure of visual input without attachments to the eye (Myers & Sperry, Beh. Res. Meth. & Instr. 14: 305-308, 1982). Three complete commissurotomy subjects (NG, LB and AA) were asked to verbally describe selected pictures and printed names of foods, animals, and other items presented to the right hemisphere through the left hemifield. Subsequent responses were also obtained to oral follow-up prompts and questions. The verbalizations of the patients were assessed for evidence of interhemispheric transfer taking into account other available cues and possible elaboration by the left hemisphere.

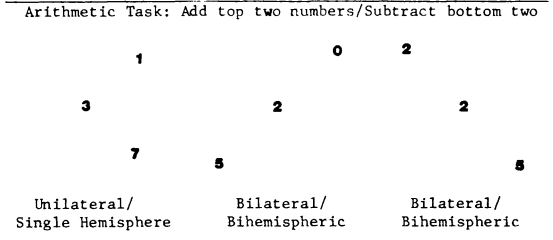
All three commissurotomy subjects, although unable to name or precisely identify the left hemifield stimuli, were nonetheless able to orally provide relevant information regarding the pictures and words presented to the right hemisphere. This information was generally connotative in nature and included evaluations, category and likely context of the stimulus, and extended to tastes, smells and other specific associations.

Taken together with occasional facial responses and cuing behaviors, the findings further demonstrate the capacity of the commissurotomized right hemisphere to comprehend verbal material and make logical mental associations.

The results affirm that some aspects of right hemisphere cognition can cross through subcortical channels permitting the interhemipheric integration of emotional, orientational, semantic and other cognitive information at a moderately high level. Such brainstem communication presumably has functional significance for normal cognitive performance, as, for example, in helping to establish the proper context or mental set for the retrieval of memories or for the reception and interpretation of callosal transmissions.

96.7 CONTRADICTORY COGNITIVE TASKS ARE PERFORMED MOST EFFICIENTLY WHEN PRESENTED TO DIFFERENT CEREBRAL HEMISPHERES. J. Liederman* (SPON: M. Albert). Psychology Department, Boston University, Boston, MA 02215

This research links neuro- and cognitive psychology by asking whether performance of concurrent cognitive tasks can be facilitated by presentation of each task to a different hemisphere. 48 right-handed subjects were required to perform two contradictory arithmetic problems simultaneously presented for 100 msec. One problem required addition; the other subtraction. The numbers were arranged so that a digit at fixation had to be added to a top number and subtracted from a bottom number. In the Bilateral/Bihemispheric conditions the addition problem was presented to one visual field and the subtraction problem was presented to the other visual field (2° from center). In the Unilateral/Single Hemisphere conditions, the addition and subtraction problems were projected to one visual field/hemisphere combination (see figure below). The answers to the problems were restricted to the numbers 2, 3, and 4. The subject responded to the two problems by pressing buttons on a keyboard. Which hand responded to each problem was varied between subjects. In terms of speed of response, the Bilateral/bihemispheric condition was associated with significantly faster reaction times than the Unilateral/single hemisphere condition for the subtraction problems (F (1,44) = 7.63, $p < .01$). This increase in speed was not at the expense of accuracy. A greater number of problems were correctly solved during the Bilateral/bihemispheric trials than during the Unilateral/single hemisphere trials. These data suggest that dividing input so that each hemisphere is confronted with a task requiring one kind of cognitive operation facilitates performance, perhaps by minimizing inter-task interference.



96.8 TRANSMISSION TIME BETWEEN HEMISPHERES: EVALUATION BY VIBROSENSORY EVOKED POTENTIALS. P.S. Gott,* E.C. Hughes and R.L. Binggeli*. Depts. of Neurology, Otolaryngology and Anatomy and Cell Biology. Univ. of Southern California Sch. of Med., Los Angeles, CA 90033.

Vibratory somatosensory evoked potentials (VSEP) were recorded to delineate normal interhemispheric transmission time (ITT) and to assess the overall characteristics of the procedure as a possible clinical test. Two different vibratory sources, an audiometer bone oscillator or an Optacon were used to stimulate each index finger independently. ITT was calculated by subtracting the latency of the first major peak over the sensory-association area contralateral (CL) to the stimulated finger from the latency of the corresponding peak over the homologous ipsilateral (IL) area (IL-CL=ITT).

Readily identifiable aberrant values were observed and rejected from the measurements leaving ITT with a mean of 14.1 and 16.9 and S.D. of 5.5 and 5.8 for the auditory bone oscillator and Optacon, respectively. Data classified as aberrant were in three categories: ITT $\leq$ -4, ITT 0+3 msec, and poorly-defined contralateral peaks. Clinical correlations of the VSEP were demonstrated by abnormal ITT in cases of agenesis of the corpus callosum and of Attention Deficit Disorder with Hyperactivity. Results suggest that important information relating to diagnosis and therapy of brain pathologies affecting interhemispheric transmission can now be made available in both experimental and clinical situations.

96.9 EVOKED POTENTIAL AND REACTION TIME MEASUREMENT OF INTERHEMISPHERIC TRANSFER TIME IN HUMANS. C. Seron*, P. D. Reiner* and R. J. Davidson* (SPON: C. Williams). Dept. of Psychology, SUNY Purchase, Purchase, N.Y. 10577.

Interhemispheric transfer time [IHTT] has been estimated by examining time differences between ipsilateral and contralateral responses to unilateral stimuli. Reaction time [RT] and evoked potential peak latency shifts have been used to measure IHTT.

This study utilized both measures simultaneously to compare them in the same group of subjects. Visual evoked potentials [VEP] and RT to hemiretinal stimuli were recorded in a group of nine right-handed males. A VEP index of IHTT was found to be more stable than RT differences.

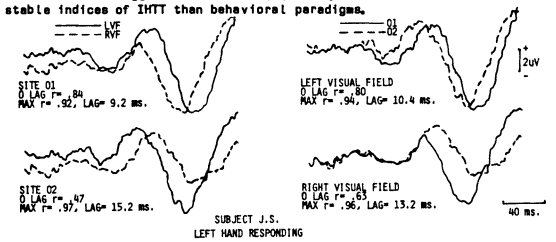
3.6° [H] by 3.0° [W] checkerboards were presented 2.9° to the left or right of central fixation for 10 ms. with an ISI of 1.5-3.5 sec. 100 stimuli were presented to one visual field at a time. Subjects lifted their left or right index finger [LH or RH] in response to each stimulus in separate trial blocks.

VEP's were derived by averaging EEG recorded from scalp sites O1 and O2 referred to linked ears for 200 ms. [0.4 ms. per point] following the onset of each stimulus. EOG was recorded from the external canthi, and from supraorbit to suborbit. Epochs confounded with eye movement were not averaged. RT was measured from stimulus onset to finger lift for the same trials. Median RT was computed for all conditions.

VEP latency differences between sites within a condition and between conditions within a site [see figure] separately by hand were examined by using a lag to maximum correlation procedure on selected portions [80 - 120 ms. and 120 - 175 ms.] of the waveforms. The max r lag time was taken as the index of IHTT if the cross-correlation exceeded 0.6 and if the zero r and max r values differed. 82% of the waveform comparisons met these criteria. The lag value for the earlier window was used when data from both windows met criteria (76% of the included comparisons). RT estimates of IHTT were computed as differences within a hand between visual fields.

Mean (SD) VEP IHTT estimates across all subjects, collapsed across all conditions, separately by hand were: LH VEP IHTT = 12.2 ms. (4.9 ms.) [zero lag r = 0.59 (0.36), max r = 0.93 (0.07)]; RH VEP IHTT = 11.2 ms. (4.4 ms.) [zero lag r = 0.58 (0.30), max r = 0.92 (0.08)]. Mean (SD) RT IHTT estimates were: LH RT IHTT = 15 ms. (18 ms.); RH RT IHTT = 2 ms. (18 ms.).

These data suggest that electrophysiological methods can provide more stable indices of IHTT than behavioral paradigms.



96.10 CEREBRAL UNILATERAL DRUG ADMINISTRATION: PHARMACOKINETICS OF HALOPERIDOL AND AMPHETAMINE. J.F. Hyde and T.P. Jerussi. Dept. of Pharmacology and Toxicology, Rutgers University College of Pharmacy, Piscataway, NJ 08854.

The pharmacokinetics of unilaterally administered 3H-haloperidol and 3H-amphetamine were investigated. The right common carotid artery of female Sprague-Dawley rats was cannulated using polyethylene (PE-50) tubing. The day following surgery, 3 μCi (10 μg) of either radiolabeled haloperidol or amphetamine were infused in a volume of 10 ul. Animals were decapitated 1, 10, 30, 60, 120, 240 or 480 minutes following 3H-haloperidol, and 1, 10, 30, 60, 90, 120 or 180 minutes following 3H-amphetamine. Right and left striata, anterior forebrains, posterior forebrains and cerebella were dissected, digested with Protosol, and counted by liquid scintillation spectroscopy.

One minute following haloperidol infusion, the cerebella had a 3-fold right/left difference, whereas a bilateral difference greater than 70-fold existed between the anterior forebrains. Moreover, a 90-fold difference was evident between the left and right striata and the left and right posterior forebrains. At later time points the right/left differences declined primarily as a result of the efflux of the drug from the right cerebral hemisphere. The pharamacokinetic parameters of the distribution and elimination phases in the right hemisphere were similar for each forebrain area. Amphetamine attained approximately a 40-fold right/left difference at one minute in all the forebrain structures, and the pharmacokinetic parameters were similar in these regions. These results indicate that preferential drug delivery by cerebral unilateral drug administration (CUDA) may serve as a useful technique to study functional and biochemical interhemispheric relationships as well as lateralized behaviors.

This research was assisted by NIMH grant 37488-01, Biomedical Research Support Grant PHS RR 07058-18, and Rutgers University Research Council Award 2-02214.

96.11 THE DEVELOPMENT OF ASYMMETRIES IN HUMAN FETAL BRAINS. M. Christine de Lacoste & D.J. Woodward, Dept. Cell Biology, U.T.H.S.C. at Dallas, TX. 75235

In this study, computer assisted 3-D reconstructions and quantitative morphometric techniques were used to: 1) investigate the pattern of pre-natal development of regional volumetric asymmetries in the human brain, and 2) determine if there are characteristic sex differences in these patterns. Our previous work has shown clear regional volume asymmetries in the adult human brain.

Sections of fetal brains from the Yakovlev collection (gestational age (GA) 15 wks. to birth; brain weight 4g to 450g) were photographed. The prosencephalic portions of every 30th section (either 15μ or 35μ in thickness) from both right (R) and left (L) hemispheres were digitized and their areas calculated. Volumes (LVOL and RVOL) were obtained by multiplying the areas by the appropriate z-dimension and summing them. Regional volumes, delineated on the basis of certain anatomical landmarks, were computed and analyzed using SAS and BMDP statistical packages. These landmarks varied with GA, but included the insula, hippocampus, genu of the corpus callosum, calcarine/parieto-occipital junction, etc.

All fetal brains examined so far were found to exhibit some degree of asymmetry such that the mean difference between R and L sectional volumes was statistically significant (p=.0001). However, a preliminary analysis of regional volumes suggests that in males, at least, the degree of R/L asymmetry increases with age. By 36 wks GA, there appear to be consistent R/L differences in the volumes of all regions (for $\bar{X}$ DIFF1 p=.004; DIFF1=$\bar{X}$ ABS(VOL LREG-VOL RREG); REG= region). Two of the regions manifesting a striking degree of asymmetry at 36 wks GA include the R peri-uncal to mid-hippocampal region (R>L by 16-17%) and the L parieto-occipital region (L>R by 14-21%). However, even at 36 wks GA the male fetal brain resembles neither the male or female adult brain in terms of its asymmetries. This preliminary finding of an increasing asymmetry beginning in males after 15 wks GA, suggests possible R/L differences in the rate of cell proliferation, or in other developmental processes such as cell death, growth in cell size, etc. Furthermore, this mechanism may operate differentially on male and female brains. To resolve these questions, future studies will implement of differential cell counts in regions with pronounced asymmetries. Supported by NSF #BNS 8316764 and Biological Humanics.

96.12 THE RELATIONSHIP BETWEEN CEREBRAL HEMISPHERE SPECIALIZATION AND COGNITIVE ABILITY IN YOUNG ADULTS OF SUPERIOR ACADEMIC ABILITY: I. LANGUAGE FUNCTIONS. Richard S. Lewis & Lauren J. Harris*. Dept. of Psychology & Neuroscience Program, Michigan State University, E. Lansing, MI 48824.

A prominant assumption in neuropsychology is that the pattern of cerebral lateralization of cognitive functions is related to cognitive ability. For example, Levy & Gur (1980) proposed that bilateral representation of either verbal or spatial functions leads to incomplete specialization of the other function, resulting in superior ability of the bilaterally represented function and a relative deficit of the asymmetrically represented function.

To date, many studies have investigated this hypothesized relationship, but most have been beset with methodological problems (Lewis & Harris, submitted). One problem may be the failure to control for the subjects' reasoning level. Recent evidence suggests that the relationship between sex, handedness, and cognitive performance is stronger in individuals with high than with low reasoning ability (Harshman, Hampson, & Berenbaum, 1983).

As part of an ongoing investigation of these questions, the present study investigates the relationship between cerebral lateralization and cognitive ability for language functions in subjects with high achievement as indexed by performance on the ACT.

Subjects are 80 Michigan State University (MSU) freshman, including equal numbers of right- and left-handed men and women. All students had ACT composite scores in the upper 15% of entering MSU freshman (ACT score of 27 or above). Lateral organization of language functions was indexed by a tachistoscopically projected lexical decision task. Tests of verbal ability included: 1) the Controlled Oral Word Association Test of verbal fluency; 2) the Vocabulary subtest of the WAIS; 3) the Similarities subtest of the WAIS, a test of verbal abstract reasoning.

Results of these and further tests will be presented.

96.13 COMPUTED TOMOGRAPHY CRITERIA OF HEMISPHERE ASYMMETRY AND LANGUAGE LATERALITY IN CROSSED APHASIA. V.W. Henderson. Dept. of Neurology, Univ. of Southern California School of Medicine, Los Angeles, CA 90033

Morphologic and radiologic asymmetries between the two cerebral hemispheres are well documented, but the functional significance of such asymmetries is unknown. Although it has been suggested that "atypical" asymmetries might reflect atypical interhemispheric or intrahemispheric patterns of language representation, this association has not been convincingly demonstrated. Our previous computed tomography (CT) study of crossed aphasics (right-handers with aphasia from right cerebral hemisphere lesions), which used 4 measures of CT asymmetry, failed to confirm that CT asymmetries correlate with language laterality (Henderson, V.W., et al, Neurology, 33-Suppl. 2:104, 1983; Neurology, in press). Other investigators, however, have used different criteria of CT asymmetry.

In the present study, 24 different linear measures of CT asymmetry were determined from CTs of 18 crossed aphasics. 4 CTs were from personal cases; others were generously provided by colleagues. Measures from this right hemisphere language-dominant group were compared to corresponding measures published by other investigators for right-handed adults known or presumed to be left hemisphere language-dominant.

Several comparisons differed significantly between right and left hemisphere language-dominant groups (Fisher's Exact Test), but there was considerable overlap between groups on all measures. Results suggest that there are radiographically discernable differences between the brains of right and left hemisphere language-dominant adults. However, exact neural structures responsible for any group differences remain unknown. Moreover, present findings support our previous conclusion that for individual subjects language laterality cannot be reliably inferred from CT criteria of hemisphere asymmetry.

Among crossed aphasics, atypical asymmetries may have occurred more often in patients who did not show signs of unilateral neglect, but the number of such cases was too small to permit conclusions concerning CT correlates of hemispheric dominance for visuospatial and attentional functions.

96.14 LEFT HANDEDNESS IN COMPULSIVE GAMBLER SOCIOPATHS E. Ziskind and I. Maltzman, Gateways Community Mental Health Center and University of California at Los Angeles

The data we have which are new are the Handedness findings in three separate sets of compulsive gambler sociopaths. The findings in three separate studies with matched controls are that 18%, 22 percent and 23 percent of the compulsive gamblers are left handed or ambidextrous.

The experimental population consists of compulsive gambler male members of Gamblers Anonymous softball teams who play ball out-of-doors on Sundays. They are individuals who are matched for age (18 to 35), race (Caucasian), and are gamblers currently on the GA program, not gambling, who are left handed or ambidextrous matched against those right handed, as checked on the ball field and by the Edinburgh Handedness Inventory. They conform to the items of our research definition of sociopaths (antisocial personality disorders). The findings demonstrate a statistically significant increase in the incidence of left handed and ambidextrous individuals as compared to right handers.

To avoid the diagnostic complexity of much of the modern studies of handedness, e.g., the Nobel Prize-winning awards of Roger Sperry and others on the split-brain preparation, for this report we are restricting ourselves to items which we believe add to the accuracy of our findings. These items are:

(1) Slantedness of the writing specimens and the history of their ontogenetic development, and

(2) The ability to perform as southpaws on the baseball team.

The remarks are of considerable moment to our research group for we have as our goal the cure of the compulsive gamblers.

Neither we nor others have as yet found the cure for compulsive gambling. The best result comes from Gamblers Anonymous, which is a maintenance therapy, not a cure. We are looking forward to the analysis of enkephalins as a possible therapeutic approach to the gambling addiction. Endorphin assays on our patients point in that direction.

HUMAN NEUROPSYCHOLOGY AND BEHAVIORAL NEUROBIOLOGY I

97.1 **A COMPARISON OF ELECTROPHYSIOLOGICAL EFFECTS OF METHYLPHENIDATE AND SODIUM VALPROATE IN HYPERACTIVE CHILDREN.** M. Morag, Y. Frank* and M.S. Myslobodsky. Psychobiology Research Unit, Dept. Psychol. and Dept. Neurol., Meir Hospital, Tel-Aviv University, Israel.

Although children with hyperactivity and poor academic performance, designated as Attention Deficit Disorder (ADD), seem to represent a heterogenous group, little effort was made to differentiate between ADD subtypes. The EEG and neurological screening conducted by the present authors showed that among 20 children referred due to hyperactivity and/or learning disability, 6 (30%) had epileptiform abnormalities (neuroepilepsy [NE] group). None of the children had clinical seizures nor were any on antiepileptic medications. All 20 children subsequently underwent a routine examination of Visual Evoked Potentials (VEP) recorded bilaterally from the occiput. In 65% of these, VEPs differed from controls owing to enhanced amplitude of components N2, N3 (250 and 400-600 msec, respectively) and a simplified pattern approaching spike-wave shape described in some epileptic patients. Subsequent examination of children of the same age (8-12 yrs), with ADD and without neurological signs yielded spike-wave shaped VEP in 7 (35%) cases. EEG in this group was normal except for occipital slowing, nonreactive alpha rhythm (n=1) and lateral alpha asymmetry of more than 50% (n=1). Hence, this study yielded two groups matched for age, sex, learning problems and brain reactivity. We then compared the pharmacological reactivity of these groups under conditions of an open single-dose trial with either an indirect dopamine agonist, methylphenidate (10 mg, p.o., n=7) or the GABAergic agent, sodium valproate (10 mg/kg, p.o., n=6). Both groups showed a suppression of the negative waves at 250 msec and 400-600 msec. The effect was most reliable over the right hemisphere within 90 min after drug administration. Hence, regardless of the electrophysiological profile of ADD, some patients appear to show identical VEP abnormalities and similar responses to compounds acting through different neurochemical systems. It is proposed that ADD is associated with a complex abnormality in both catecholaminergic and GABAergic systems.

This work was partially supported by a grant from the Martin and Vivian Levin center, Jerusalem, Israel, to M. Morag.

97.2 LATERALITY IN CHILDREN ASSESSED BY THE EDINBURGH HANDEDNESS INVENTORY. G.N.O. Brito, L.C. Stopp* and F.J.R. Paumgartten*. Instituto Biomedico, Universidade Federal Fluminense, Niteroi, RJ 24210, Brazil.

There are two main hypotheses concerning the development of hemispheric lateralization. The progressive lateralization theory states that the higher functions of the brain are initially represented bilaterally and are not fully lateralized until several years after birth. Conversely, the invariant lateralization theory states that higher brain functions are lateralized at birth. Since there is a relationship between lateralization of brain function and handedness, studies of hand preference in children provide tests of the theories discussed above.

In the present study, we investigated the laterality of 159 boys and 182 girls ranging in age from 2 1/2 to 7 years. Hand, foot and eye preferences were assessed by the Edinburgh Handedness Inventory (Oldfield, Neuropsychologia, 1971, 9, 97) through direct observation of each child. Children were classified as left, mixed or right-handed according to procedures modified from Annett (Br. J. Psychol., 1970, 61, 303).

The present study showed that girls tended to be more dextral and less mixed handed than boys. Although there was no sex difference for eye preference, girls preferred to kick with the right foot more often than boys. When the handedness distribution for children was compared to the distribution that we obtained for adults (20-69 years), we found that children tended to be less dextral and more mixed handed than adults. Additionally, children preferred to use the left eye more often than adults, whereas we found no difference in foot preference between children and adults.

These results suggest that girls are more lateralized than boys and that children are not as fully lateralized or have reverse laterality (e.g., eye preference) when compared to adults. The results from the present study are consistent with the progressive lateralization theory of the development of higher brain functions. Supported by CNPq and PROPP-UFF.

97.3 DIFFERENTIAL HEMISPHERIC SENSITIVITY TO SPATIAL-FREQUENCY COMPONENTS OF VISUAL PATTERNS. J. Sergent, Montreal Neurological Institute, Montreal PQ H3A 2B4, Canada, and E. Switkes*, University of California, Berkeley CA 94720.

The efficiency of cerebral visual processing is dependent on the particular characteristics of the representations of information generated by the visual system, and on the stimulus attributes required in performing specific operations. Different tasks do not make the same demands in terms of stimulus components that must be processed, and the differential capacity of the cerebral hemispheres at carrying out certain operations may depend on their respective ability to process particular ranges of spatial-frequency spectral components of the input.

In a first series of experiments, a set of high-resolution black-and-white photographs of faces known to the subjects was used in 3 different reaction-time tasks with lateral tachistoscopic presentation in a within-subject design: verbal identification, professor/non-professor manual categorization, and male/female manual categorization. A right-visual field advantage prevailed in the identification and the first categorization, while the male/female categorization yielded a non-significant left-visual-field superiority. The results indicate differential efficiency of the hemispheres as a function of the stimulus attributes that must be processed.

In a second series of experiments, the stimuli were either digitized unfiltered (0 to 32 c/deg) faces or digitized low-pass filtered (0 to 2 c/deg) faces, all other experimental aspects being the same as in the first series. Digitized unfiltered faces yielded the same pattern of results as that obtained previously, whereas low-pass filtered faces were processed more slowly overall, and relatively more efficiently in left than in right field presentations. The latter effect was significant in all tasks, but not all subjects displayed the left field advantage in verbal identification.

The results are consistent with the view that the right hemisphere may be more sensitive than the left hemisphere to the outputs of low spatial-frequency channels of the visual system, and this may have predisposed the right hemisphere to a greater involvement in visuospatial operations which essentially require the processing of the low frequencies of a display.

97.4 RESIDUAL VISION IN AN AREA OF BLINDNESS DUE TO A LESION IN THE GENICULO-STRIATAL PROJECTION SYSTEM: ANOTHER REFUTATION OF THE LIGHT SCATTER HYPOTHESIS

P. Stoerig*, E. Pöppel, and M. Hübner*.

Institut für Medizinische Psychologie, 8 Munich 2 FRG

Scotomata in human patients resulting from lesions to the optic radiation or to the occipital lobe are not necessarily absolute (Pöppel etal., Nature 243, 1973). To test again whether residual vision is a genuine phenomenon or whether it is due to light scatter produced by the visual target (as suggested by Campion et al, Behavior. Brain Sci., 6:3, 1983) we studied a patient who had suffered a right occipital lobe infarction which led to an incomplete hemianopsia in the left visual field. The patient, while sitting at a Tübinger perimeter and fixating a central fixation spot with his right eye covered, was shown circular targets (44', 32 cd/sq.m.) for 200 msec each in his scotoma. The patient is informed that a randomly interspersed number of the targets consists of 'blank trials', and he is asked to guess every time he hears an auditory signal whether the target has been present or not (Yes-No-Procedure). By varying the ratio of the light-on to the light-off condition we obtained ROC-curves at four positions within his blind hemifield (20°, 30°, and 40° eccentricity on the 180° meridian, and the Blind Spot within the scotoma). We could show that with the exception of the Blind Spot discrimination was above chance. Thus, light scatter cannot account for an explanation of residual vision.

As to the mediating structures of this performance at least three possibilities have to be discussed none of which can be excluded so far: a) there could be a residual visual field representation of the striate cortex itself (Pöppel, Neurosc.Res.Pro.Bull., 15:3, 1977); b) the retinal projection via the superior colliculus and pulvinar system to extrastriate cortex (usually claimed to be responsible for 'Blindsight') might be responsible; c) the projection that directly connects the lateral geniculate body with the extrastriate cortex (Fries, Proc.R.Soc. London, B 213, 1981) might be involved.

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97.5 **FACTORS IN THE PERSISTENCE OF COGNITIVE DEFICITS 15 YEARS FOLLOWING PENETRATING BRAIN WOUNDS.** **J. Grafman, A. Salazar*, H. Weingartner*, and S. Vance*. Vietnam Head Injury Study, Walter Reed Army Medical Center, Washington, DC 20307.**

We examined the relationship of a preinjury variable (a summed score of intelligence), a lesion severity variable (lesion volume loss), and lesion location to the persistence of cognitive deficits in Vietnam veterans with penetrating brain wounds. We hypothesized that a summed score of intelligence would share a significant amount of the variance on postinjury cognitive testing, being greater for tests requiring a number of complementary cognitive processes, being less for tests measuring a specific cognitive process (e.g., face recognition). Volume loss was predicted to play a larger role when a global cognitive measure was utilized but a smaller role when a specific cognitive process was measured. Finally, lesion location was thought to play a major role only for specific cognitive processes. The statistical procedures we utilized to test these hypotheses were multiple and logistic linear regression.

Our findings supported our hypotheses. For example, preinjury AFQT (F=406.92, p=.0001), total brain volume loss (F=47.69, p=.0001), and to a lesser degree, left temporal-occipital white matter involvement (F=15.95, p=.0001) all contributed to postinjury intelligence test performance as represented by a global score (total R^2=.64). However, when regressed upon total errors from a face discrimination test, total brain volume loss did not contribute to the regression model (F=.06, p=.81), preinjury AFQT made a smaller contribution (F=32.30, p=.0001) to this model than when the dependent variable was a general intelligence measure, while several cortical structures including the right mesencephalon (F=19.92, p=.0001) made a relatively larger contribution to the model.

In conclusion, total brain volume loss and preinjury intelligence play a much larger role in the persistence of cognitive deficits when a postinjury intelligence measure is used, whereas lesion location plays a relatively larger role when a postinjury measure that evaluates a specific cognitive process is used. Measurement problems caused by the interaction of volume loss and lesion location will also be discussed.

97.6 MEMORY FOR SEMANTIC PICTORIAL ORGANIZATION IN PATIENTS WITH POSTERIOR HEMISPHERIC LESIONS. Dahlia W. Zaidel. Dept. of Psychology, UCLA, Los Angeles, CA 90024.

Successful retrieval of newly presented material depends, among others, on pre-existing experience-dependent long-term semantic memory (LTSM). The question of asymmetries in the storage of LTSM or retrieval from it was investigated in stroke patients with hemispheric damage.

The patients studied had damage in the parietal, occipital or occipital-parietal regions of either the left (N=7, $\bar{X}$ age=52) or right (N=4, $\bar{X}$ age=54) hemisphere. None suffered from sensory or motor deficits in the upper limbs nor from signs of dysphasia.

Ten pictorial stimuli composed of familiar figures but representing either organized (real life arrangement) or unorganized (random arrangement) scenes were presented to the patients and normal control subjects (N=13). The task was to recognize a single detail occurring in each stimulus as well as the whole pictorial scene in separate 5-choice arrays. There was a total of 20 responses, 10 for details and 10 for whole scenes.

In the control group, a significant dissociation between mean percent correct recognition reflected better memory for organized than for unorganized scenes. In the patient groups, only left-sided patients showed a similarly significant dissociation. Furthermore, both right-sided patients and normal control subjects recognized whole scenes better than details, regardless of organization, whereas left-sided patients had a poorer memory for whole unorganized scenes.

The results suggest that there exist asymmetries in storage or retrieval from LTSM. This extends to patients with posterior lesions conclusions reached earlier from temporal-lobe patients and from normal subjects about asymmetries in long term representation of experience. The results also suggest that left posterior regions play a role in processing patterned schemata representing unfamiliar organization.

97.7 METAMEMORY OF ALCOHOLIC KORSAKOFF PATIENTS. R. H. Bauer, D. Kyaw* and M. M. Kilbey. Department of Psychology, MTSU, Murfreesboro, TN 37132.

Awareness of one's own learning and memory abilities is referred to as "metamemory." Some researchers have suggested that alcoholic Korsakoff patients (AKP) are less aware of their own learning and memory abilities than are normal adults. Furthermore, confabulation may be a result of AKP distorted view of their memory abilities. However, others have suggested that AKP are aware of their learning and memory abilities. One purpose of the present study was to compare metamemory in AKP, alcoholic controls (AC), and nonalcoholic controls (NAC).

In many situations the learner is allowed to present the to-be-remembered information at their own pace (self-paced), whereas in other situations the material is presented at a rate determined by someone or something else (experimenter-paced). A number of experiments have compared recall by AKP and NAC in experimenter paced tasks, but there appears to be no research comparing recall of AKP and NAC in self-paced tasks. A second purpose of the present study was to compare recall of AKP, AC, and NAC in experimenter- and self-paced tasks.

The subjects were AKP, AC, and NAC (n = 8 per group) matched for age, sex, race, education, and IQ. Each subject was given 16 trials in which lists of 9 words each were presented by a slide projector. Immediately after the last word of each list, the subjects recalled the words in any order. On one-half of the trials the experimenter presented the words at the rate of one word per 2 sec. and on one-half the trials the subject presented the words at their own pace. Immediately prior to one-half the experimenter- and subject-paced trials the subjects were asked to estimate the number of words they could recall on that trial, whereas on the remaining trials they were not required to estimate the number they could recall.

Analysis of the difference scores between the actual number of words recalled and the estimated number of words recalled showed that AKP tended to over estimate the number they could recall. These findings suggest that AKP are less aware of their memory abilities. On both experimenter- and subject-paced trials AKP recalled fewer words than AC and NAC. Allowing AKP to present the information at their own rate did not improve their recall, suggesting that AKP do not attempt to compensate for their memory problems by studying the information for a longer time.

97.8 LEVELS OF PROCESSING BY ALCOHOLIC KORSAKOFF PATIENTS. D. Kyaw*, R. H. Bauer, M. M. Kilbey. Department of Psychology, MTSU, Murfreesboro, TN 37132.

The levels of processing hypothesis proposes that alcoholic Korsakoff patients (AKP) store verbal information at a lower level, e.g., on the basis of sound, whereas nonalcoholic controls (NAC) store verbal information at a deeper level, e.g., on the basis of word meaning and associations among words. On the basis of this hypothesis, recall of words that rhyme would be expected to be comparable in AKC and NAC whereas recall of words that are associated (cigar, smoke, puff) and words from the same category (trout, bass, tuna) would be expected to be higher in NAC than AKP. The major purpose of the first experiment was to compare recall of rhyming, associated, and category words by AKP, NAC, and alcoholic controls (AC).

The subjects were AKP, AC, and NAC that were matched for age, sex, race, education, and IQ (n = 8 per group). Each subject was given 16 trials of 9 words each. Each list had 3 words that rhymed, 3 words that were associated, and 3 words from the same category. Words of each type were distributed randomly within each list. The subjects were not informed about the nature of the word lists. The words were presented on a screen at the rate of one word per 2 sec. Immediately after the last word of each list the subjects recalled as many words as possible in any order. The number of words recalled was significantly lower in AKP than AC and NAC. In accord with the levels of processing hypothesis, recall of rhyming, associated, and category words was comparable in AKP, but recall of associated and category words was significantly higher than recall of rhyming words by NAC. There was no significant difference in recall of rhyming, associated, and category words by AC.

Interviews after testing revealed that AC and NAC became aware that the word lists had words that rhymed, were associated, and came from the same category, but AKP were often only aware the same words rhymed. In Experiment 2 the subjects were informed that each list had 3 words of each type, and they were then given 16 additional trials. Recall of rhyming, associated, and category words was comparable in AKP and recall of associated and category words were higher in NAC than recall of rhyming words. AC recalled more category words than rhyming or associated words. Informing AKP about the word lists did not increase recall of associated and category words suggesting that lower recall by AKP is not due to lack of awareness concerning the word lists.

97.9 DEFICITS IN OLFACTORY,BUT NOT VISUAL OR VERBAL RECOGNITION IN EARLY AFFECTED HUNTINGTON'S DISEASE PATIENTS. P.J. MOBERG*, G.D. PEARLSON, L.J. SPEEDIE*, J.R. LIPSEY*, S.E. FOLSTEIN*. Johns Hopkins Med. Inst., Depts. of Psychiatry and Medical Psychology, Balto., MD 21205

9 early affected Huntington's disease (HD) patients and 10 age matched normal controls were compared on 3 similarly structured recognition tasks. All HD patients were Stage I or II (Shoulson, 1981, Ann. Neurol.). Patients and controls were tested with the Mini-Mental State Exam (MMSE) (Folstein, Folstein and McHugh 1975, J. Psych. Res.), a verbal recognition paradigm derived from the Rey Auditory Verbal Task (Rey, 1971), and a visual design recognition task derived from Kimura's figures (used with permission).

Subjects and controls, were all screened with and performed normally on an odor discrimination task, and were then tested with an olfactory memory task using 10 target odorants presented in identical masked bottles. Participants were asked to remember, but not to name the target odors; and were presented 5 minutes later with 20 odorants including the original 10, plus 5 odors similar to 5 of the target odors, and 5 which were dissimilar. Subjects were asked to identify, without naming the odors initially presented. Recognition accuracy was calculated using the d' score for all 3 recognition tasks.

All participants scored in the normal range ($\geq$25) on the MMSE. Patients and controls did not score significantly differently on the verbal or visual recognition tasks, but HD patients scored significantly worse on the olfactory memory task (1.41±0.26 vs 2.69±0.62: $p < .001$, 2-tailed). A d'cutoff of$\leq$1.80 on the olfactory memory task correctly identified all 9 HD patients, and misclassified only 1 normal control.

97.10 SUBGROUPS OF ALZHEIMER'S PATIENTS: NEUROPSYCHOLOGICAL AND CEREBRAL METABOLIC PROFILES. A. Martin*, P. Brouwers*, F. Lalonde*, C. Cox*, N.L. Foster*, T.N. Chase, and P. Fedio* (SPON: H. Lansdell). Intramural Research Program, NINCDS, NIH, Bethesda, MD 20205

Patients given the presumptive diagnosis of Alzheimer's disease (AD) were evaluated on measures of neuropsychological functioning, cerebral glucose metabolism, and cognitive decline after a 1 to 2 year interval to determine whether qualitatively different subgroups could be identified. Data from 43 AD patients on three verbal (word production) and three nonverbal (visuoconstructive and pattern discrimination) tests were factor analyzed to obtain individual factor scores which were utilized in a cluster analysis. Five patient groups were identified and then verified by a discriminant analysis which correctly reclassified 42 of the 43 patients. In comparison to matched normal controls (N=18), the majority of patients (N=25) fell into three groups characterized by relatively equal impairment on all tasks, with group differences ascribable to overall severity of deficits. In addition, two other groups, each with 9 patients, were identified. One group was characterized by severely impaired verbal abilities, but with intact perceptual and constructive skills; the other group by greater impairment on nonverbal than on verbal tasks. In contrast to these qualitatively different cognitive profiles, all patients presented with memory deficits which were usually not material specific. Thus, memory and learning, per se, were poor indicators of group membership.

Preliminary analysis of positron emission tomography data ([^{18}F]FDG), from 19 patients indicated that, in comparison to normals (N=7), patients with homogeneous cognitive deficits exhibited bilateral and relatively symmetrical hypometabolism in the temporal and parietal cortex. Patients with primarily perceptual and constructional deficits had greater hypometabolism of the right temporal and parietal regions, while patients assigned to the language impaired group exhibited decreased metabolism primarily of the left temporal lobe. Frontal areas were less affected and did not discriminate among subgroups.

Finally, test-retest comparison of the cognitive data revealed differential patterns of decline. Specifically, while the majority of patients showed progressive deterioration on all measures, patients in the language impaired group exhibited continued worsening of verbal abilities with negligible decline on nonverbal tasks.

97.11 POST-STROKE MOOD DISORDERS IN LEFT HANDED PATIENTS. R.G. Robinson, J.R. Lipsey*, and T. R. Price*. Depts. of Psychiatry and Neuroscience, Johns Hopkins Univ Sch Med, Baltimore, MD 21205, Depts. of Psychiatry and Neurology Univ of Maryland Sch Med Baltimore, MD 21201.

Previous studies of the effect of lesion location on post-stroke mood disorders in right handed patients have demonstrated that symptoms of major depression occur most frequently in patients with left anterior lesions (Robinson, RG et al, Brain 107:81, 1984). In the present study, we examined the relationship between handedness and post-stroke mood disorder. Twenty-one left handed patients having either a left (N=12) or right hemisphere (N=9) ischemic infarct or intracerebral hemorrhage were examined. The two groups of patients were not significantly different in background characteristics, time since stroke, severity of functional physical impairment, or intellectual impairment (measured by Mini-Mental Exam). The diagnosis of depression was based on DSM III symptom criteria. Clinically diagnosable depression was significantly more common in left hemisphere lesion patients (major depression=4, minor depression=3, non-depressed=5) than in right hemisphere lesion patients (major depression=1, minor depression=0, non-depressed=8) (x^2=4.93 df=1, p<.05). Based on previously published findings in right handed patients (Robinson, RG et al, Brain, ibid.), we predicted the likelihood of patients with right or left hemisphere lesions having depressive disorders by assuming that 60% of left handed patients would have the usual left hemisphere dominance pattern but that 40% of the patients would have a reversal with right hemisphere dominance for both language and depression. Based on this assumption, for example, we would expect that 2.0 patients with left hemisphere lesions would have major depression. The observed frequency was significantly different than the expected distribution x^2=6.07 df=2, p<.02). In contrast, however, the expected distribution of major, minor and no depression for right handed, left cerebral dominant patients was not significantly different than the observed distribution for left handed patents (x^2=3.8, df=2 p>.1). Of the left hemisphere lesion patients, 4 of 12 were aphasic while none of the right hemisphere lesion patients had this finding. Although this may mean that these left handed patients did not have right hemisphere dominance, the results suggest that the emotional response to unilateral cerebral injury may be independent of cerebral dominance for language.(Supported by NIH Grants NS15178, NS18622, RSDA MH00163.)

97.12 CATECHOLAMINE BRAIN LEVELS IN PHYSICAL AND PSYCHOLOGICAL STRESS. C. McChesney*[1,2], F. Petty[1,2] and G. Kramer*[2]. [1]Dept. of Psychiatry, Univ. of Iowa Col. of Med. and [2]Veterans Administration Medical Center, Iowa City, IA 52240.

In an attempt to distinguish physical and psychological stress by biochemical means, we measured brain catecholamine levels in rats exposed to either physical stress (foot shock), psychological stress (new environment), and a mixture of physical and psychological (re-exposure to an environment previously associated with physical trauma).

We initially used four groups of four Sprague Dawley rats. The control group (C) received no experimental manipulation. The boxed group (B) was placed in a new environment -- a shock-box but received no shock. The shocked group (S) was boxed and received 20 minutes of pulsed foot-shock as per Herman et al (Life Sciences, 1982). The re-exposed group (R) was shocked on day one identical as group S and on day 2 was re-exposed to the same environment but received no shock. Three brain regions (frontal neocortex, hypothalamus, septum) were dissected. Three neurotransmitters, norepinephrine, dopamine (DA) and serotonin, along with some metabolites, were analyzed by HPLC. In addition, the frontal neocortex region was analyzed for catecholamines by two groups of four rats: one received lorazepam (LZP) 1 mg/kg IP and 20 minutes of pulsed foot-chock as per Herman et al (1982) and the other group received LZP 1 mg/kg) and were killed 20 minutes later.

There was no change in serotonin by any of the three stressors in the three brain regions. 5HIAA was nonspecifically elevated by all three stressors (B, S, R) in the neocortex and septum. 5HIAA/5HT ratio was nonspecifically elevated in all three brain regions by all three stressors. Shock (S) -- physical stress -- elevated DA in hypothalamus, increased DOPAC in hypothalamus and septum, and increased DOPAC/DA in neocortex. All three stressors (B, S, R) elevated DOPAC/DA in the hypothalamus. Norepinephrine was increased in the hypothalamus and septum in the R group but decreased in the hypothalamus only in the S group. The LZP + shock and LZP alone groups had significantly lower DOPAC/DA levels in the neocortex than did the shock alone group and significantly lower 5HIAA levels than B, R, or S groups in the neocortex.

97.13 BIOCHEMICAL CHANGES DURING A DACRYSTIC REGIMEN. D. A. Goodman. Newport Neuroscience Center, Culver City, CA 90230.

The neurobiology of human dacrystic states (weeping and agonized crying) is a research area incompletely studied in the laboratory. The present study examined the biochemical sequelae of patients in a 12-month dacrystic regimen.

Patients (n=27)admitted successively to a clinic were classified according to their induced behavioral responses. A cartography of dacrystic states was organized according to the perceived intensity of weeping. Responses varied from a mild suffusion of the eyes to infant-like automatized thrashing of limbs associated with agonized crying that is the most intense dacrystic response. In addition,at selected intervals -- during weeks 1,4, 13,26 and 52 -- blood samples were taken and analyzed for a number of circulating neurohormones including human growth hormone, testosterone (in males) and catecholamines by radioimmunoassay.

Data analysis revealed three distinctive relationships between early onset of an intense dacrystic response and blood biochemistry. Those patients capable of agonized crying by the end of the 4th week of the clinical regimen showed a significant increase in hGH levels not evident in the non-weepers. Early onset and persistence of agonized crying was related to a fall of an average of 30% in circulating epinephrine levels (p=.05). Another finding of interest is that among the male patients (n=18) induced weeping and agonized crying tended to normalize serum testosterone. As a result of the regimen, those patients with low initial levels tended to show a rise in testosterone, while patients with high initial levels showed a fall in values towards the population mean.

These findings suggest that intense weeping/agonized crying by patients is accompanied by changes in circulating levels of serum hGH, testosterone and a catecholamine. The biochemical data, at least tentatively, support the hypothesis also borne out by blood pressure measurements and patient self report that a dacrystic regimen of 12 months in duration may be useful in shifting the nervous system from a relative ergotropic to an increased endophylactic or trophotropic mode.

97.14 PREMENSTRUAL SYNDROME: SCIENTIFIC AND ETHICAL CONCERNS. S.J. Bird. Center for Policy Alternatives and Science, Technology, and Society Program Massachusetts Institute of Technology, Cambridge, MA 02139.

Premenstrual syndrome (PMS), a complex cluster of somatic and/or psychological symptoms associated with the menstrual cycle, is an increasingly active focus of neuroscience research. This is in part due to the growing appreciation of the role of reproductive hormones in brain regions not considered to be directly or solely involved in reproductive function.

Like all neuroscience research examining the relationship between neurobiology and behavior, the complex, dynamic interactions between biological, psychological, and social factors that result in PMS pose difficulties in the development of appropriate experimental design. These problems are compounded by some confusion in the clinical definition of PMS, the relatively high incidence of its milder forms, and widespread misunderstandings and preconceptions in the general perception of the syndrome. The general knowledge and unconscious assumptions of individual investigators and their personal views and experience determine the direction of their research. These factors influence the questions posed, the hypotheses framed, the kinds of data collected and the methodology employed, how such data are interpreted, and recommendations, either stated or implied, for the application of research findings. Current research, which will be discussed, is designed to investigate the dynamic relationship between PMS and its examination, and to explore the impact of research in this area on the development of treatment programs and the formulation of health and public policy. (Supported by NSF and NEH grant #RII-8318803.)

97.15 NEUROCONSCIOUS BEHAVIOR (ncb): A GENERAL HYPOTHESIS. DESHMUKH, V., 3600 Rustic Lane, Jacksonville, Florida 32217

The current neuroscience and cognitive science lack a general brain-conscious behavior theory. (Szentagothai, J., Ann. Rev. Neurosci., 1984: 7:1-11, Norman, D. A., in "Perspectives on Cognitive Science ", 1981). The crucial dilemma is to develop a reasonable model within the general framework of natural sciences including the laws of thermo-dynamics.

The present hypothesis proposes a general dynamic model with the introduction of the following concepts:

The organism (including humans) is the essential unit of ncb. The organism is defined as an individual biomagnetic system constituted to carry on the bioconscious (life) activities by means of functionally separate but mutually dependent and integrated organ subsystems. Conscious activity i.e. to know and to act knowingly is an intrinsic function of all organisms. The organism itself is an integral part of ecosystem. The ecosystem as a whole is relatively stable. Several natural ecosystems conflux into each other and form the suprasystem of the natural universe.

The organism-environment form a structural-functional binary ecosystem with constant exchange (transaction) of matter-energy-information (mei). A single transaction of mei forms the ncb unit event. A sequence of ncb events in space-time form a ncb activity, which can be analysed by the discrete event and weight vector analysis. Time variant analysis of ncb events can be mapped in organismic and environmental space-time. Basic ecological principles including the quality-quantity of energy concept apply. what is lost in quantity is gained in quality of functional energy.

The ncb work is the work done by an organism on its environment. The ncb energy is the organisms capacity to perform ncb work. The ncb event mapping can lead to measurment of (a)ncb event energy,(b)ncb energy density,(c)ncb energy flux,(d)ncb flux density at surface-ncb luminence,

The general thermodynamic equation for open systems with control volume concept (Holman, J. P. in "Thermodynamics"; p. 129, 1980) is applicable to ncb systems (organisms) with the addition of ncb energy term. The control or regulatory volume is the organisms experiential (or memory) subsystem. The experiential subsystem tends to constantly intervene between the current organismic state and the biophysical environment. It acts as an experiental environment in addition to the biophysical environment for the organism.

To overcome the problem of entropy in physical systems, a concept of syntropy in bioconscious systems is introduced. Syntropy means a concurrent integrative change with emergence of new properties/functions.

Finally, a concept of natural homeotropy is introduced. Homeotropy is maintenance of overall order in nature. The total natural order is conserved. The entropy in physical systems is compensated by syntropy in biophysical systems to conserve the natural homeotropy.

DEVELOPMENT AND PLASTICITY: DESCENDING PATHWAYS AND CEREBELLUM

98.1 POSTNATAL ADMINISTRATION OF α-DIFLUOROMETHYLORNITHINE IMPAIRS DEVELOPMENT OF CEREBELLAR CORTEX. J.V. Bartolome*, L. Schweitzer*, T.A. Slotkin and J.V. Nadler (SPON: D.R. Armstrong). Depts. Pharmacology and Anatomy, Duke Univ. Med. Ctr., Durham, NC 27710.

α-Difluoromethylornithine (DFMO) specifically and irreversibly inhibits the enzyme ornithine decarboxylase (ODC). ODC catalyzes the initial step in the synthesis of polyamines, which are thought to play an essential role in the proliferation and differentiation of many mammalian cells. Daily administration of DFMO to rats during the first three weeks postnatally inhibits the growth of the brain well before the onset of general growth retardation. If DFMO inhibits the division and differentiation of neurons, one might expect to find especially profound effects on the development of brain regions, such as the cerebellar cortex, in which neuronal populations continue to divide after birth.

Sprague-Dawley rat pups were given DFMO (500 mg/kg, s.c.) daily for 21 days beginning the day after birth. Control littermates received an equal volume of saline. Effects of DFMO treatment became evident by postnatal day 10. DFMO-treated rats suffered a drastic deficit in the number of cerebellar granule cells. Many of the remaining granule cells became arrested in the molecular layer during migration and remained in this ectopic location until at least four months of age. DFMO administration did not appear to affect the number of Purkinje cells. However, the mean diameter of Purkinje cell bodies was 38% less than in controls. In addition, Purkinje cells failed to form a discrete layer; many of these neurons remained scattered throughout the molecular layer. The final size of the cerebellum as a whole and of individual folia was markedly subnormal. In general, the cerebellar cortex of DFMO-treated rats failed to progress much beyond the stage of development reached in control rats during the first postnatal week. These results indicate that ODC activity and/or polyamines play an obligatory role in cerebellar neurogenesis and histogenesis. (Supported by NIH grants HD 09713 and NS 06233.)

98.2 SEX DIFFERENCES IN RECOVERY OF LOCOMOTION FOLLOWINC CORTICAL DAMAGE IN RATS. S.L.Caulder* and A.M.Gentile. (SPON: M.T.Moffreid). Teachers College, Columbia Univ. New York, NY 10027.

Several experiments have demonstrated that the consequences of subcortical damage are often sexually dimorphic in rats (Studelska & Beatty, 1978; Beatty & Siders, 1977). Few studies have examined sex differences in response to cortical lesions (Goldman et al., 1974; Teitelbaum, 1973), none of which focused specifically on skilled motor behavior. The purpose of the present studies was to examine locomotor performance of male and female rats following removal of sensorimotor cortex.

In the first two experiments, mature rats (6 male and 7 female) were trained to transverse a narrow, elevated runway. Bilateral removals of sensorimotor cortex, known to disrupt locomotor performance (Gentile et al., 1980), were carried out on all animals. Testing was initiated 21 da after surgery and continued until preoperative criterion was achieved. The second experiment followed the same basic procedures except that some rats were gonadectomized 14 da prior to cortical lesions. Thirty rats were used in this study: 6 males and 6 females sustained gonadectomy and cortical damage, 6 of each sex sustained only cortical damage, and 3 males and 3 females served as sham operated controls. In addition to behavioral running time measures, cinematographic analysis of high speed film data was used to examine movement patterns in order to elucidate recovery processes.

In both experiments, cortical removals in females resulted in little, if any, impairment. In contrast, a pronounced locomotor deficit and slow recovery was evident in all cortically damaged males. The effects of cortical damage were not influenced by prior ovariectomy. For males, however, gonadectomy reduced initial deficits and facilitated recovery.

It appears that the presence of circulating ovarian hormones in adult females cannot account for the sexually dimorphic response to brain damage we observed. Of course, earlier hormonal influence on female brain organization cannot be ruled out. Our data shows, however, that the presence of testosterone in adult males may be detrimental to recovery.

98.3 THE DISTRIBUTION OF CORTICOSPINAL PROJECTIONS IN ADULT AND POUCH YOUNG OPOSSUMS (DIDELPHIS VIRGINIANA), T. Cabana* and G.F. Martin (SPON: A.L. Humbertson). Dept. of Anatomy, School of Medicine, The Ohio State University, Columbus, Ohio 43210.

Degeneration and autoradiographic experiments in adult opossums have indicated that corticospinal axons course in the dorsal and lateral funiculi of the cervical and upper thoracic cord and distribute to laminae III-VI, especially in medial portions of those laminae (Martin et al., Brain Beh. Evol., 12:270-310, 1975). The distribution of cortical axons has been re-evaluated with the orthograde tracer horseradish peroxidase conjugated to wheat germ agglutinin (WGA-HRP). Labeled cortical axons were found in the areas previously shown to contain them. In addition, a few labeled axons were found in the sulcomarginal and ventral funiculi and terminal labeling extended to laminae VII-IX, part of lamina X and the medial edge of laminae I-II. Labeling in laminae I-II and IX was sparse and restricted to the cervical enlargement.

With the same method in pouch young opossums, labeled cortical axons were traced into the dorsal and lateral funiculi just caudal to the pyramidal decussation by about postnatal day (PND) 26 (40 days after conception, 50mm S-R length), to upper cervical levels by about PND 28 (53mm S-R length) and to upper thoracic levels (their caudal extent) by about PND 34 (62mm S-R length). At PND 34, labeled axons were also found in the sulcomarginal and ventral funiculi. Growth of cortical axons into the gray matter followed a rostrocaudal gradient and was first evidenced at about PND 38 (67mm S-R length). By about PND 40 (70mm S-R length), labeled axons were present in most of the areas containing them in the adult animal. Labeling density increased markedly in the following weeks but subsequently diminished to that seen in the adult animal.

Our results suggest that: 1) corticospinal innervation in the adult opossum is more extensive than indicated from degeneration and autoradiographic experiments, 2) cortical axons do not significantly overgrow their terminal targets during development and 3) the time when cortical axons first grow into spinal gray as well as the time when their terminal density is particularly robust correspond, respectively, to the time when cortical labeling was first produced by spinal injections of HRP and when transient labeling was observed (Cabana and Martin, Soc. for Neurosc. Abst., 9:61, 1983). Supported by U.S.P.H.S. Grant BNS-8309245 and NS-10165-10.

98.4 ORGANIZATION OF AFFERENT PROJECTIONS TO THE CEREBELLAR PARAMEDIAN LOBULE IN NEONATAL KITTENS. D. L. Tolbert*, S. S. Schneider* and M. G. Murphy* (SPON: P. A. Young). Murphy Neuroanatomy Research Laboratory, Depts. of Anatomy and Surgery, St. Louis Univ., St. Louis, MO 63104.

At the time of birth the kitten cerebellar cortex is relatively immature, consisting of an external granule cell layer, an attenuated molecular layer, a Purkinje cell layer and an internal granule cell layer. To determine if afferent projections to the cerebellar cortex are appropriately organized in neonates, injections of WGA-HRP were made into rostral or caudal folia of the paramedian lobule (PML) in 1 to 21 postnatal day old kittens. The distributions of retrogradely labeled neurons in the inferior olive (IO), the lateral reticular nuclei (LRN) and in the pontine nuclei were plotted. Similar experiments were also carried out on adult cats. Following injections of the rostral PML in kittens, retrogradely labeled neurons in the IO were localized contralaterally in the middle part of the medial accessory olive (MAO) and in the medial 1/2 to 1/3 of the dorsal accessory olive (DAO). Injections of the caudal PML labeled neurons contralaterally in the lateral 1/3 of the MAO and the DAO and in the caudomedial dorsal lamella of the principal olivary nucleus. In 2 and 4 postnatal day old kittens a few HRP labeled neurons were also present in the ipsilateral MAO. In the LRN, neurons in the magnocellular division of the nucleus were labeled after injections in the rostral PML; whereas, after caudal PML injection, neurons were mainly labeled in the parvocellular division of the nucleus. Lateral reticulocerebellar projections to the PML were bilateral, but with an ipsilateral predominance. In the contralateral PN, discrete clusters of labeled neurons were medial, dorsomedial and ventrolateral to the pyramidal tract following rostral PML injections and medial, ventral and dorsal to the tract following caudal PML injections. Ipsilateral to these injections in the PML neurons were retrogradely labeled in homologous areas of the PN and, in addition, there was a discrete cluster of labeled neurons in the dorsolateral PN following rostral PML injections.

The results from these experiments in neonatal kittens were comparable to the data obtained from similar experiments in adult cats indicating that, even though neurogenesis and maturation of the cerebellar occurs postnatally, projections to the PML from the IO, LRN and PN are adult-like in their topographical organization at the time of birth. Supported by NIH grant NS-20227.

98.5 PAW USAGE AND PAW PRINT ANALYSIS OF LOCOMOTION AFTER NEONATAL OR ADULT HEMISPHERECTOMY IN CATS: J.R. Villablanca and J.W. Burgess: Mental Retardation Research Center; Depts. Psychiat. & Anatomy; UCLA School of Medicine; Los Angeles, CA 90024.

In a continuing study of recovery of function (companion abstracts) limb motor responses were analyzed in cats with removal of the left cerebral hemisphere as neonates (8-15 days; N=4) or adults (N=4), and in intact adult controls (N=12). Paw movements were assessed in a box with a clear plexiglas front under which food-deprived cats reached for pieces of meat (400 responses/cat). Five attitude-movement categories were scored: 1) club-footed pronated; 2) same with claws protruding; 3) club-footed but sideway swipe; 4) same with protruding claws; 5) sideways swipe with wrist and digits-claws flexed (normal). Adult-lesioned cats showed mostly 1 and 2 responses; neonatal-lesioned showed a significantly different pattern of mostly 1, 2, and 3, with some 4 responses (P=.003). Category 1 made up 45.4% of adult-lesioned, but only 36.7% of neonatal-lesioned responses (P=.004). To assess locomotion, cats' pawpads were dyed with stamppad ink and the cat walked through a narrow wooden chute lined with ruled chart paper. The measures were: paw width for all 4 paws; stride length, measured between successive prints of right and left hind feet; step width, measured between the two foot prints; and angle of limb abduction, measured between a hind foot and successive print of the opposite hind foot. About 20 observations were average for every measure to (160 total scores/cat). Measures showed that neonatal-lesioned cats walked more like normals than adult-lesioned (P=.007). Adult-lesioned cats splayed their toes or slipped, such that impaired hind pawprint was significantly wider (X 7 mm) than intacts (P<.01). Also the hind stride length on the impaired side was significantly shorter (X, 56 mm) than intacts (P<.05). In adult-lesioned cats the impaired hind limb tended to adduction, whereas in neonatal-lesioned it tended to abduction. Neonatal-lesioned cats showed no significant difference from intacts except in unimpaired forepaw, which averaged 5 mm wider than controls (P<.01). Thus, adult-lesioned exhibited more paw-use postures and locomotor abnormalities than neonatal-lesioned animals. These results show that in this model, enhanced early-lesion recovery is seen for complex limb motor patterns as well as for simpler tests previously reported. These functional results parallel the more extensive neuroanatomical reorganization which we find in neonatal hemispherectomized cats. (USPHS Grants HD-05958 and HD-04612).

98.6 RECOVERY OF ACTIVITY, AGGRESSION, SOCIAL BEHAVIOR, AND HOLE-BOARD PERFORMANCE AFTER NEONATAL OR ADULT HEMISPHERECTOMY IN CATS. J.W. Burgess and J.R. Villablanca; Ment. Ret. Res. Ctr. Depts. Psych. and Anat.; UCLA Sch. of Med.; Los Angeles, CA 90024.

In a continuing study of recovery of function (companion abstracts), complex, spontaneous behavioral patterns were analyzed in cats with removal of the entire left cerebral hemisphere either as neonates (8-15 days of age; N=10) or as adults (N=11), and in intact adult controls (N=24). Activity was tested in a rectangular open field (4X3 m), using continous 5 sec time-samples. Compared to normals, adult-lesioned cats were hypoactive in 3 measures: Locomotion, rearing, and sniffing (P<.05). Lesioned kittens showed deficits in the same 3 measures at 100 days of age (P<.05), but by 150, 200, and 300 days of age they were equivalent to intact littermates. Social responses to a normal cat in the open field were scored continously from an ethogram for 30 min. In the presence of another cat, adult-hemispherectomized cats violated species-typical body-buffer space (P<.05), approached with abnormal frequency (P<.05), and attacked other cats significantly more often than controls (P<.05). By comparison, normal cats never attacked and seldom approached in the open field. Neonatal-lesioned adults showed only occasional approach and a greater tendency to sit (P<.05) or "slow-walk" (P<.05) than controls; attacks were rare. Search behavior was tested in food-deprived cats with a 2.5 m diameter radial maze and 30-chamber holeboard placed in a 70x70x70 cm observation box. All hemispherectomized animals showed a spatial bias for investigation, turning to the left in the maze (P<.001), and approaching the holeboard from the left side (P<.01); however, there was no evidence of gross sensory neglect in either group. Adult-lesioned cats exhibited a search deficit in the holeboard, missing more food than control or neonatal-lesioned adults (P<.01), which were equivalent. Overall, the behaviors of neonatal-lesioned cats were significantly more normal than in adults receiving the same lesion. These results demonstrate that the "Kennard Effect" of enhanced early-lesion recovery in this animal model not only applies to neurological recovery (previous abstracts) but also to the complex responses analyzed here. This functional recovery parallels the more extensive neuroanatomical reorganization which we have seen in the neonatal hemispherectomized cat. (USPHS Grants HD-05958 and HD-04612).

98.7 THE EXUBERANCE OF YOUTH: AN ANALYSIS OF CORTICOTHALAMIC, CORTICOSPINAL AND CORTICORUBRAL PROJECTIONS IN ONE DAY OLD CATS. C.T. Leonard, G.A. Robinson and M.E. Goldberger. Dept. of Anatomy, The Medical College of PA, Phila., PA 19129.

Postnatal development of motor behavior in cats is likely to be related to maturational changes in descending cortical pathways. In order to examine these potential changes, HRP/WGA (3%) was injected into left sensorimotor (SM) cortex of 1 day old and adult cats. At birth, there is dense diffuse anterograde labeling in the ipsilateral dorsal thalamus. Labeled axons stream through the massa intermedia and throughout the contralateral thalamus including lateral nuclei. The adult animal does contain some labeled fibers in the massa intermedia and central medial nucleus but fibers do not extend much beyond the midline. The corticospinal tract (CST), in the one day old kitten, is well developed. In the cervical region there is dense labeling in the contralateral lateral funiculus with considerably less dense labeling in the ipsilateral lateral and ventral funiculi. The contralateral CST was labeled densely throughout the cord. The ipsilateral ventral CST could not be found below the cervical level but the ipsilateral lateral CST could easily be identified as far as mid-lumbar levels. CST axons had penetrated the grey matter throughout the neonatal spinal cord and were found in both dorsal and ventral horns with no apparent laminar organization. In the adult animal, the crossed CST is found throughout the cord. The ipsilateral lateral and ventral CST were very sparse and only seen extending to thoracic and cervical levels respectively. The neonatal corticorubral pathway is bilateral with the densest labeling occurring ipsilaterally in the ventrolateral, magnocellular portion of the nucleus. The adult animal exhibits a large ipsilateral corticorubral projection with only a few axons labeling contralaterally. Our findings indicate the existence of several cortical projections which either retract, undergo metabolic changes and fail to label as adults, or with development cells of origin undergo cell death. The onset of hindlimb motor function does not seem to correlate with the arrival of the CST or other corticofugal pathways as these pathways exist prior to the expression of the behavior.

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98.8 ELECTRICAL STIMULATION INCREASES (^{14}C) 2-DEOXYGLUCOSE UPTAKE IN NON-FUNCTIONAL FETAL MOTOR CORTEX TRANSPLANTS. F.R. Sharp and M.F. Gonzalez. Dept. Neurology, SFVAH, San Francisco, CA 94121

We have recently shown that patterns of (^{14}C) 2-deoxyglucose (2DG) uptake during motor cortex electrically stimulated vibrissae movements (Sharp and Evans, JCN, 208:255) were mostly different from the patterns of 2DG uptake during motor cortex stimulated forelimb movements in normal rats (Sharp, JCN, in press). We have also shown that it is possible to transplant parts of fetal frontal (motor) cortex into lesioned adult rat motor cortex (Sharp and Gonzalez, Neurology, in press). This report describes our initial attempts at electrically stimulating fetal motor cortex transplants. Thirty Sprague-Dawley rats weighing 150-200 grams had the right motor-sensory cortex removed under deep anesthesia. One week later these host animals were anesthetized and the right frontal cortex from 17 or 18 day embryonic brain was then transplanted into the host brain cavities. The cavities were sealed. After a three month survival, the host animals were anesthetized and the cavities carefully examined for the presence of a surviving fetal transplant. If a transplant was found, a bipolar electrode was cemented into its geometric center. Animals were restrained, allowed to recover from anesthesia for 12 hours, and 2DG injected after initiation of transplant electrical stimulation.

Electrical stimulation of three surviving large transplants in three host rats produced no observable motor movements or other behavioral changes. Electrical stimulation did increase 2DG uptake about the stimulating electrode within the fetal frontal cortex transplant. There were no definite increases of (^{14}C) 2DG uptake within the host brain during transplant stimulation. Histological examination of the transplants revealed many surviving cells within the transplant, but almost complete separation of transplant and host brain by a space or membrane. Our results show: the transplants can increase glucose metabolic rate; the transplants probably were non-functional since transplant stimulation did not produce motor movements and did not activate host brain motor pathways; and our assay system appears to be adequate for testing whether fetal motor cortex transplants are functional or non-functional.

98.9 DEVELOPMENT OF CORTICOSPINAL PROJECTION FROM THE MURINE BARREL FIELD. J.E. Crandall, J.M. Whitcomb* and V.S. Caviness, Jr. Dept. Neuropathol. and Dept. Neurol., E.K. Shriver Ctr., and Mass. Gen. Hosp., Boston, MA 02114

We have studied the changes from birth (P0) to maturity in the pattern of distribution of corticospinal projection neurons in the barrel field of murine SI cortex. The retrogradely transported fluorescent dye, true blue, was injected into the spinal-medullary junction. Survival times from 48-96 hrs allowed optimal retrograde neuronal filling. At all ages labeled neurons are confined to layer V. Between birth (P0) and P3, label virtually fills the soma and the entire dendritic arbors of individual cells except for the terminal arborization in the molecular layer.

With injections at P0-P3 the majority of neurons of layer V are filled in this way. Thus, there is dense labeling of radially adjacent neuronal somata aligned in columnar fashion throughout the full width of the layer. Only exceptionally are there gaps in the array of labeled cells within layer V.

Dramatic changes occur in the pattern of labeling over the succeeding week and the adult pattern is approximated by P10. Within the individual neuron, the dye can be visualized only within the soma and proximal dendrites. The density of labeled neurons falls rapidly and their distribution pattern changes. Labeled neurons are restricted to the midzone of layer V, i.e., layer Vb. Radially adjacent cells above or below in Va or Vc are not labeled. Tangentially adjacent cells within layer Vb are less likely to be labeled.

In principle, both neuronal death and pruning of "exuberant" axons may contribute to this apparent reduction in the projection. In order to estimate the relative effect of cell death, a series of animals injected on P1 were allowed to survive for varying ages through adulthood. To estimate the contribution of axonal loss, a series of animals injected at varying ages from P1 were allowed to survive to P20. In both groups the number of labeled cells were counted through an arc of 500 um within the center of the PMBSF. In agreement with Heumann and Leuba ('83), the present method suggests cell death accounts for at least 50% of the revision of the system. In addition, axon pruning occurs in at least 40% of the surviving population.

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98.10 EFFECTS OF ENVIRONMENTAL EXPOSURE ON LOCOMOTOR IMPAIRMENTS FOLLOWING CORTICAL LESIONS IN RATS. J.M. Held, Z. Beheshti*, & A.M. Gentile., Teachers College, Columbia Univ., New York, NY 10027.

Exposure to an enriched environment before or after damage of sensorimotor cortex has been shown to ameliorate locomotor deficits in rats (Held, Gordon & Gentile, 1981). The present study compared the effects of physical activity alone with the potentially broader effects of perceptual-motor enrichment; i.e., group activity on a variety of tasks. As preoperative enrichment was found previously to be most potent, this study varied environmental exposure prior to damage.

Locomotion on a narrow elevated runway was tested pre- and postoperatively for all animals. In addition, groups of rats were: 1)exposed to an enriched environment (n=12), 2)given access to an activity wheel (n=12), or 3)individually housed in wire mesh cages-impoverished(n=9). Rats were exposed in groups of four to the enriched environment or placed individually in the activity wheel for 2 hr per da for 25 da preoperatively. Within each exposure group, rats were given bilateral removals of sensorimotor cortex (18mm^2 each side); the remaining six animals within each group were sham operated controls. Postoperatively, testing was initiated 17 da after surgery throughout which time all animals were maintained under impoverished conditions.

Locomotor deficits following cortical damage were a function of preoperative exposure: environmentally enriched rats were least impaired, impoverished rats most impaired. Activity wheel rats initially showed the same marked deficits as impoverished animals but recovered more rapidly. The opportunity for physical activity afforded wheel animals preoperatively may have enhanced peripheral motor capabilities that aided recovery. However, physical activity alone did not yield the same protective effects from initial impairment that enrichment did. Greater elaboration or organization of neural structures associated with perceptual-motor enrichment probably accounted for the initial sparing of the enriched group.

98.11 THE EFFECTS OF MEDULLARY PYRAMIDOTOMY ON MOVEMENTS EVOKED BY INTRACORTICAL MICROSTIMULATION. ANALYSIS OF THE ANOMALOUS IPSILATERAL CORTICOSPINAL TRACT IN THE RAT. G. Kartje Tillotson, D.L. O'Donoghue and A.J. Castro. Dept. of Anat., Loyola Univ., Stritch Sch. of Med., Maywood, IL 60153.

Intracortical microstimulation in normal rats evokes contralateral forelimb (FL) movements at low current intensities (12μamps) and ipsilateral movements at high currents (44μamps). Stimulation of the intact cortex in adult rats that sustained neonatal unilateral sensorimotor cortical (SMC) lesions results in low current contralateral (15μamps) as well as ipsilateral (22μamps) FL movements (Kartje-Tillotson, G., et al, Brain Res., in press, 1984). Neonatal SMC lesions in rats also result in the development of an anomalous ipsilateral corticospinal tract from the unablated hemisphere (Castro, A.J., Exp. Neurol., 46:1-8, 1975). The present study was undertaken to determine if low-threshold ipsilateral movements are mediated through the anomalous corticospinal tract, thereby indicating a functional role for this aberrant pathway. Right SMC aspiration lesions were made on two day old rat pups (n=4) under hypothermic anesthesia. Unoperated pups underwent hypothermic anesthesia and served as controls (n=5). At maturity, under ketamine anesthesia, intracortical microstimulation(0.25msec pulses, 350hz, 300msec train, 5-100μamps) was applied at a depth of 1.7mm in the FL motor area of the intact (left) cortex. Mean threshold values for contra- and ipsilateral FL movements were 15μamps and 21μamps, respectively. Following microstimulation, the left medullary pyramid was exposed using a parapharyngeal approach, and cut with a #11 scalpel blade. After pyramidotomy, cortical electrode penetrations were made at points where FL responses had previously been evoked. Mean threshold values had increased for both contralateral (61μamps) and ipsilateral (76μamps) forelimbs. Animals were sacrificed by anesthetic overdose and vascular perfusion with saline followed by 10% buffered formalin. The brains were removed, photographed, frozen sectioned at 40 microns, and stained with cyanine R/neutral red to facilitate inspection of the lesions. These data indicate that acute medullary pyramidotomy abolished cortically-evoked low-threshold contra- as well as ipsilateral FL movements, indicating that the aberrant ipsilateral corticospinal pathway is capable of contributing to the motor control of the ipsilateral limb. (Supported by NIH grant NS 13230).

98.12 MOTOR CORTICAL PLASTICITY AFTER HEMICEREBELLECTOMY IN NEWBORN RATS. THE STRUCTURES MEDIATING ABNORMAL CORTICALLY-EVOKED FORELIMB MOVEMENTS. D.L. O'Donoghue, G. Kartje-Tillotson and A.J. Castro. Dept. of Anat., Loyola Univ. Stritch Sch. of Med., Maywood, IL 60153.

Intracortical microstimulation (0.25msec pulses, 350Hz, 300msec train, 125μm exposed tip, 1.7mm depth) in normal rats evoked low-threshold contralateral (contra-, 12μamps) and high-threshold ipsilateral (ipsi-, 44μamps) forelimb (FL) responses. After hemicerebellectomy (hcb11m) by aspiration (hypothermic anesthesia) at 2 days of age, cortical stimulation at maturity evokes low-threshold ipsi-FL (21μamps), as well as contra-FL movements. The present lesion experiments were designed to determine the neuronal structures that contribute to or mediate the abnormal low-threshold ipsi-FL movements. In adult animals after neonatal hcb11m, electrode penetrations were made (under ketamine anesthesia) to determine the area of FL motor cortex. Next, animals sustained only one of four secondary lesions involving the following structures: 1. cerebellar tissue spared by the neonatal lesion; 2. callosal fibers; 3. the opposite cerebral hemisphere; or 4. the medullary pyramid. Intracortical stimulation after secondary cerebellar or callosal lesions showed FL movements at pre-lesion current intensities indicating that these structures do not mediate abnormal responses. Secondary lesions of the opposite cerebral cortex caused a marked increase in the current needed to evoke ipsi-FL movements (43μamps, with only 30% of the electrode penetrations showing ipsi-FL movements) without effecting thresholds for contra-FL responses (8μamps). These data indicate that the contralateral cortex is indirectly needed for ipsi-FL responses. Pyramidal lesions, ipsilateral to the stimulated cortex, increased the threshold for ipsi-FL (54μamps), as well as for contra-FL (55μamps) movements. In addition, only 50% of the penetrations that showed pre-lesion FL responses showed post-lesion responses. These data indicate that abnormal cortically-evoked ipsi-FL responses are mediated by direct corticospinal projections.

(Supported by NIH grant NS 13230).

RETINA II

99.1 ELECTROPHYSIOLOGICAL EVIDENCE THAT ALL MALE SQUIRREL AND CEBUS MONKEYS HAVE DICHROMATIC RETINAS. G. H. Jacobs and J. Neitz*. Dept. of Psychology, Univ. of California, Santa Barbara, CA 93106

Behavioral and microspectrophotometric measurements strongly suggest that although the retinas of many female squirrel monkeys (Saimiri sciureus) contain three classes of cone photopigments which support trichromatic color vision, all males of this species have only two classes of photopigments and are dichromatic. To further substantiate this conclusion we have developed an electrophysiological measure that provides a rapid, noninvasive assessment of the complement of cone photopigments in individual animals. The technique is based on the principle of flicker photometry in which electroretinographic (ERG) responses elicited from two interleaved trains of light flashes are compared. One of these sources is a fixed reference light, the other a monochromatic light variable in intensity and wavelength. The responses from two rapidly flickering (62 Hz) lights are electronically filtered and subtracted one from another. Variation in the intensity of the monochromatic light permits the establishment of a photometric equation between the two lights and, thus, the determination of a spectral sensitivity function for the underlying cone mechanism(s).

Using the flicker photometric technique, we have examined a large sample (>50) of squirrel monkeys, and have verified that whereas individual female squirrel monkeys have either two or three types of cone photopigments, all males have only two types. The males can be further subdivided according to the spectral location of their middle to long wavelength cone. Three classes were found: (a) mean peak sensitivity = 536.3 nm (N = 11), (b) mean peak sensitivity = 549.0 nm (N = 10), (c) mean peak sensitivity = 559.3 nm (N = 15).

With this same technique we also tested 8 males from another South American primate species (Cebus apella). Again, all of the males had only a single middle to long wavelength cone. Two classes were found: (a) mean peak = 549.0 nm (N = 3), (b) mean peak = 560.2 nm (N = 5).

A clear implication of these results is that the inheritance of color vision in these species differs from the arrangement believed characteristic of humans. It is likely that in both squirrel and Cebus monkeys there is only a single photopigment locus on the X-chromosome. (Supported by EY02052).

99.2 PHOTORECEPTOR CONTRIBUTIONS TO VISUAL MECHANISMS IN TURTLE. D.F. Sisson and A.M. Granda. Institute for Neuroscience, Univ. of Delaware, Newark, DE 19716.

Both absolute and increment thresholds were determined for red (650 nm) and green (540 nm) test stimuli in turtles trained to retract their heads to target flashes of light. Spectral sensitivities of visual mechanisms were derived from measurements of inverse background intensities, at various wavelengths between 450 and 650 nm, required to produce values 0.5 log unit above the respective absolute thresholds according to the methodology of Stiles.

Four visual mechanisms so far have been isolated with respect to absolute sensitivity and spectral position. When matched to corrected absorption spectra for photopigments resident in this retina, the contributions of particular photoreceptors to the several mechanisms could be assayed.

At low background intensities with red, test stimuli, Mechanism I appears to be mediated by rods and red-sensitive, single cones with a sensitivity difference of 0.8 log unit for the two components of the spectral curve. The difference accords well with flash sensitivity data and the known physiological linkage that exists between rods and red-sensitive cones in this eye.

A second mechanism at intermediate background intensities also showed a two-component, spectral curve. Both short- and long-wavelength components were equally sensitive with the short-wavelength component some 0.95 log unit less sensitive than the rods that also peak in this spectral region. Green-sensiitve, single cones are not implicated as their spectra show the influence of filtering action by yellow oil droplets and that does not obtain here. The long wavelength component was fit by a function derived from a red-sensitive photopigment modified by an orange oil droplet, a characteristic of chief members of double cones.

At high intensity backgrounds with red light flashes, a third mechanism was isolated that conformed to the absorption spectrum of red-sensitive, single cones acting together with red oil droplets. Mechanism's III sensitivity is consistent with intracellular flash sensitivity determined for these structures by Baylor and Hodgkin. A fourth mechanism of equal sensitivity was isolated with green light and correlated with the flash sensitivities of green-sensitive, single cones.

99.3 SYNAPTIC TRANSMISSION FROM RODS TO DEPOLARIZING BIPOLAR CELLS IN THE TIGER SALAMANDER RETINA. Samuel M. Wu. Cullen Eye Institute, Baylor College of Medicine, Houston, Texas 77030.

The first synapse in the vertebrate visual system is formed between the photoreceptors and the bipolar cells. In most vertebrate species, bipolar cells receive synaptic inputs from both rods and cones, and the mechanisms of signal transmission of these two synapses may not be identical. It is therefore important to isolate one synapse from the other and to study them separately.

In this report, the synapses between rods and depolarizing bipolar cells (DBC) were selectively studied, using simultaneous recording techniques in the living slice preparation of the tiger salamander retina. Pairs of intracellular microelectrodes were inserted into rod-DBC pairs under visual control when the preparation was illuminated with infrared rays. Injection of -1 nA current into a rod elicited a sign-inverting, sustained depolarization of about 2 mV in the DBC, indicating that the synapses between rods and bipolar cells were intact in retinal slices. Voltage 'tails' after the termination of a bright flash (λ = 500 nm, spot diameter = 400 μm) were observed in dark-adapted rods and DBCs, but not in cones. These simultaneously recorded voltage 'tails' were used to isolate rod input from cone input, and to study the input-output relation of the rod-DBC synapse. Within the voltage range between 0 and -10 mV from the rod dark potential (-39 ± 1.2 mV), the input-output relation of the rod-DBC synapse is approximately linear, with an estimated voltage gain of about 3.7.

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99.4 ROD/DEPOLARIZING BIPOLAR CELL TRANSMISSION IN THE GOLDFISH RETINA. S. Nawy* and D.R. Copenhagen (SPON: K.T. Brown). Div. of Neurobiol., Dept. of Physiol., Univ. of Calif., San Francisco, CA 94143.

We have recorded from depolarizing bipolar cells (DBC) in the superfused isolated goldfish retina. Fish were dark-adapted for a minimum of 2 hrs., and the dissections performed under infra-red (950 nm) illumination. Histological examination of retinas isolated in this way revealed the relative absence of cone outer segments. All DBC responses to maintained bright stimuli (about 10 photons/um^2/sec) were similar to rod-dominated responses desribed elsewhere (Saito, T., et. al., J. Gen Physiol., 73:73, 1979) and had a peak spectral sensitivity between 520 and 540 nm. We found no deviation from univariance in these cells. For linear range responses to brief (10 msec) flashes, the flash sensitivity was approx. 100 mv/Rh**, and the response waveform could be fitted with a model of n stages of low-pass filtering (n=12-18) and a time to peak of about 350-400 msec. Responses recorded from retinas of light-adapted fish had peak sensitivities at about 615 nm and a much faster time to peak. We therefore believe that by carefully dark-adapting the retina we have isolated the rod input to these cells.

Cells demonstrated voltage fluctuations in the dark which were suppressed by bright illumination. The voltage variance was generally twice as large in the dark as in the light. Power density spectra for both the light and dark fluctuations were computed and the light spectrum subtracted from the dark to yield a difference spectrum. An equation for this spectrum could be fitted by using 2 stages of low-pass filtering with equal time constants of about 35 msec.

We tested the possibility that this dark noise is composed of spontaneous isomerization-like events in the rod outer segments. Baylor (Baylor, D.A., et. al., J. Physiol., 309: 597, 1980) has shown in toad retina that spontaneous events in the rod outer segments have a similar power spectrum to that of the photoresponse. We averaged a series of responses to dim (.01 photons/um^2/flash) 10 msec flashes, and then computed the power spectrum of these responses. Assuming that spontaneous and photo-events have the same power spectrum in fish as well as toad rods, then it appears that there is an additional light-suppressible component of noise in the rod/depolarizing bipolar cell pathway. (Supported by EY01869)

99.5 ANALYSIS OF VOLTAGE FLUCTUATIONS IN DEPOLARIZING CELLS OF TIGER SALAMANDER RETINA J. Belgum* and D.R. Copenhagen. Depts. of Ophthalmology and Physiology, University of California, San Francisco, CA 94143.

The membrane potential of depolarizing bipolar cells (DBC) fluctuates continuously in the dark. The effects of light and extrinsic current on the amplitude of these fluctuations (variance) were studied. A strong correlation was observed between the light or current-induced membrane potentials and the variance.

Bright spots of light (400 μm dia.) depolarized these cells and reduced the variance of the membrane fluctuations. Addition of an annular light pattern to the bright spot reduced the depolarization and increased the variance. Annular patterns alone hyperpolarized the DBCs below the dark potential and increased the variance above the dark value. Depolarizing currents injected into these cells in the dark reduced the variance. Hyperpolarizing currents injected while the cells were depolarized by bright spots could increase the variance. Changes in the measured input resistances as a function of membrane polarization were insufficient to account for the changes observed in the variance of the fluctuations.

Since injected current appears to modulate the variance independent of light-controlled synaptic inputs, we conclude that a major source of the fluctuations is a voltage-dependent process. The light-induced changes in variance are consistent with this interpretation.

Power spectra of the fluctuations were obtained under each of the above experimental conditions using standard FFT techniques. The spectrum of fluctuations added or suppressed by a given experimental manipulation was then obtained as a difference spectrum. For a given cell difference spectra were of the same shape regardless of the means by which a change in noise was obtained. These spectra could be fitted by either a single Lorentzian function or the product of two such functions with time constants ranging from 35 to 80 msec. Supported by EY 01869.

99.6 MEASUREMENT OF BIPOLAR CELL RECEPTIVE FIELD CENTER SIZE FOR TIGER SALAMANDER RETINA. S. Borges* and M. Wilson (Spon: P. Pappone). Department of Zoology, University of California, Davis, CA 95616.

An optical system allowing a spot of light to be placed accurately on a single receptor, or small group of receptors visualized on a TV monitor, has been used to determine the receptive field sizes for cells of the tiger salamander retina.

The extent of bipolar cell receptive field centers has been determined by recording the responses of these cells in isolated, dark-adapted retinae, to stimulation of different groups of rods. These experiments reveal roughly circular receptive field centers that exceed 350 μm in diameter and within which bipolar responsiveness falls off radially.

Previous reports of bipolar cell morphology have shown their dendritic spread in the outer plexiform layer to be considerably less than 350 μm, with one bipolar cell typically contacting 10-15 receptors only (Lasansky, A., J. Physiol., Lond., 285:531, 1978), whereas our measurements show a functional connection to at least 300 rods. The discrepancy between morphological and functional field size is thought to be too great to be attributed to signal spread through the receptor network. Dye filling of receptor cells indicates that terminal processes do not extend far enough to account for the discrepancy.

Signal spread between bipolar cells or the involvement of another cell type in establishing the bipolar receptive field center might be implied by these results.

99.7 ANOMALOUS RECTIFIER CHANNELS IN HORIZONTAL CELLS. Ryuuzo Shingai*, Fred N. Quandt, and William K. Stell (Spon: Q. Pittman). Lions' Sight Centre, University of Calgary, Fac. of Medicine, Calgary, Alta., Canada T2N 4N1.

Recently, it has become possible to dissociate cells from the vertebrate retina. This preparation has become useful for studies which characterize the ion channels of various retinal cell types. We have used single channel analysis to determine the properties of channels responsible for inward rectification in horizontal cells. Single horizontal cells were enzymatically isolated from goldfish retina. Currents were recorded from intact or inside-out configurations of plasma membrane using the gigaohm seal patch clamp technique. Membrane currents were measured under conditions similar to those used by Ohmori, Yoshida, and Hagiwara (1981) to study the inward recitification of myotubes. The recording pipette contained 125 mM KCL. Inward current steps could be measured when the pipette also contained 100 uM Ba^{2+}. The mean duration of these inward currents was typically 214 msec (-20 mV relative the resting potential, 13°C). The frequency of inward current steps increased as the membrane was hyperpolarized. For excised patches in symmetrical isotonic KCl solutions, currents were seen only when the membrane potential was negative to E_K, and the amplitude increased with hyperpolarization. The slope conductance measured between -20 to -60 mV was 16-18 pS (13°C) and the zero current potential extrapolated to 0 mV. K^+ appears to be a dominant current carrier for this channel, since the extrapolated zero current potential shifted to more positive values when a portion of internal K^+ was replaced by tetramethylammonium. The observations are most consistent with the hypothesis that the inward current steps are due to anomalous rectifier K^+ channels gated open at potentials negative to E_K, and becoming periodically unblocked by Ba^{2+}. The distribution of inward rectifier channels, judged by positions of the recording pipettes, appeared to be uniform over the cell body. In contrast currents from other gated channels, including L-glutamate activated channels were only infrequently recorded. Our measurements of the gating behavior and permeation properties of anomalous rectifier channels should help to clarify their physiological role in horizontal cells.

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99.8 VOLTAGE-DEPENDENT AND SYNAPTIC CONDUCTANCES IN AMACRINE CELLS STUDIED WITH WHOLE-CELL PATCH CLAMP IN TIGER SALAMANDER RETINA. Steven Barnes* and Frank Werblin* (SPON: Richard Van Sluyters) Graduate Group in Neurobiology, University of California, Berkeley, CA 94720.

The network of transient-responding amacrine cells in many vertebrate retinas mediates a form of lateral interactions activated by movement or change. Amacrine cells respond to the onset and termination of a light stimulus with a characteristic EPSP and large, single spike, and activity can propagate through the network via synaptic coupling between neighboring amacrine cells. The network properties emerge from the interactions of 1) the voltage- and calcium-dependent conductances of individual cell membranes, 2) synaptic currents from more distal cells, and 3) synaptic inputs from neighboring amacrine cells. Using the 'gigaseal' whole-cell voltage clamp technique we have evaluated the active currents by studying enzymatically isolated amacrine cells. Then we measured the synaptic currents by whole-cell patch clamping amacrine cells in retinal slices. These studies lead to a characterization of the individual amacrine cell response and to an evaluation of signal propagation in the network mediated by the specific synaptic and voltage-dependent conductances.

99.9 DIFFERENTIAL EFFECTS OF BLOCKING POTASSIUM CONDUCTANCE UPON ERG B-WAVE AND SLOW PIII. B. Oakley II and H. Shimazaki. Depts. of Elect. Eng'g. & Biophysics, & Bioeng'g. Program, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801.

The trans-retinal electroretinogram (ERG) was measured in the isolated retina preparation of the toad, Bufo marinus. Extracellular potassium ion concentration, $[K^+]_o$, also was measured, using K^+-selective microelectrodes. Müller (glial) cells seem to have a large potassium conductance, g_K, and it has been hypothesized by others that both the b-wave and the slow PIII components of the ERG are generated by responses of Müller cells to light-evoked changes in $[K^+]_o$. Superfusion with pharmacological substances (including Ba^{2+}, Cs^+, Rb^+, 4-AP, & TEA^+) known to block g_K in a variety of cell types was used in an attempt to block any responses of Müller cells to light-evoked changes in $[K^+]_o$.

Under control conditions, slow PIII and the light-evoked decrease in $[K^+]_o$ in the distal retina had identical waveforms for all stimuli tested, supporting the idea that slow PIII results from the response of a K^+-sensitive cell to the light-evoked decrease in $[K^+]_o$. Each blocker of g_K abolished slow PIII, yet none of these substances had a significant effect on the light-evoked decrease in $[K^+]_o$. Thus, it seems that these substances blocked g_K in the K^+-sensitive cells that generate slow PIII. However, none of these substances had a significant effect on the ERG b-wave at the concentrations that blocked slow PIII, consistent with effects observed previously by Winkler and Gum [Invest. Ophthalmol. Vis. Sci. 20 (Suppl.):183, 1981].

Overall, the results are inconsistent with the hypothesis that both slow PIII and the b-wave are generated by the Müller cells' responses to light-evoked changes in $[K^+]_o$. Intracellular recordings from Müller cells now are being made in an attempt to understand the cellular basis for these results.

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99.10 THE ELECTROANATOMY OF THE RABBIT ON-OFF AMACRINE CELL. P.A. Coleman* and R.F. Miller. Department of Ophthalmology, Washington University School of Medicine, St. Louis, MO 63110

Intracellular recordings have been obtained from neurons which present with transient, depolarizing responses at both light on and off, occasionally associated with spike activity. When intracellularly stained with horseradish peroxidase the somas of these cells are found in the inner nuclear layer without evidence of an axon. These ON-OFF amacrine cells typically have small perikarya (5-7 μm) but large dendritic fields (0.5-1.0 mm). Examination of the arrangement of these processes shows that all branching occurs close to the soma, with the majority of the dendritic field comprised of long (400 μm), yet thin (0.2-0.3 μm) projecting dendrites. The branching is generally non-decremental.

We have obtained both anatomical and electrical measurements (input resistance $\{R_n\}$ and time constant $\{\tau_o\}$) from the same neurons . Using a recursive computer program (which analyzed the complex impedence of the cell) we determined the value of the specific membrane resistance $\{R_m\}$ which duplicates our measured R_n. In one neuron a R_m of approximately 7500 ohms-cm^2 was needed to account for the measured R_n of 115 megohms. The average electrotonic length of the dendrites (from soma to terminal) is 1.55 lambda. Calculations of steady state electrotonic current spread suggests reasonable electrical communication between cell body and dendrites.

Application of 40 mM Mg^{++}, which blocked the light evoked response, increased both the R_n and τ_o. This effect is assumed to be mediated by the block of the tonic release of neurotransmitter by presynaptic neurons to the ON-OFF amacrine cell.

At the present time we do not know whether these amacrines, like those of lower vertebrates generate dendritic spikes, but we see many similarities between the ON-OFF amacrines of rabbit and those seen in the mudpuppy.

99.11 LOCAL, NON-SPIKING INTERNEURONS IN THE LATERAL INHIBITION PATHWAY OF SUSTAINING FIBERS IN THE CRAYFISH VISUAL SYSTEM. B. Waldrop and R. M. Glantz, Dept. of Biology, Rice Univ., Houston, TX 77251.

Sustaining fibers (SFs) are tonic ON interneurons in the crayfish visual system which have been identified on the basis of their corneal receptive fields. They have their dendrites in the second optic lobe (Medulla), and receive excitatory inputs via a columnar array which represents a retinotopic map of visual space. Lateral inhibiton is well documented for the SFs, and has the following properties: 1) inhibition is tonic, 2) it covers the entire cornea, 3) is proportional to the log intensity of the inhibitory light, and 4) it is associated with synchronous bursting in the SFs. It has previously been shown that lateral inhibition produces little or no post-synaptic inhibition of SFs (Waldrop et al, Neurosci. Abstr. 7:251, 1981) and thus is produced by a decrease in the excitatory columnar input.

A class of local, non-spiking interneurons has been examined which share many properties with SF lateral inhibition. These amacrine (axon-less) cells have biplanar dendritic trees in the Medullary neuropile, and respond to light ON with a phasic/tonic depolarization without action potentials. Both response phases are proportional to the log of the light intensity. The receptive field of each amacrine cell covers the entire cornea. Depolarization of an amacrine cell with extrinsic current in the light depresses the firing frequency of extracellularly monitored SFs, and hyperpolarization increases the SF frequency. In some cases, amacrine cell depolarization produces synchronous bursting of SF output. The similarities between amacrine cell response properties and SF lateral inhibition, and the direct demonstration of SF inhibition with depolarization of amacrine cells, lead to the conclusion that amacrine cells form part or all of the SF lateral inhibitory system.

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99.12 MICROCIRCUITRY OF THE ON-ALPHA (Y) CELL CONTRIBUTES TO VELOCITY TUNING. R.G. Smith*, M.A. Freed, P. Sterling, Dept. Anat., Univ. of Pa. Med. Sch., Phila., PA, 19104, M.L. Hines* and J.W. Moore, Dept. Physiol., Duke Univ. Med. Ctr., Durham, NC, 27710.

The on-alpha (Y) cell has the largest dendritic tree (200-1000 μm) of any ganglion cell in the cat retina. It responds to stimuli moving from 1-1000°/sec with a peak response at velocities near 100°/sec; larger cells prefer higher velocities. (Cleland, B.G. and Harding, T.H., J.Physiol. 345, 47-63, 1983).

We used a compartmental model to ask whether the velocity sensitivity of the alpha cell could be accounted for by the arrangement of excitatory inputs on its dendritic tree. An important source of input comes from a regular array of CBb_1 narrow field, excitatory cone bipolar cells which have a depolarizing transient (Freed, M.F. and Sterling, P., Neurosci. Abs. 1983; Nelson, R., and Kolb, H., Vis. Res. 23, 1183-1195, 1983). Each compartment simulated a small portion of the alpha cell dendrite and the synaptic inputs from one bipolar cell. A compartment generated an epsp while the stimulus remained within its simulated receptive field. The psp had a large initial transient limited to less than a preset duration. The effect of this arrangement was that moving stimuli sequentially activated a row of simulated bipolar synaptic contacts along the dendrite.

One possible velocity tuning mechansim might be temporal summation of epsp's propagating centripetally along a dendrite from successively illuminated bipolars. We found the psp propagation velocity to be more than 1000°/sec, an order of magnitude faster than the optimal stimulus velocity. While the integrated psp from a moving stimulus was temporally summed at a stimulus velocity equal to the propagation velocity, the effect was insignificant because slower moving stimuli gave longer total psp on-time, thus larger responses at the soma.

Another possible mechanism for velocity tuning might be that the waveshape of the CBb_1 psp determines the extent of temporal summation. For a small range of epsp shapes, we found that the simulated firing rate of the alpha cell had a peak near 100°/sec, as observed in physiological recordings. At higher velocities, the stimulus remains in the alpha cell receptive field for a shorter time leading to reduced psp amplitude. At lower velocities the increasing time between psp transients allows psp's to decay. Alpha cells with larger receptive fields show higher velocity preferences because their longer dendrites allow greater psp integration times. This result implies that specificity in pattern of synaptic contact and timing together with dendritic length contribute to alpha cell velocity tuning.

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99.13 TRANSIENT EFFECTS OF SMALL SPOT EXPOSURES ON RHESUS SPATIAL VISION. D.O. Robbins, H. Zwick*, K.R. Bloom* and M.G. Leedy* Ohio Wesleyan Univ., Delaware, OH 43015 and Letterman Army Institute of Research, San Francisco, CA 94925.

Maximum visual acuity in the primate retina is usually thought to follow the distribution of foveal photoreceptors. As the distance from the foveola increases, acuity is thought to decline rapidly under maximum photopic conditions. We have tested this notion in the rhesus using both Landolt rings and square wave gratings. On and off-axis assessments of visual acuity and contrast sensitivity were made following punctate exposures of selected regions of the retinal mosaic with coherent light of different wavelengths and exposure energies. Visual performance was tracked prior to, during and immediately following exposure.

For exposure energies ranging from near morphological damage level to energies several log units below this level, brief foveal exposures (100 msec) of spot sizes from 20 to 50 microns in diameter produced transient changes in both visual acuity and contrast sensitivity beyond that which would be expected solely on the basis of expected local adaptation of a limited number of foveal photoreceptors. Longer duration exposures (5 to 15 minutes) of even lesser energy levels positioned in the center of the fixation point produced similar transient shifts in visual performance. Placement of the flash exposures at various degrees of eccentricity (1 to 5 degrees off the fixation point) produced approximately the same magnitude of acuity deficits and the duration of these recovery processes were essentially similar to those observed for foveola exposures. Transient changes in contrast sensitivity were not restricted to the highest spatial frequencies, but were observed across a wide range of spatial frequencies (38 to 2 cycles/degrees). An analysis of the time required for full recovery showed that longer recovery times were necessary when lower frequency stimuli were used to follow shifts in sensitivity than when intermediate or high spatial frequencies were employed.

While we have not ruled out the contribution of stray light in these findings, the extensive range of exposure levels and spatial frequencies over which similar deficits were observed moderates acceptance of this conclusion. Alternatively, physiological foveal receptive fields may be larger than previously considered or anatomically derived.

99.14 DIFFERENT RESPONSES OF CAT GANGLION CELLS TO ISCHEMIA AND EYEBALL DEFORMATION. O.-J. Grüsser, U. Grüsser-Cornehls,* R. Kusel* and A. Przybyszewski.* Dept. of Physiology, Freie Universität, Berlin, Germany (West)

Eyeball deformation of 2 – 20 sec duration in deeply anaesthetized cats performed in total darkness leads to an activation of on-center ganglion cells (X – and Y-type) and an inhibition of off-center ganglion cells (X– and Y-type). Release of deformation leads to a delayed activation of off-center neurons, while in on-center neurons a short post-deformation activation is found mainly in Y-neurons. The other on-center neurons returned to their normal dark activity after an inhibition period of several seconds. Eyeball indentation causes ischemia due to increased IOP (up to the level of arterial blood pressure) and retina stretch due to the enlargement of the surface. Stretch is believed to especially affect horizontal cells leading in these neurons to an increase in sodium-conductance and a depolarization. This would explain the different responses of the on– and off-neuron system to eyeball deformation.

Increase in intraocular pressure (IOP) by means of a cannula inserted into the anterior chamber leads to an ischemic interruption of the spontaneous and evoked activity of on-center and off-center ganglion cells when the perfusion pressure is lower than 15 mm Hg. The recovery to normal activity after normal IOP is attained depends on the duration of the preceding ischemic period. On-center neurons only respond in total darkness to increased intraocular pressure with a modest activation of several seconds duration before the ischemic depression of neuronal activity is observed. This might be due to the slight increase in retinal surface caused by the high IOP.

Eyeball deformation in a hydrodynamic open condition (only short transitory increase in IOP), however, also evoked an activation of on-center neurons and an inhibition of off-center neurons. Thus the activity of the retinal ganglion cells during eyeball deformation (closed eye) consists of two response components: activation of the on-system and inhibition of the off-system by retinal stretch, and inhibition and decrease in spontaneous activity in both neuronal systems caused by acute retinal ischemia. Ischemic effects had a latency of 6 – 10 seconds, stretch effects of 0.2 –2 seconds.

It will be demonstrated that the stretch effects mentioned can be "titrated" with light-evoked excitation or inhibition and also affect the responses of retinal ganglion cells aroused by sinewave modulated electrical current applied to the eye.

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99.15 RADIAL PROFILE OF RESISTIVITY IN FROG RETINA. C.J. Karwoski, D.A. Frambach*, & L.M. Proenza. Vision Res. Lab., Univ. of Georgia, Athens, GA 30602, and Eye Res. Inst., Boston 02114.

Measurements of tissue resistivity are of importance for determining sources and sinks of extracellular (EC) current and the volume fraction (α) of EC-space. These in turn are necessary for understanding the processes underlying the generation of field potentials (ΔV_o) and the accumulation and clearance of solutes in neural tissue.

In the isolated frog retina, resistance of clamped sections without optic disc was measured at 75 ohm-cm^2, which results in an average resistivity between inner and outer limiting membranes (ILM & OLM) of 5208 ohm-cm. In frog eyecups, radial profiles of retinal light-evoked ΔV_o, changes in extracellular potassium concentration, and responses to constant current pulses (ΔV) were obtained at 10 μm increments with double-barreled K^+-selective microelectrodes. Physiological criteria were developed for locating the ILM and OLM in retinas of eyecups, and layer resistivities were computed on the assumption that ΔV between ILM and OLM in each retina represented 5208 ohm-cm.

The resistance of the pigment epithelium was 330 ohm-cm^2. Resistivities (in ohm-cm) for layers of the neural retina include: subretinal space, 1310; inner and outer nuclear layers (INL & ONL), 6180; and inner plexiform layer (IPL), 1870. The ganglion cell (GC) and optic nerve fiber (ONF) layers were too thin to resolve with the 10 μm increments used in these experiments, but the resistivity of the two layers combined was 7270. The outer plexiform layer (OPL) was also too thin to reliably resolve, but its resistivity is likely the same as the IPL.

The volume fraction, α, was calculated as $(\rho_e\lambda^2)/\rho_t$, where ρ_e is the resistivity of the EC-fluid, ρ_t is the EC-component of the tissue resistivity, and λ^2 is the tortuosity of EC space. Taking ρ_e as equal to ρ of the superfusate (78 ohm-cm), and λ^2 as 2.4 for the nuclear and plexiform layers (as has been found in brain: Nicholson & Phillips, 1981) and as 1.4 for the subretinal space, gives the following values for α: subretinal space, 0.09; ONL and INL, 0.028; IPL (and OPL), 0.10; GC/ONF, 0.026.

The decreased resistivity and increased α of the IPL and subretinal space, compared to that of the nuclear layers, are expected from anatomical considerations. Inhomogeneity of resistivity and α are probably not unique to the retina, and thus need to be considered in analyses of ΔV_o, or changes in EC ionic concentrations, anywhere in the nervous system.

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99.16 A SIMPLE RELATIONSHIP FOR THE MICHAELIS-MENTEN TO WEBER-FECHNER TRANSITION IN VERTEBRATE VISION. R.L. Chappell and K.-I. Naka. Hunter College of CUNY, NY, USA & National Inst. for Basic Biology, JAPAN

The Weber-Fechner (W-F) relationship and Michaelis-Menten (M-M) equation are important laws governing the visual process in man and animal. Experimentally, the two relationships have been reported even in data from the same cell although logically it has been difficult to describe how they can coexist. Our results from studies of incremental sensitivities in horizontal cells (H-cells) of turtle and catfish have suggested an approach by which the two may be closely reconciled.

Using Wiener kernels which are the incremental sensitivity of the units investigated, we found turtle luminosity H-cell incremental sensitivity to be W-F-like at higher intensities even though the cell's step response ON peak follows the M-M relationship:

$$V/Vmax = I(I + I_{\frac{1}{2}}) \quad \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

where $I_{\frac{1}{2}}$ is the intensity giving a peak response of 1/2 Vmax. For catfish, however, external H-cell incremental sensitivity was simply the M-M relationship's local slope obtained by differentiating eqn.(1):

$$dV/dI = I_{\frac{1}{2}} \cdot Vmax/(I + I_{\frac{1}{2}})^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Noting the prominent change from peak to plateau in turtle H-cell step responses compared with catfish, we reasoned that since I remains unchanged during steady illumination, the only way to form a plateau if eqn.(1) holds during the plateau is for the curve to shift laterally along the I axis to give a plateau amplitude (V') which is some fraction (ϕ) of the peak response (V), that is:

$$V' = \phi\, V \quad \ldots\ldots\ldots\ldots\ldots\ldots(3)$$

Substituting V' for V in eqn.(1) we determined $I'_{\frac{1}{2}}$, the new half amplitude intensity for the laterally shifted M-M curve. dV/dI for the plateau phase was then calculated using this $I'_{\frac{1}{2}}$ in eqn.(2) for the corresponding I.

For the Weber-Fechner relationship to hold, the product of I times dV/dI must remain constant. Our calculations showed that this condition is nearly met (<10% change per log I increment) at intensities $>I_{\frac{1}{2}}$ of the ON peak's M-M curve for any plateau having $\phi \lesssim 0.9$. Such is the case for turtle H-cells where ϕ values of 0.6 are common. For catfish, ϕ is $\sim$1.0 so I times dV/dI changes $\sim$90% per log increment over this range and increment sensitivity is simply the local slope of the M-M curve. We cannot claim new insight into how the sift of the curve is physically produced and acknowledge other models for M-M to W-F transition. Our simple rationale may at least be useful for quantifying possible contributions of peak to plateau transitions to the effect.

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99.17 SYNAPTIC ORGANIZATION OF SUBSTANCE P-LIKE IMMUNOREACTIVE AMACRINE CELLS IN GOLDFISH RETINA. S. Yazulla, K.M. Studholme* and C.L. Zucker. Department of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

Substance P is an undecapeptide which has been suggested to have a role as a neurotransmitter in many neural systems, especially those mediating pain. A class of amacrine cells in the goldfish retina exhibit substance P-like immunoreactivity (SPIR). We studied the ultrastructural appearance and the synaptic organization of SPIR amacrine cells by electron microscopical immunocytochemistry. Amacrine cells showing SPIR form a single class which have their cell bodies in the proximal portion of the inner nuclear layer and give rise to one or two descending processes which arborize in a very narrow band at the 45-50% (sublamina a) level of the IPL. SPIR is restricted to large dense-core vesicles (DCVs), which are distributed throughout the dendrites. Processes labeled with SPIR contain a mixture of DCVs and numerous small agranular vesicles. Of 88 synaptic contacts analyzed, SPIR processes occurred as the presynaptic element 57 times and as the postsynaptic element 31 times. SPIR processes contacted amacrine and ganglion cell dendrites with equal frequency, and received synaptic input from both amacrine and bipolar cells, also with equal frequency. The stratification of SPIR amacrine cells in sublamina a suggests that their synaptic interactions are restricted to "off" neurons. However this is in contrast to published electrophysiological data where the application of substance P to the retina had a long excitatory effect on the majority of ganglion cells with an "ON" or "ON-OFF" component. In many cases, this effect persisted in the presence of cobalt chloride, indicating a direct synaptic action of substance P. It is suggested that, after release from boutons, which would produce a local effect, substance P could diffuse throughout the IPL and affect neurons in both sublaminas a and b. The centrally located processes of SPIR amacrine cells are ideally suited for such an action.

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100.1 PERIPHERAL STIMULATION PRODUCES PERSISTENCE OF POSTURAL ASYMMETRY IN SPINALIZED RATS. A.L. Beggs, J.E. Steinmetz, and M.M. Patterson. College of Osteopathic Medicine, Ohio Univ., Athens, Ohio 45701.

Previous studies have shown that postural asymmetry produced by cerebellar lesions will outlast spinal transection if an appropriate time interval (1-2 hr) is allowed to elapse between brain lesion and cord section (e.g., Digiorgio, 1929). More recently, we have found that similar effects can be produced by peripheral stimulation. Additionally, a study employing animals that were spinalized prior to peripheral stimulation not only showed that persistent asymmetry could be induced but also indicated that a shorter period of stimulation (30 min) was required in spinalized rats (Steinmetz et al., 1982). Two preparations were utilized in the present study to determine the minimum stimulation period required to produce the fixation effect in intact and spinalized rats. In the first experiment, 5 mA of constant current was delivered through wound clips to the thigh skin of rats for either 10, 15, or 20 min (Ns=7). Postural asymmetry was measured immediately following termination of the stimulus, the spinal cord was sectioned, and a second measurement was taken 5 min following cord section. The results of this experiment revealed that 10 min of stimulation was not sufficient to produce the fixation effect. However, flexion of the stimulated limb persisted after spinal section in rats receiving either 15 or 20 min of stimulation. In the second experiment the fixating stimulus was delivered directly to the severed tibial nerve in the left hind limb of spinalized rats and responses to test pulses were recorded from the peroneal motor nerve. Rats were first given test pulses to establish base levels and then either 10, 15, or 20 min (Ns=7) of 1.2 mA current at 60 Hz to the tibial nerve. Stimulation was interrupted briefly (9 sec) once every 60 sec to record a response to a test pulse. The stimulation times were followed immediately by a 30 min period in which only test pulses were given at 60 sec intervals. Following stimulation, increased responsiveness was evident in the 15- and 20-min groups and not in the 10-min group. These results demonstrate that the fixation effect can be obtained in a completely neural preparation with 15 min of stimulus input.

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100.2 A ROLE FOR PROPRIOSPINAL NEURONS IN THE TONIC NECK REFLEX. E.E. Brink, I. Suzuki*, S.J.B. Timerick, and V.J. Wilson. Rockefeller University, New York, N.Y. 10021.

We have investigated pathways of the tonic neck reflex in decerebrate, acutely labyrinthectomized cats. There are at least three possibilities. First, neck afferents are known to run caudally in the dorsal columns to the rostral part of the cervical enlargement. However, transection of the dorsal columns at the C4-C5 border (present results) has no effect on the reflex in the forelimbs other than causing an apparent increase in gain. Second, a pathway may reach forelimb motoneurons via the brainstem. Such a pathway obviously contributes to the reflex, but Magnus observed the tonic neck reflex in spinal cats and we have previously observed neck-evoked modulation of spinal interneurons in spinal preparations (Wilson, Ezure & Timerick, 1984). Third, part of the substrate of the tonic neck reflex may consist of cervical propriospinal neurons. Neurons located medially in the grey matter of C4 respond to sinusoidal rotation of the head about a roll axis. We now show that many are propriospinal neurons and that a substantial fraction of these have axons that terminate in the cervical enlargement.

We recorded from 146 neurons in C4 whose activity was modulated by head rotation in roll: 44 were type I (excited by chin rotation to the ipsilateral side) and 102 type II. 57 could be activated antidromically from C5, and were therefore propriospinal neurons (11 type I, 46 type II). The axons of most neurons tested were crossed. Neurons were mainly located medially in laminae 7 and 8: the population overlaps with the medial propriospinal neurons studied by Lundberg and his colleagues (Sasaki, Alstermark and Lundberg, 1983). In many instances dynamics were checked with sinusoidal rotation within the range 0.05-2.0 Hz, and they were similar to tonic neck reflex dynamics. Stimulation at different rostro-caudal levels revealed that 60% of the neurons projected beyond the cervical enlargement, but that 40% terminated in the C6-Th1 segments. Since most of these were crossed type II neurons, they would produce a type I signal in the contralateral cervical enlargement.

While the segmental connections of propriospinal neurons remain to be studied, these results suggest that the neurons are part of the neural substrate of the forelimb tonic neck reflex. Supported in part by NIH NS 02619 and NASA NSG 2380.

100.3 VESTIBULAR RESPONSES OF NEURONS IN THE CERVICAL SPINAL CORD OF THE CAT. R.H. Schor, S.J. B. Timerick and V.J. Wilson. The Rockefeller University, New York, NY 10021.

The responses of neurons in the C7-C8 segments of the spinal cord to electrical (0.1 msec pulses, round and oval window) and natural (whole body tilt) stimulation of the labyrinth were investigated in the decerebrate cat. Tilt-sensitive neurons were characterized by the direction of tilt which produced maximal excitation (response vector). This was determined by rotating the direction of a constant angle of tilt around the animal at a frequency of 0.1 Hz ("wobble", Schor et al. 1984). The response dynamics of the cell in the plane of the response vector were investigated using 0.01-2 Hz sinusoidal tilts.

Vectors were found for 42 cells in 6 cats. 37 were within 30 deg of the roll plane; the remaining 5 cells had response vectors close to the planes of the vertical semicircular canals. At low frequencies phase was near position. Most cell responses had flat or slightly advancing phase at higher frequencies. Gain increased steadily with frequency from an average of 1.3 impulses/sec/deg at 0.02 Hz to 3.9 impulses/sec/deg at 0.5 Hz.

Of 23 cells tested with electrical stimulation of the labyrinth, 10 were excited at short latency (<5 ms), 8 disynaptically. Six cells had short latency input from the ipsilateral labyrinth only, 1 from the contralateral labyrinth only and 3 from both labyrinths. Longer latency effects were also observed. Cells having a short latency labyrinthine input could not be distinguished from those lacking such an input on the basis of response vector orientation, dynamics or sensitivity. Tilt-sensitive cells were found in laminae 4-8 and those having a short latency input tended to be most ventral in this region.

These tilt-sensitive cells may be involved in vestibular-evoked forelimb reflexes. It is surprising, regardless of their function, that no cells were found having response vectors in the pitch plane. Our results suggest no specific role for tilt-sensitive neurons receiving short-latency input, since they had similar tilt responses to the population as a whole. Our natural stimulation would not activate neurons responding primarily to stimulation of the horizontal canals or sacculus; the presence of short-latency input might have more significance for this subpopulation.

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100.4 THE EFFECT OF CHRONIC SPINALIZATION ON MEMBRANE PROPERTIES AND SYNAPTIC INPUTS TO GASTROCNEMIUS MOTONEURONS LL Baker, SH Chandler, LJ Goldberg, Dept. of Kinesiology & Brain Research Institute, UCLA, Los Angeles CA 90024 & Dept. Physical Therapy, USC, Downey, CA 90242.

The goal of the present study was to evaluate the resting membrane properties of extensor motoneurons (mn) and the synaptic effect on those mns from single shocks to the cutaneous sural nerve in acute and chronic spinal cats.

The spinal cord of adult cats was severed at the T12 level and these cats (chronic) were maintained from 4-6 months. Intracellular recordings from identified medial and lateral gastrocnemius mns were performed on acute and chronic spinal cats after decerebration. Post synaptic potentials (PSP) evoked by single shocks to the ipsilateral sural nerve were recorded. Sixty-two mns were recorded from chronic cats, and 31 mns from acute cats. No differences in the mean resting potential (RP), conduction velocity, afterhyperpolarization (AHP), input impedance or rheobase were observed between chronic and acute spinal cats. It was observed that for AHP durations, chronic cats showed a unimodal frequency distribution while acute cats showed a bimodal distribution.

The PSP patterns of 37 mns in chronic and 33 mns in acute cats, were evaluated. We observed mns demonstrating exclusively excitatory, inhibitory, or mixed PSP patterns in both acute and chronic cats. When mns were grouped according to the RP, there was no difference in the occurrence of early PSP patterns between the acute and chronic cats. The number of mns showing a late excitatory PSP (EPSP) with onset longer than 10ms and duration greater then 15ms was markedly increased in the chronic cats. The late EPSP was observed in 78% and 39% of the mns recorded from chronic and acute cats respectively. The duration of the late EPSP was also significantly longer in the mns from chronic cats (89±34ms) when compared to the mns in the acute cats (53±26ms). The increased occurrence of long latency, long duration EPSPs elicited by sural nerve stimulation in the chronic cats without a concurrent change in membrane properties suggests that a change in the interneuronal network(s) mediating this multi-synaptic cutaneous input to gastrocnemius mns might underlie the well known increase in cutaneous reflex excitability observed in chronic spinal cats.

100.5 DISTRIBUTION OF MONOSYNAPTIC Ia EPSPs IN THE MOTOR NUCLEUS OF CAT LATERAL GASTROCNEMIUS MUSCLE. S. Vanden-Noven, T. M. Hamm and D. G. Stuart. Department of Physiology, University of Arizona, Tucson, AZ 85724.

Homonymous monosynaptic Ia EPSPs are not distributed homogeneously throughout the motor nucleus of lateral gastrocnemius (LG; Vanden-Noven, Hamm and Stuart, Neurosci. Abstr. 9:528, 1983). The present study tests the hypothesis that this heterogeneity is attributable to "own-branch" EPSPs being larger than "other-branch" EPSPs (Botterman et al., Neurosci. Letters 24:35-41, 1981). The cat LG muscle is innervated by four primary nerve branches which supply anatomically separate compartments: LG1, LG2, LG3 and LGm (English, A., J. Neurophysiol., In press, 1984).

Intracellular recordings from LG motoneurons were made in anesthetized low-spinal cats during periods of electrical stimulation of the four LG nerve branches and the nerve to soleus. Measurements were made of each cell's composite homonymous own- and other-branch monosynaptic Ia EPSPs evoked by stimulation of the test-nerve branches. A normalization procedure (Vanden-Noven et al., 1983) was done to factor out variations in EPSP amplitude due to cell "type" (i.e., S, FR, FF) and variable afferent content among the nerve branches.

An analysis of variance of EPSPs produced by stimulation of each nerve branch between the different cell groups indicates a significant localization of Ia EPSPs for three out of the five pathways studied. Stimulation of the LG2 and LGm nerve branches produced significantly larger EPSPs in their own motoneurons. There was a tendency for LG1 and LG3 cells to receive larger EPSPs from stimulation of their own nerve branches, although these differences were not statistically significant. LG2 motoneurons also received larger EPSPs from soleus than did LG1, LG3 and LGm. In conclusion, there appears to be a gradation in the extent to which monosynaptic Ia EPSPs are localized in different spinal motor nuclei. The extent of localization varies not only between motor nuclei (Botterman et al., J. Physiol. 338:355-377 and 379-393, 1983), but also between components of an individual motor nucleus (e.g., LG). Supported in part by USPHS grants NS 07888, HL 07249 and NS 17887.

100.6 CHARACTERISTICS OF SPINAL INTERNEURONS THAT RESPOND TO PRIMARY SPINDLE AFFERENT ACTIVATION. W.T. Rainey, K.G. Buahin* and W.Z. Rymer, Dept. of Physiology and Neuroscience Program, Northwestern University Medical Center, Chicago, IL 60611.

The responses of many lumbosacral spinal interneurons to longitudinal tendon vibration and to Group I strength electrical stimulation of triceps surae nerves have provided evidence that these cells receive mono- or poly-synaptic input from primary (Ia) spindle afferents. In addition, although Golgi tendon organs are vibration responsive in contracting muscle, similar findings were obtained in two animals with ventral root section, in which tendon organ input would not be significant. In decerebrate cats, we have identified three types of Ia recipient interneurons. However, putative Ia inhibitory interneurons have not been extensively analyzed. The two other types of cells can be differentiated by their responses during and after a vibratory stimulus and the strength and latency of their responses to nerve stimulation. One group of interneurons in the intermediate grey apparently has strong monosynaptic Ia input, because their firing rates can be partially or completely locked at the frequency of longitudinal tendon vibration, especially when the muscle is stretched to near maximum physiological length. During ramp and hold stretche the transition between the peak dynamic firing and the tonic rate is more gradual than that seen in Ia afferents. Four-fifths of the cells in this category respond to selective mechanical stimulation of muscular free nerve endings, although the apparent strength of the input from these endings varies with the state of descending input to the lumbosacral cord. After partial to complete transections of T12, almost all neurons in this category respond to the activation of free nerve endings.

A second group of neurons that responds to Ia afferent activation continues firing above background levels for tens to hundreds of milliseconds after cessation of longitudinal tendon vibration. Most of these cells, especially the neurons with medium to long duration after discharges, are at best poorly driven by Group I electrical stimulation. These cells usually have weak dynamic responsiveness to ramp stretch and at most weak responses to mechanical stimulation of free nerve endings. These neurons may receive predominately polysynaptic Ia input and may be responsible for the prolonged increase in EMG and force activity following vibration. (Supported by NIH NS 14959 - NINCDS.)

100.7 RENSHAW CELL DESYNCHRONIZATION OF MOTOR OUTPUT. Kwame G. Buahin* and W. Zev Rymer. (SPON: Y. Geinisman). Neuroscience Program and Dept. of Physiology, Northwestern Univ., Chicago Ill. 60611

Renshaw interneurons (RC) have previously been shown to decorrelate discharge patterns among motoneurons (MNs) isolated from sectioned ventral root filaments (Adam et al, Biol. Cyb. 29: 229 1978). We have investigated whether blocking the cells with cholinergic antagonists produces discernible alterations in EMG and force which might be attributed to increased synchrony of motoneuronal discharge. Synchronization of motor unit (MU) activity leads to reduced tetanic fusion and lowered force production (Rack et al, J. Phys. 204: 443 1969). Hence, more EMG activity will be needed to generate the same force levels when MU synchronization is increased. The shapes and amplitudes of peaks on tremor force power spectra are also modified by synchronization (Allum et al, JNP 41: 557, 1978).

The cat soleus muscle was studied in decerebrated preparations in which force was generated via the crossed extensor reflex. Intramuscular EMG and DC and AC-coupled high-gain force (tremor force) were recorded before and after blockade of RC activity. Reduction of the cells' response was produced by intravenous and intrathecal administration of atropine (ATR, 0.5mg/kg) and mecamylamine (MEC, 4mg/kg). Single unit recording data indicate that these drugs, with the dosages used, abolish RC activity (Adam et al, ibid).

In 13 experiments, slopes of linear plots of rectified EMG versus force consistently showed increases after RC blockade, indicating that more EMG activity was required to generate the same force levels after cessation of RC activity. Power spectra of tremor force had bandwiths of 20-25Hz, from DC, and always showed peaks between 3-8Hz, both before and after drug administration. However, power amplitudes for the same DC force levels were augmented by as much as 5-10 fold after RC blockade. Depending on the preparation, tremor spectra for the same force levels broadened at either or both low and/or high frequency ends after ATR/MEC, although no consistent trend has been established yet. Our findings of slope changes in EMG-force relations and augmentation of amplitudes of tremor force spectra are consistent with a decorrelating role for RCs. RCs, thus, appear to reduce tremor and improve the efficiency of the force-generating apparatus of cat soleus. (Supported by NIH R01-NS14959 - NINCDS)

100.8 STRETCH REFLEX THRESHOLD AND VELOCITY DEPENDENCE IN SPASTICITY. R.K. Powers, W.Z. Rymer and J. Marder-Meyer. Rehabilitation Institute of Chicago, Chicago, IL, 60611.

Several investigators have reported that the amount of reflex activity evoked by stretch of spastic muscle is strongly dependent upon stretch velocity. Spastic muscle is generally inactive at rest, but can be readily activated by rapid stretching.In contrast, when a stretch reflex is superimposed upon an active muscle in normal subjects, the velocity dependence of stretch evoked EMG and force can be rather modest (Geilen and Houk, J.Neurophys., in press). This difference in velocity dependence could be due to a fundamental difference in reflex dynamics or to phenomena related to the onset of the stretch reflex in quiescent muscle. We are therefore investigating the velocity dependence of the stretch reflex of the elbow flexors of spastic hemiplegic subjects by measuring reflex threshold and total reflex activity evoked by ramp and hold extensions of the elbow at different ramp velocities.

Fourteen hemiplegic subjects with mild to severe spasticity in the elbow flexor muscles have been studied to date. A torque motor configured as a postion servo applied 1 radian angular extensions about the elbow at four different constant angular velocities (0.25, 0.50,1 and 2 rad/sec). A PDP 11/23 computer controlled the presentation of stretch stimuli and the recording of joint angle, torque and surface EMG from elbow flexor and extensor muscles.

Many subjects showed considerable variability in the amount of torque generated in response to repeated presentations of a given stretch, both within a single session and between sessions. Increases in reflex torque were generally associated with decreases in the reflex thresholds of elbow flexors (measured as the joint angle at which reflex activity begins). Reflex threshold was inversely related to stretch velocity. Reflex EMG generally showed a linear dependence on stretch velocity, as previously reported for spastic patients (Ashby and Burke, JNNP,34:765, 1971), but in contrast to the low fractional power dependence reported for normal subjects (Geilen and Houk,ibid.). We are currently investigating whether this difference may be partly due to threshold related phenomena by examining stretch evoked responses when the subject is asked to actively contract the flexor muscles prior to and during the stretch.

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100.9 REFLEX GAIN, MUSCLE STIFFNESS AND VISCOSITY IN NORMAL CATS. J.A. Hoffer, T.R. Leonard*, N.L. Spence* & C.L. Cleland. Department of Clinical Neurosciences, University of Calgary Faculty of Medicine, Calgary, Alberta T2N 4N1, CANADA.

The poor regulation of muscle stiffness in decerebrated cats has been attributed to the low gain of force feedback in that preparation (Hoffer & Andreassen, Muscle Receptors and Movement p. 311, 1981). Consequently, we have examined how reflex gain differs in normal and decerebrated cats.

Using the approach described last year (Hoffer et al., Soc. Neurosci. Abstr. 9:470), we delivered calibrated perturbations to the ankle extensors of quietly standing, unrestrained cats and measured the length, force and EMG changes in the lateral gastrocnemius and soleus muscles. We then repeated the experiment after decerebration at the premammillary level. Perturbations were produced by electrical stimulation of the common peroneal nerve (1 Hz), causing the ankle flexors to briefly stretch the ankle extensors about 0.3 mm. Alternatively, we pushed downward on the cat's back to stretch the ankle extensors by 1-2 mm.

To insure that differences in the reflex responses were only due to changes in the activity of descending pathways, we carefully matched the muscle length and force preceding the perturbation, as well as the amplitude of the imposed stretch. The temperature of the hindlimb, monitored with implanted thermistors, was restored to normal values with radiant heat after decerebration.

We found that 0.3mm stretches caused an early EMG peak (10-40ms) of unchanged amplitude but doubled area after decerebration. There was also an increase in the reflexly-mediated force. After decerebration, 1-2mm pushes caused large, poorly damped oscillations in EMG, force and length, whereas in normal cats responses would quickly stabilize.

The abnormal reflex behavior following decerebration could be due to 1) altered motor unit activation patterns, 2) altered fusimotor drive, 3) enhanced gain of excitatory reflex pathways and/or 4) reduced gain of inhibitory reflex pathways. The abrupt termination of the early EMG peak and increased damping in normal cats are consistent with reflex inhibition being stronger in normal than decerebrated cats.

We propose that the balance between force and length feedback (Houk et al., J. Neurophysiol. 33:784, 1970) may prove to be appropriate for the segmental regulation of both stiffness and viscosity (i.e., the mechanical impedance of muscles) in normal, though not in decerebrated, cats.

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100.10 REFLEX COMPENSATION IN TIBIALIS ANTERIOR AND SOLEUS MUSCLES COMPARED IN THE DECEREBRATE CAT. T.R. Nichols. Dept. of Physiology, Emory Univ. School of Med., Atlanta, GA 30322.

Autogenetic reflex action in the soleus muscle (SOL) of the decerebrate cat is known to compensate for yielding and other nonlinear behavior, to enhance the stiffness of the muscle and to constrain the muscle to obey characteristic relationships between stiffness and force and between stiffness and amplitude of length change (Nichols and Houk, 1976. J. Neurophysiol., 39:119-142). In the present study, mechanical consequences of reflex action in a flexor (tibialis anterior, TA) were investigated and compared with reflex action in SOL. The tendons of TA and SOL were connected to the pulley of a position-controlled printed motor so as to preserve reciprocal mechanical coupling. The cat was decerebrated at the premammillary level and the muscles activated selectively by peripheral nerve or brainstem stimulation. Ramp and hold length changes .5 to 8 mm in amplitude were applied to the muscles. Muscle temperature was held at either room temperature or 36°C. Before an experiment was terminated, the muscles were excited through their respective nerves at 20-50 Hz and perturbations applied to measure the response properties of arreflexive muscle.

In most preparations, characteristic relationships were similar in shape for both SOL and TA. However, the magnitudes of regulated stiffness for TA were generally, although not always, smaller. Comparison of reflex responses and responses of arreflexive muscle showed that this quantitative difference was due to a smaller enhancement of stiffness by reflex action in TA. The stiffness of both regulated and arreflexive TA decreased with amplitude and increased somewhat with force. Arreflexive TA showed only a small amount of yield which became slightly more accentuated at the lower temperature. The yield was largely compensated by reflex action.

These results indicate that TA is an inherently more linear muscle than SOL under these conditions and that improvements in linearity brought about by reflex action are therfore more subtle than in the case of SOL, as expected for a regulatory system. In the case of TA, responses to perturbations are dominated by the underlying mechanical component of the response.

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100.11 ADAPTIVE PLASTICITY IN THE PRIMATE SPINAL STRETCH REFLEX: SPECIFICITY OF EFFECT. J.A. O'Keefe*, R. Dowman and J.R. Wolpaw (Spon: K.D. Barron). Center for Labs and Research, NYS Dept. of Health, and Depts. of Neurology and Anatomy, Albany Medical College, Albany, NY 12201.

Monkeys can change the amplitude (amp) of the initial, purely segmental response to sudden muscle stretch, the spinal stretch reflex (SSR), when reward is contingent on such change (Wolpaw et al., J. Neurophysiol. 50: 1296-1319, 1983). Over weeks, SSR amp can increase to >150% of control or decrease to <50%. Change is relatively specific to the agonist muscle. We studied concurrent effects on post-SSR EMG and the somatosensory evoked potential (SEP) to further define the specificity of SSR adaptive change.

Five monkeys (Macaca nemestrina) were chronically implanted with fine-wire EMG electrodes in biceps and triceps and screw electrodes over contralateral primary somatosensory cortex and frontal sinus. Each was trained by computer to keep elbow angle at 90° (±1.5°) against steady extension (or flexion) force. If this angle was maintained for a randomly selected 1.2-1.8 sec period, and if the average absolute value of biceps (or triceps) EMG for the final 100 msec was within a preset range, a brief torque pulse extended (or flexed) the elbow 3-4° eliciting the SSR and SEP. The task functioned under one of three modes. Under the control mode, reward always occurred 200 msec after pulse onset. Under the SSR↑ or SSR↓ mode, reward occurred only if the average absolute value of EMG during the SSR interval (14-24 msec after pulse onset) was greater (SSR↑), or less (SSR↓), than a specified value.

Under all 3 modes, M2 and M3 components were very small or absent. When SSR amp increased (SSR↑ mode), the post-SSR silent period was often more marked; while when it decreased (SSR↓ mode), the silent period was often less marked. This compensatory change was much less than the SSR amp change.

The first SEP peaks were a 15 ms positive peak and a 25 ms negative peak. When SSR amp changed under the SSR↑ or SSR↓ mode, little or no change occurred in SEP peak amps.

These results suggest that SSR adaptive change is not due to change in afferent input such as might be produced by alteration in muscle spindle sensitivity. Furthermore, the effect of the process underlying SSR change seems mainly confined to the wholly segmental, largely monosynaptic arc of the SSR. Function of closely-related pathways appears unchanged. At present the most probable site of action of the alteration responsible for SSR adaptive change is the Ia synapse on the alpha motoneuron.

100.12 CENTRAL AND PERIPHERAL MODIFICATION OF THE UNLOADING REFLEX IN MAN. J.D. Cooke and S. Spencer*. Dept. of Physiology, University of Western Ontario, London, Canada.

Following the sudden removal of a maintained force, a characteristic sequence of changes in EMG activity occurs in the unloaded or shortening muscle. This sequence, called the unloading reflex, consists of a decrease in EMG drive (silent period) followed by a phasic burst of activation. The latencies of the silent period and the later phasic response (25-30 and 70-80 msec respectively) are similar to those of the 'short' and 'long' latency EMG responses to sudden muscle stretch (loading) in man. In the present study we compared the effects of instruction and peripheral input on the unloading reflex with their known effects on the loading reflex.

Experiments were performed on 8 normal humans, both male and female. Subjects maintained a constant arm position (approx. 90 deg elbow joint angle) opposing a steady load applied by a torque motor. The load could oppose either flexion (flexion load) or extension (extension load) of the forearm. The applied force was removed at random times to elicit an unloading reflex in the unloaded muscle. The duration and rate of the unloading were varied as well as the force level during unloading.

As seen with the loading reflex, the late response in the unloading reflex (phasic excitation) was affected by the instruction given to the subject. Excitation was progressively decreased as the subject was instructed to 'assist' the movement on unloading, to 'let go' or not respond, to 'stop' the movement or to 'return' the handle back to the initial position. The early response (silent period) was relatively resistant to instruction. In contrast, both the early and late responses in the unloading reflex were affected by the amount and rate of change of the load. Both responses depended on the initial rate of arm movement produced by the load change, being greater the more rapid the initial rate of arm movement.

The unloading reflex thus bears strong parallels with the loading reflex. In both cases the long latency components are sensitive to the instruction given to the subject and both short and long latency responses are determined by the nature of the perturbation of the arm position.

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100.13 EXCITABILITY CYCLE OF A HUMAN FLEXION REFLEX: AN EXAMPLE OF LEARNING IN THE NERVOUS SYSTEM. K.L. Robinson*. (SPON: R. Butler). Program in Occupational Therapy, Univ. of Western Ontario, LONDON, Ontario, Canada N6A 5A5. (RESEARCH DONE AT: Neurosciences Dept., McMaster University, Hamilton, Ontario, Canada. L8N 3Z5)

Cutaneous reflexes have long interested neurologists and neurophysiologists; most often they have been used in humans to investigate pathological conditions (Dimitrijevic and Nathan, 1968; Shahani and Young, 1971). Hagbarth (1960) applied strong electrical stimuli to different skin areas of the lower limbs during weak voluntary muscle condtractions and found early and late excitatory components which reflected a topographical pattern of muscle involvement. The purpose of the present study was to investigate the excitability changes in the upper extremity cutaneous flexion reflex circuit of normal subjects over a month-long period of regular testing.

The results indicated the early and late excitatory changes to low threshold stimulation, which have been attributed to group II cutaneous fibres, and the prominent late increase in excitability thought to be mediated by group III fibres (Caccia et al., 1973; Faganel, 1973). The intervening silent period has been attributed to Renshaw cell inhibition and other spinally-generated mechanisms modulated by supraspinal influences (Caccia et al., 1973; Jenner and Stephens, 1982). This silent period was found to deepen and lengthen when followed over the one month period. As well, between day variability of reflex responses decreased with time. These results will be discussed.

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100.14 SPINAL REFLEXES IN THE ATLANTIC STINGRAY, DASYATIS SABINA. C.A. Livingston and R.B. Leonard. Marine Biomedical Institute and Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston Texas 77550

The study of spinal cord reflexes can reveal basic features of spinal cord organization. It has been demonstrated that the spinal cord generates the locomotor rhythm in many species including stingrays. It is likely that the neuronal circuitry underlying locomotion and reflexes share some common elements. Here we describe the segmental reflexes in the stingray as a step in identifying circuits related to the locomotor pattern generator.

The animals were anesthetized, decerebrated, and then allowed to recover from anesthesia. The reflexes were examined in paralyzed animals while decerebrated and again after spinal transection. In stingrays, each segmental peripheral nerve divides to innervate the elevator and depressor muscles of the pectoral fin. Both elevator and depressor branches were separated into motor and sensory divisions and all four branches were mounted on bipolar electrodes. The sensory nerves were stimulated with single pulses or brief trains. The stimulus intensities were set to recruit only large fibers or both large and small fibers while monitoring the sensory volleys with an additional electrode.

In spinal animals, the responses to stimulation of either the elevator or depressor sensory nerves was a simultaneous burst (8-15 msec latency) on both motor nerves. The burst on each motor nerve had two parts with a total duration of 25-40 msec on the elevator and 10-20 msec on the depressor motor nerve. The burst on the depressor motor nerve was followed by a quiet period that typically outlasted the burst on the elevator nerve. The burst on each motor nerve and the duration of the quiet period increased with increased stimulus intensity. Occasionally the depressor nerve burst was absent, but the quiet period still occurred now with a latency similar to the elevator nerve burst. Prior to spinalization, the reflexes were variable and depended upon shifting levels of spontaneous activity and the occurrence of spontaneous fictive swimming. When reflex bursts were clearly present, they were similar to those in the spinal animals although distinctly less vigorous. In addition, the depressor quiet period was significantly shorter.

Neither reciprocal inhibition nor local sign is evident in the short latency reflexes. The quiet period on the depressor nerve may represent reciprocal inhibition and be significant for locomotor pattern generation. (Supported by Grant No. NS11255)

100.15 THE FUNCTIONAL SIGNIFICANCE OF THE PERIORAL REFLEX IN VOLUNTARY MOTOR CONTROL OF THE LIPS. M.P. Caligiuri* and J.H. Abbs* (SPON: R. Bleier). Speech Motor Control Labs., Waisman Center, Univ. of Wisconsin, Madison, WI 53705-2280.

Few motor physiologists would disagree with the general importance of muscle spindles in the control of movement. The monosynaptic connections between spindles and motoneurons and the powerful projections of these afferents to cortical areas support their potential significance. Paradoxically, the facial muscles, which are important for facial expression and speech, are devoid of spindles, at least in primates. Moreover, while a perioral reflex can be elicited by select mechanical stimuli applied to the lips, its functional significance in control of movement is equivocal. Unlike stretch reflexes in the jaw closing muscles or in the limbs, the perioral reflex does not appear to be monosynaptic although it is mediated in the lower brain stem. In this context, determining the potential role of the cutaneous perioral reflex represents an interesting problem in the sensorimotor regulation of movement.

One question regarding the contribution of the perioral reflex to motor control is its relative sensitivity to stretch stimuli with velocities and accelerations in the same range as voluntary lip movements. Some observations suggest that a percussive tap is the most effective stimulus. Another issue in demonstrating a functional role for the perioral reflex concerns the differential excitation of antagonistic muscles. For this reflex to be functional for a given motor task, compensatory excitation in the agonist muscle should be accompanied by inhibition of the antagonistic muscle.

In this study, stimuli were selected to include velocities and accelerations (1) within and (2) outside the range of voluntary labial movement. These labial stretch stimuli were introduced during different isometric lip closure postures. EMG from four perioral muscles was recorded: two lower lip muscles (o. oris inferior and its antagonist, depressor labii inferior) and two upper lip muscles (o. oris superior and its antagonist, levator labii superior). Stimuli with velocities and accelerations within the range of voluntary lip movements did not elicit a perioral reflex, whereas stimuli outside of this functional range elicited excitatory responses in both the agonist and antagonist muscles. These results suggest that the perioral reflex may not play a prominent role in the motor control of the lip. Research supported by grants from NIH (NS-13274 and HD-03352).

100.16 THE SOURCES OF EFFERENT VAGAL FIBERS IN CANINES. N. L. Strominger, A. P. Knox*, D. O. Carpenter and D. B. Briggs*. Dept. of Anatomy, Albany Medical College, Albany, NY 12208 and New York State Department of Health, Albany, NY 12201.

The distribution of perikarya emitting axons into the vagus nerve was studied in a series of dogs using horseradish peroxidase histochemical techniques. Enzyme (25-33%) was injected directly into the cervical portion of the vagus nerve unilaterally in one group of animals; the vagus was ligated below the level of the injection. The nerve was cut and dipped for the duration of the experiment in a capsule containing the enzyme in a second group. After 2-4 days dogs were perfused with phoshate buffered saline followed by 8% gluteraldehyde. Blocks through the bulb and cervical spinal cord were cut at 50 μm on a freezing microtome, reacted with tetramethyl benzidine and stained with neutral red. The spatial disposition of labeled perikarya was plotted on projection drawings.

Label was exclusively ipsilateral to the injection. The great majority of labeled perikarya were in the dorsal motor nucleus of the vagus (DMX). Most cells of DMX were labeled in some cases, though there was a wide range in intensity of reaction product. Some labeled cells of DMX appeared to extend into the adjacent area postrema (AP). A few labeled cells were seen in some cases within the AP at a distance from DMX. Also, labeled cells were located in a subependymal position immediately rostral to AP. A rare labeled cell was seen just ventral to the solitary tract. The nucleus ambiguus was well-labeled. Occasional labeled cells were present in the tegmentum between nucleus ambiguus and DMX. Another group of labeled cells conformed to the retrofacial nucleus. These observations are in agreement with those of Chernicky et al. ('83), and in addition show that a few cells of the AP give rise to fibers in the vagus nerve.

101.1 ADAPTATION OF HUMAN WRIST MOVEMENTS TO INERTIAL LOADS. S.L. Lehman* and J.C. Houk. Rehabilitation Inst. of Chicago and Dept. of Physiology, Northwestern Univ., Chicago, IL 60611

Surprise changes in inertial loads bring about adaptive modification of wrist muscle forces. During a series of fast, practiced flexions and extensions of the wrist, a large (24 Kg) inertial load was suddenly imposed, without prior cues to the subject. Force, velocity,and position of the wrist were recorded by the same apparatus that imposed the load. We tabulated force extrema, their timings, and the durations of force pulses (time between zero crossings) as subjects adapted to the new load.

Timings and magnitudes of forces adapted independently, in quite different ways. Force durations changed drastically and adaptively during the first loaded movement. Agonist force was applied for about twice as long as for the unloaded movements. The duration of antagonist force also increased dramatically during the first loaded trial. Both durations exceeded their fully adapted values during this movement. In the sequence of successive loaded trials, force timings were further tuned, but over a smaller range. Force durations converged on values somewhat smaller than those for the first loaded trial, and much longer than the comparable durations for unloaded movements. Force magnitudes, by contrast, changed only moderately in the first loaded trial. The slight increase in agonist force is probably accounted for by decreased velocity of movement (the force-velocity curve). Force magnitudes then adapted during the course of several trials, increasing gradually to larger values.

We confirmed the independence of force duration and amplitude adaptations for different sizes of wrist movements (0.5-6.0 cm, or about 5 to 50 degrees), and for a smaller inertial load (4 Kg). Electromyograms (EMG) of wrist flexors and extensors show qualitative agreement with the force duration changes. High agonist EMG activity occurs over a longer period in the first loaded trial, as it does in later trials. Also, the first burst of antagonist EMG appears to be shortened in the first loaded trial.

Transitions from large to small inertias showed drastic changes in both impulse timing and force magnitude in the first unloaded movement. Initial agonist duration dropped to about half its loaded value, then continued to decline appropriately during succeeding movements. The agonist pulse height also dropped precipitously, in contrast to the loading transition.

101.2 CONTROLLED VARIABLES IN STEP-TRACKING MOVEMENTS OF THE WRIST. D.S. Hoffman and P.L. Strick. VA Med. Ctr. and Depts. of Physiol. and Neurosurg., SUNY-Upstate Med. Ctr., Syracuse, NY 13210.

Step-tracking movements are characterized by a distinct pattern of muscle activity. A burst of activity in agonist muscles precedes movement onset. This burst is followed by a burst of activity in antagonist muscles. We sought to determine what kinematic parameters of movement these bursts of muscle activity might control.

Human subjects performed a step-tracking task which required them to make different amplitudes of wrist movements (radial deviation). On some trials subjects were instructed to move as fast as possible and on others they were to move at slower speeds. Muscle activity was recorded from an agonist muscle, extensor carpi radialis longus (ECRL) and an antagonist muscle, extensor carpi ulnaris (ECU).

We examined the correlations between the magnitudes of the agonist and antagonist bursts and the following kinematic parameters: distance, velocity, acceleration, the rate of change of acceleration (jerk) and movement duration. When movement amplitude and intended speed were varied, the magnitude of the agonist burst was best correlated with the initial peak of acceleration or the initial peak of jerk. The magnitude of the agonist burst showed the lowest correlation with movement duration. In contrast, the magnitude of the antagonist burst was best correlated with the reciprocal of movement duration and showed the lowest correlation with movement amplitude. Furthermore, the product of peak acceleration (or jerk) and movement duration was linearly related to the peak amplitude of movement.

Our observations lead us to propose that the two bursts of muscle activity control two different movement parameters: 1) agonist burst: the initial peak of acclera-tion (or jerk); 2) antagonist burst: movement duration. Furthermore, we propose that the central nervous system determines the initial trajectory of step-tracking movements by controlling these two movement parameters.

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101.3 A MATHEMATICAL ANALYSIS OF MOVEMENT FOLLOWING PERTURBATION DURING HOLD-RAMP-HOLD WRIST MOVEMENTS IN THE MONKEY. S.A. Kane*, J.W. Mink, and W.T. Thach (SPON:W.M. Landau). Departments of Anatomy & Neurobiology and Neurology & Neurosurgery and The McDonnell Center for the Study of Higher Brain Function, Washington University School of Medicine, Saint Louis, MO 63110.

The oscillatory behavior of a monkey's wrist was modeled as a classical spring-dashpot system, successfully quantitating the differences in damping and stiffness associated with tremor (r^2 of .98 to .99+ for each task).

Rhesus monkeys were trained to insert their hands, with fingers extended, into a wedge-shaped manipulandum and to perform hold-ramp-hold movements by flexing and extending about the wrist while tracking a visual target. Trajectories at velocities of 28 deg/sec were performed in each direction through 70 deg of wrist arc with and against uniform torque loads (.10 and .14 N-m). Each task was performed in blocks of 10 trials, two of which included a 50 msec loading or unloading torque pulse (.077 and .11 N-m), one delivered during the hold, .5 sec before movement began, the other during the ramp after the monkey had moved 15 deg from the hold position. Position was measured by a potentiometer circuit, averaged for 6-10 trials, and stored in 6 msec bins.

Position data were chosen to begin after the torque pulse discontinuity and end 100-300 msec later, when either the oscillations died away or the monkey made a volitional, non-oscillatory movement. The damping coefficient g (1/sec) and and natural frequency f_0 (hz) were determined by fitting the position as a function of time to $f(t)=Ae^{xp}(-gt/2)\cos(wt+a)$ for the 8 task conditions and averaging for holds and ramps:

	g-hold	g-ramp	f_0-hold	f_0-ramp
#1	76.8 +/- 10.8	59.1 +/- 7.9	10.2 +/- .7	8.74 +/- .39
#2	54.3 +/- 7.4	64.6 +/- 8.7	7.49 +/- .17	9.17 +/- .47
#3	20.8 +/- 3.5	20.0 +/- 3.6	7.37 +/- .41	7.30 +/- .34

Monkeys #1 and #2 had more damping than monkey #3 (paired t-test, $P<.01$) and had little physiological tremor. Monkey #3 had relatively poor damping (oscillating for periods up to 300 msec after perturbation), relatively more compliance (proportional to f_0^{-2}), and a large amplitude, 8-10 Hz tremor. The calculated parameters were constant over the 100-300 msec time range considered for each task. The role of changing EMG, spindle afferent, and cerebellar activities in the modulation of damping, compliance, and tremor is currently being investigated. (Supported by Grants #5 T32 GM07200, #NS12777-09, and The McDonnell Center)

101.4 AGONIST-ANTAGONIST MUSCLE ACTIVITY IN OSCILLATION AFTER TORQUE PULSE PERTURBATIONS OF THE MONKEY'S WRIST. J.W. Mink, S.A. Kane*, and W.T. Thach. Departments of Anatomy & Neurobiology and Neurology & Neurosurgery and The McDonnell Center for the Study of Higher Brain Function, Washington University Medical School, St. Louis, MO 63110.

Rhesus monkeys were trained to perform a hold-ramp-hold tracking task by flexing and extending at the wrist. The tasks were performed in blocks of 10 trials, two of which included a 50 msec torque pulse that was delivered either during the initial hold or after the monkey had tracked the first 15 degrees of the trajectory (See Kane, et al, Soc. for Neurosci. Abstr., 1984). EMG activity was recorded from the wrist flexor and extensor muscles by electrodes placed on the skin surface. EMG activity was monitored while the monkey performed rapid alternating movements to insure that the electrodes were not picking up activity of the antagonist muscles.

Following torque pulse application, the monkey's wrist position exhibited a pattern of damped oscillation which was accompanied by periodic bursting in the EMG that continued for 100 to 300 msec after the end of the torque pulse. Three EMG patterns were seen in three monkeys: 1) agonist bursts with no phasic activity in the antagonists, 2) agonist bursts alternating with antagonist bursts, and 3) agonist and antagonist bursts occurring simultaneously. All three patterns were seen to some degree in each monkey, but one had a clear predominance of pattern 1, and another of pattern 3. The monkey with periodic bursting after torque pulses in the agonist without periodic bursting in the antagonist had the best damping of the oscillations after the torque pulse and the least amount of physiological tremor in holds and ramps. The monkey with periodic bursting after torque pulses simultaneously in both agonist and antagonist had the worst damping of the oscillations after the torque pulse and the largest amplitude 8-10 Hz physiological tremor in holds and ramps. The relationship between the EMG pattern and the degree of damping was seen within each monkey as well as between monkeys, e.g. when the monkey with the worst damping of oscillations used pattern 1, the degree of damping increased. What CNS mechanisms control timing and amplitude of agonist - antagonist muscle interactions in damping and tremor? (Supported by Grants #5 T32 GM07200, #NS12777-09, and the McDonnell Center)

101.5 CEREBELLAR PURKINJE CELLS HAVE BIDIRECTIONAL DISCHARGE DURING RAMP TRACKING AND PHASIC MODULATION DURING OSCILLATION OF THE MONKEY'S WRIST. W.T.Thach, J.W.Mink, S.A.Kane*, and M.K.Horne*, Departments of Anatomy and Neurobiology, Neurology and Neurosurgery, and The McDonnell Center for Higher Brain Function, Washington University Medical School, St.Louis, Mo. 63110.

The discharge of cerebellar Purkinje, cortical interneuronal, and deep nuclear cells was recorded during hold-ramp-hold tracking of a monkey"s wrist performed in flexor and extensor directions with and against maintained torque load. Wrist flexor and extensor EMG, position and force were monitored during all recordings. Loading and unloading torque pulses were applied on occasional trials during hold or ramp. The monkey had a marked 8-10/sec "physiological" tremor, and after torque pulses poorly damped oscillations of position and force accompanied by periodic EMG bursts simultaneous in agonist and antagonist (despite loading) muscles (Kane, et al., and Mink, et al., Soc. for Neurosci. Abstr., 1984).

During holds, discharge was poorly if at all related to position or load direction. During ramp movement, discharge at or before movement onset increased (most cells) or decreased (some cells) for the duration of movement, independent of direction or load. After torque pulses during holds and ramps, discharge increased or decreased usually with periodic fluctuation related to wrist oscillation. Cells changing discharge in relation to ramp movement and torque pulse often underwent periodic changes in relation to tremor as well. Complex spikes of Purkinje cells bore no strict time relation to oscillation.

Phase relations of periodic cell discharge, EMG, position and force will be determined to see if the cerebellar activity is so timed as to support a possible damping mechanism (through modulation of stretch reflexes--Schieber and Thach, and Thach and Schieber, Soc. for Neurosci. Abstrs. 1980; and Elble et al., Soc. for Neurosci. Abstr. 1981) for limb oscillation (Supported by grant #5 T32 GM07200, #2 RO1 NS12777-09, and The McDonnell Center).

101.6 MODULATION OF EMG ACTIVITY BY HIGH FREQUENCY TORQUE FORCING. J. S. Thomas. Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

Torque motors were used to produce high frequency (5-40 Hz) periodic torque changes during a postural fixation task in which human subjects were required to maintain the position of a freely moving handle against a net torque offset.

Periodic torque variations equal to 50-80% of the net torque offset were typically capable of inducing almost total entrainment (100% modulation) of the surface EMG signal recorded from the prime mover during tasks involving fixation about finger, wrist, or elbow joints. Variations in forcing frequency and periodic torque waveform (sinusoid, asymetrical sinusoid, square, and triangle) suggest that both joint velocity and acceleration are the forcing parameters. Transport delays (reflex latencies) were examined by means of step changes in perturbation amplitude or frequency and by the response to individual "missing cycles". The results of these experiments suggest that both M_1 and M_2 latency components contribute to the EMG modulation.

High frequency periodic torque modulations delivered to homologous muscles during bilateral fixation tasks produced modulations of their respective EMGs which were identical to those seen during unimanual forcing regardless of the phase or frequency match of the separate bilateral forcing torques.

101.7 **STIFFNESS OF THE RELAXED HUMAN ELBOW.** W.A. MacKay and D.J. Crammond*. Dept. of Physiology, University of Toronto, Toronto M5S 1A8, Canada.

The hypothesis that shifts in limb position are produced by the regulation of muscle stiffness, has several versions some of which make one or both of the following assumptions. First, elastic restoring torques increase linearly with angular displacement, and second, the ratio of spring constants in antagonistic muscle pairs changes systematically with different maintained positions. These assumptions were examined in normal humans, whose right forearm was snugly strapped into a lightweight manipulandum coupled to a torque motor. Angular displacement about the elbow was monitored by a potentiometer, and torques at the wrist were monitored by strain gauges. Forearm motion was limited to the horizontal plane. Elbow stiffness of the relaxed arm, with a resting elbow angle of about 90°, was calculated in two ways: imposed sinusoidal oscillation to find the natural frequency of the forearm, and imposed trapezoidal torque pulses which allowed direct computation of the (torque/angular displacement) relation. Both methods were used over a range of displacement magnitudes and gave very similar results. Elbow stiffness was found to decline drastically with displacement. Over the range tested, 0.006 to 0.2 radians, the relationship of stiffness to displacement was a power function with an exponent of about -2/3. Therefore, elastic restoring torques increased approximately as the cubic root of angular displacement. This means that the elastic restoring torques of the flexor and extensor muscle groups were far from linear functions of displacement, and indeed must have been sigmoidal with the greatest slope at the resting angle of the elbow. Furthermore, as subjects performed step-like flexion-extension movements of about 10° on either side of mid-point, no significant difference was measureable in elbow stiffness in the two end positions. Torque pulses were applied about 300 ms before the onset and 200 ms after the cessation of movement. The results in both positions were essentially the same as those for the resting forearm at a 90° angle. Therefore, under conditions of minimal exertion, equilibrium points for different elbow angles in the horizontal plane cannot be distinguished by ratios of flexor muscle stiffness to extensor muscle stiffness. 'Length-tension' curves are dependent on the current resting position of the limb. Supported by MRC of Canada.

101.8 SIMULTANEOUS IDENTIFICATION OF JOINT STIFFNESS, STRETCH REFLEX DYNAMICS, AND EMG/TORQUE DYNAMICS IN THE HUMAN ANKLE. R.E. Kearney, I.W. Hunter and P.L. Weiss. Biomedical Engineering Unit, Faculty of Medicine, McGill University, Montreal, CANADA, H3G 1Y6.

System identification techniques have been used to determine the dynamic relation between human ankle position and torque - the dynamic stiffness (e.g. Hunter & Kearney, J. Biomech, 1982). Furthermore, stretch reflexes in the ankle dorsiflexors and plantarflexors have been characterized in terms of the dynamic relation between ankle angular position and surface EMG activity (e.g. Kearney & Hunter, Exp. Brain Res., 1983). However, the torques resulting from reflex activity could not be determined because the EMG/torque dynamics were not known and could not be estimated since both EMG and torque were strongly correlated with position. This report describes an multiple-input experiment in which dynamic ankle stiffness, stretch reflex dynamics, and EMG/torque dynamics were identified simultaneously.

Two concurrent inputs were used. The first was a stochastic perturbation of ankle angular position generated by an electro-hyraulic actuator. The second input was a random perturbation of central drive to the ankle muscles. This was generated by having the subject voluntarily track a target which varied randomly about a mean level with a low-pass filtered version of ankle torque. The two driving signals were chosen to be statistically independent so that multiple-input multiple-output system analysis techniques permitted the effects of each input to be determined.

Analysis of response components correlated with the position input yielded estimates of joint stiffness and stretch reflex dynamics consistent with our previous results. Analysis of response components correlated with the voluntary input provided a simultaneous estimate of the EMG/torque dynamics. EMG/torque dynamics were found to be well modeled by a second-order low pass system having a natural frequency near 2 Hz and a damping parameter less than one. The natural frequency tended to decrease and the damping parameter tended to increase as the level of activation increased. This behaviour is consistent with previous isometric studies on both isolated and intact muscle. These results provide the basis for the evaluation of the mechanical consequences of stretch reflex activity at the human ankle.

Supported by the Canadian Medical Research Council.

101.9 STIFFNESS-TORQUE-EMG RELATIONS AT VARIOUS TIBIALIS ANTERIOR AND TRICEPS SURAE CO-CONTRACTION LEVELS OBTAINED BY REAL-TIME FEEDBACK OF ELASTIC-STIFFNESS. I.W.Hunter, R.E.Kearney and P.L.Weiss. Biomedical Engineering Unit, Faculty of Medicine, McGill University, Montreal, Canada H3G 1Y6.

In previous studies (Hunter and Kearney, J. Biomech., 1982) we have shown that provided co-contraction is avoided there is a linear relation between human ankle elastic stiffness and the torque generated by either tibialis anterior (TA) or triceps surae (TS). We have further demonstrated that the elastic stiffness increases as subjects progressively co-contract TA and TS while maintaining zero net ankle torque (Hunter et al., Neurosci. Abstr., 1983). However we were unable to provide subjects with continuous feedback of elastic stiffness in these previous studies because of host computer (PDP 11/23+) speed limitations.

In the present study, subjects' left feet were attached, via a cast, to a computer controlled, electro-hydraulic actuator operating in an angular-position servo-control mode. Small-amplitude, stochastic angular-displacements, about a fixed mean ankle angular-position, were delivered to subjects with power sufficient for system identification purposes to 50Hz. Angular displacement, torque and TA and TS electromyograms (EMGs) were sampled and stored for off-line analysis.

Special purpose hardware was developed to produce a real-time estimate of elastic-stiffness from the measured stochastic ankle angular-displacements and the resulting torques. Subjects observed a display whose ordinate was elastic-stiffness and whose abscissa was mean torque. A desired elastic-stiffness/torque combination was presented as one point on the display and the estimated elastic-stiffness and mean torque produced by the subject (by the appropriate TA and TS co-contraction) was displayed as a second point. Subjects were required to match a range of elastic-stiffness/torque combinations which were designed to cover the full range of voluntarily produced co-contraction levels.

A second-order dynamic model provided an adequate fit between the angular-displacement and torque data at each of the elastic-stiffness/torque points which the subjects were able to achieve successfully. The mean TA and TS EMGs were used in a simple non-linear model to make a prediction of the elastic-stiffness at each co-contraction level.

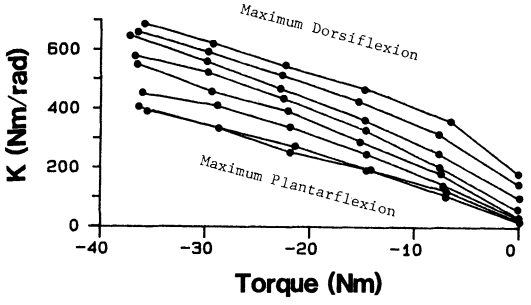
Supported by the Canadian Medical Research Council.

101.10 RELATION BETWEEN MEAN ANKLE JOINT POSITION, PLANTARFLEXOR TORQUE AND ELASTIC STIFFNESS. P.L. Weiss, R.E. Kearney and I.W. Hunter. Biomedical Engineering Unit, Faculty of Medicine, McGill University, Montreal, Canada, H3G 1Y6.

Ankle joint elastic stiffness (K) increases linearly with mean ankle torque. This relation has been previously established for the case of mean torques maintained by predominantly active muscle processes i.e. voluntary muscle contractions (Hunter & Kearney, J. Biomechanics, 1982). More recently we have shown that this relation persisted for passive torques generated by changes in mean ankle joint position (Weiss et al., CMBEC, 1984).

The purpose of the present study was to examine this relation when ankle torque was generated by both active muscle and passive joint processes. The ankle was stochastically perturbed about mean joint positions distributed over the entire range of motion while voluntary plantarflexor contractions were maintained. The linear dynamic relation between ankle position and torque was identified and estimates of K were obtained for each mean position/mean voluntary torque combination.

The figure shows the relation between K and torque generated by active plantarflexor contractions at eight mean ankle positions varying from maximum plantarflexion (bottom line) to maximum dorsiflexion (top line). It is evident that the slopes of these curves did not vary with mean position. Thus, while comparable changes in K resulted when ankle torque was increased by either voluntary muscle contractions or by joint rotation, the two processes, active muscle and passive joint, appear to be independent.



101.11 NON-MONOTONIC TORQUE-ANGLE RELATION OF HUMAN ELBOW FLEXORS: IMPLICATIONS FOR THE EQUILIBRIUM-POINT HYPOTHESIS OF MOVEMENT. Z. Hasan and R.M. Enoka. Departments of Physiology and Physical Education, University of Arizona, Tucson, AZ 85724.

The torque exerted about a joint depends upon the muscular forces as well as on the lengths of the moment arms for each of the muscles. Since moment arms generally vary non-monotonically with joint angle, the torque exerted about the joint should similarly be non-monotonic over the range of motion. There are reports in the literature of monotonic torque-angle relations in experiments that employed perturbations; such observations may be due to reflexive changes of muscle activity with joint angle (Vincken et al., Neuroscience 9: 529-534, 1983). We measured the isometric elbow-flexion torque at different elbow angles in 8 normal adult subjects. Muscle iso-activation was approximated by having each subject match the level of electromyographic activity (EMG) of an elbow flexor, the brachioradialis (BR) muscle, to a fixed target set at about 10% of the maximum-effort EMG. The peak isometric flexion torque occurred at an elbow angle that ranged between 65 and 115 degrees for different subjects. This peak indicates that for a specified level of flexor activity and a fixed external torque, **two** positions of equilibrium are possible.

The BR EMG attained by the subjects, though somewhat variable from trial to trial (< 4% of maximum), did not show a trend with elbow angle, as expected. The biceps brachii (BB) EMG, recorded concurrently, also did not systematically vary with angle in 2 subjects. In other subjects, however, the BB EMG appeared related to elbow angle, violating the functional synergism implied by the 'equivalent elbow flexor' concept (Bouisset et al., Electroenceph. Clin. Neurophysiol. 42: 543-551, 1977).

We also recorded, in the same subjects, the EMG alterations associated with ramp-and-hold elbow movements which were produced on cue without target tracking. A weight hung from a pulley applied an extension torque. Although flexion movements always involved a transient increase in the flexor EMGs, the final, steady-state EMGs could be above or below the pre-movement levels. The direction of the steady-state change depended upon the initial and final positions and was in accord with the non-monotonic, isometric data. The fact that the directions of the transient and steady-state changes in flexor EMG are not always the same does not support the equilibrium-point hypothesis of movement (Polit & Bizzi, J. Neurophysiol. 42: 183-194, 1979).

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101.12 SYNERGISTIC MUSCLE ACTIVITY IN ISOMETRIC CONTRACTIONS ABOUT THE HUMAN ELBOW JOINT. T.S. Buchanan*, W.Z. Rymer, J.L. Lewis*, D.P.J. Almdale*, and Y. Wu* (SPON: W.A. Lee). Dept. of Physiology and Rehabilitation Engineering Prog., Northwestern University, and the Sensory Motor Performance Prog., Rehab. Inst. of Chicago, Chicago, IL 60611.

A biomechanical model of the human elbow joint has been created for the study of synergistic muscle activities for up to ten elbow joint muscles. The purpose of this model is twofold: (1) to use myoelectrical information to study the central command involved in muscle coordination and joint stabilization, and (2) to examine methods for muscle force prediction.

The myoelectrical activities in up to ten muscles that act about the human elbow joint have been simultaneously recorded with bipolar, bared, fine-wire (75 μm & 125 μm) intramuscular electrodes. These recordings were made while the subject was in a fixed position, voluntarily reacting to static loads at the joint. During the experiment the subject's arm was flexed and abducted 90° at the shoulder and was at 90° flexion at the elbow. The amplified, bandpass-filtered, rectified, digitized EMGs were recorded together with the force magnitude on a PDP 11/23 micro-computer. Force was generated against a wristlet, which was connected to a load cell on a wire and pulley system. This system allowed the force to be directed in the plane orthogonal to the forearm.

To date, three different subjects have been studied, one of which was repeated, for a total of four sets of data. Analysis of external torque and EMG demonstrates invariant patterns of activity as external load direction changes (eg. flexion, introrotation, extension, exorotation). When the load magnitude is changed, these activity patterns maintain the same shape by scaling the entire activity curves up or down. The fact that these activity curves show invariant characteristics implies that the muscle synergistic relationships are independent of load magnitudes.

Analysis has also been done to determine the constants relating muscle force to EMG (assuming a linear relationship). Although three different muscle force/EMG models have been used, the values for these constants have been found to vary widely due either to simplifying assumptions in the models or to inaccuracies in the EMG estimates.

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101.13 STRATEGIES FOR FATIGUE COMPENSATION IN HUMAN ELBOW FLEXOR MUSCLES. R.F.Kirsch* and W.Z. Rymer, Biomedical Engineering Program, Northwestern University and the Sensory Motor Performance Program, Rehabilitation Institute of Chicago, Chicago, Ill., 60611.

Although the Golgi tendon organ has been shown to be a sensitive transducer of muscle force, investigation has shown its influence in reflex control of muscle contraction in decerebrate cats to be modest(Houk,et.al.J.Neurophysiol. ,33:784,1970 and Rymer and Hasan, Brain Res. 184:203,1980). Because of the limitations of decerebrate animal preparations, the present study was aimed at examining the role of force-feedback pathways in normal human subjects. Muscle fatigue, which reduces muscle contractility, is a state in which force feedback should be evident, as the nervous system attempts to compensate for muscle weakness.

We studied the reflex EMG responses of elbow flexor muscles to constant amplitude angular perturbations (1 radian at 3 rad/sec) initiated over a range of different torque levels (4-32% max.) before and after a series of fatiguing isometric contractions (20 repetitions of 25 sec. contractions at 60% max with 5 sec. rest between). A torque motor configured as a position servo applied the stretches while a computer (PDP 11/23) simultaneously recorded signals corresponding to elbow torque and angular position, as well as the EMG for several elbow flexor and extensor muscles. Analysis of the EMG power spectrum revealed that its properties returned to normal within 10 min. following completion of the exercise, allowing direct comparison of EMG responses thereafter to the pre-fatigue responses.

To date, seven subjects have been studied (5 male, 2 female), six of whom showed a significant decline in muscle contractility. Of these, 2 had augmented incremental EMG responses (to identical stretches) with comparable torque responses before and after fatigue, while the remaining 4 showed a decreased EMG increment but also a somewhat smaller post-fatigue torque response. It was also noted that the muscles could be readily potentiated after the 15 minute recovery period by forceful contraction. It appears that fatigue compensation may depend partly on muscle potentiation and partly on force-feedback when potentiation has not occurred. The finding that torque responses frequently do not completely recover indicates that force-feedback may have a limited efficacy or range of use.

Supported by the Hearst Foundation and the Sensory Motor Performance Program.

101.14 ESTIMATION OF EQUILIBRIUM POSITION, STIFFNESS, AND VISCOSITY DURING HUMAN SINGLE JOINT ARM MOVEMENT. C.G. Atkeson and E. Saund*. Department of Psychology, MIT, Cambridge, MA 02139.

Several proposals for biological movement control have emphasized the damped, springlike behavior of muscle. We have been investigating the role of equilibrium position, stiffness, and viscosity, through experimental estimation of these parameters during human single-joint forearm movement. One possible control strategy is that the nervous system generates motor output commands not in terms of desired muscle forces or joint torques, but with regard to stiffness, viscosity, and equilibrium position of springlike muscle actuators acting about the joints.

We have developed theoretical arguments for the ways in which equilibrium position, stiffness, and viscosity can be modulated so as to maintain invariant tangential velocity profiles under a variety of movement conditions, as has been previously experimentally observed. These scaling laws for equilibrium position, stiffness, and viscosity are similar to scaling laws describing how joint torques may be modulated to produce movements of various speeds.

In order to test these scaling laws as well as other ideas as to how the nervous system might exploit explicit control of equilibrium position, stiffness, and viscosity, we have developed a procedure for estimating the time history of joint stiffness, viscosity, and equilibrium position during human forearm movement. The approach is based on measuring the response of the arm to torque perturbations over a number of trials. We treat the mechanical properties of the muscles acting at the elbow joint, as well as the associated reflexes, as forming a lumped damped-spring mechanical system.

These estimated time histories of stiffness, viscosity, and equilibrium position have been used in a computer model of the forearm to simulate the actual movement. Simulated torque perturbations applied to the model yield predicted responses that correspond well with experimentally observed human arm responses to disturbance perturbations.

(Research supported by NIH grants AM27610 and NS09343 and the Office of Naval Research Contract Number N00014-80-C-0505.)

101.15 MUSCLE-REFLEX COMPLIANCE: A COMPARISON OF ELBOW AND ANKLE. Gerald L. Gottlieb and Gyan C. Agarwal, Dept. of Physiology, Chicago, IL 60612.

We have previously described compliant characteristics of the elbow to step torque perturbations (Gottlieb and Agarwal, Soc. Neurosci. Abs. 187.7, 1983). Similar experiments have been performed on the ankle in both normal and spastic subjects.

These two joints differ in a number of important respects. The range of ankle motion is only about 1/3 that of the elbow. The ankle has very active phasic stretch reflexes to dorsiflexion while its plantar stretch reflexes, like those at the elbow, are weak. Finally, the passive compliance of the elbow is very low and essentially negligible when the muscles are active while at the ankle, passive forces are always significant.

In spite of these differences, the compliant properties of both joints that are attributable to the muscle contraction are very similar. Both show a nonlinear behavior including a short-range stiffness property and symmetry in loading and unloading.

There are three mechanisms which can underly this behavior. These are intrinsic muscle properties, a tonic stretch reflex and involuntary but modifiable reactions. These mechanisms are discussed in terms of the available data. (Supported by NIH Grants NS-12877, AM-33189 and NSF Grant ECS-8212067).

101.16 ELECTROMYOGRAPHIC PATTERNS ASSOCIATED WITH DISCRETE ANKLE MOVEMENTS. D.M. Corcos, G.L. Gottlieb, G.C. Agarwal and R.D. Penn. Department of Physiology, Rush Medical College, Chicago, Illinois 60612

The relationship between the movement time (MT) for accurate and rapid discrete movements of distance A to a target of width W was quantified by Fitts (J. Exp. Psy., 47: 381-391, 1954.) and is given by the equation:

$$MT = a + b \log_2 (2A/W)$$

This relationship, known as Fitts' Law, has received considerable support for many types of movements. It also raises the interesting question: if MT is affected by distance moved and accuracy, then how do the patterns of muscle activation alter?

Recent studies on elbow joint movements indicate that for movements of different amplitudes, either the intensity of the EMG (Freund H.J. and Budingen H.J. Exp. Br. Res., 31: 1-12, 1978.) or the time course (Wadman W.J. et. al. J. Hum. Move. Stud., 5:3-17, 1979.) increases with increasing distance.

We studied how accuracy of movement affects the patterns of muscle activation. The study was performed on the ankle joint because of the asymmetrical nature of extensors and flexors. Seven subjects made accurate and rapid ankle movements of 12, 18 and 24 degrees to targets of 2, 4 and 8 degrees. The data suggest that the agonist muscle was activated for a longer time and with greater intensity for larger movements. The duration of the EMG burst increased for increases in target size but the amplitude was not affected. It appears that the pattern of activation is modified in both intensity and duration according to task demands.

Data will be presented to show the effect of adopting different movement strategies on the pattern of muscle activation and the consequent velocity profile. The interrelationship of various kinematic and EMG variables will be considered.

(Supported by NIH Grants NS - 15630 and AM - 33189)

101.17 EFFECTS OF MUSCLE TENDON VIBRATION DURING ACCELERATORY AND DECELERATORY PHASES OF HUMAN LIMB MOVEMENTS. W.G. Darling* and J.D. Cooke (SPON: M. Goodale). Dept. of Physiology, Univ. of Western Ontario, London, Canada.

Previous studies in this laboratory have shown that tendon vibration of the antagonist (lengthening) muscle during proprioceptively guided movements causes undershoot of the intended target. Agonist muscle tendon vibration, however, has no effect on attainment of the intended end position under these conditions. Since vibration is a potent stimulus to muscle spindles it was concluded that the length of the antagonist muscle is monitored by the CNS during limb movements and is used for the attainment of final limb position. This hypothesis was further examined in this investigation by applying vibration to agonist and antagonist muscle tendons during the acceleratory and decelerator phases of initial and well-practiced movements.

Six normal healthy individuals performed elbow flexion and extension movements in a visual step tracking task. Subjects moved accurately, without terminal oscillation and increased movement velocity with practice. Vibration was applied randomly during the acceleratory or decelerator phase of about 1/2 the movements.

Vibration of the antagonist muscle tendon during acceleration caused undershoot of the visual target or an inappropriately strong deceleration of the limb so that there was much slower than usual movement into the target. This occurred in both the initial and well practiced movements. Vibration of the antagonist during the decelerator phase had no effect on the movement trajectory or on end position. Vibration of the agonist muscle during acceleration usually caused increased peak velocity of movement and, in some subjects, undershoot of the intended end point of the movement. Vibration applied during deceleration sometimes produced overshoot of the end point during initial movements but had no effect on practiced movements.

These results suggest that the CNS samples the activity of muscle spindles in the lengthening muscle during the acceleratory phase and uses this information to control movement during the decelerator phase. During the decelerator phase information from the antagonist muscle spindles is largely ignored for both initial and well practiced movements.

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101.18 EFFECTS OF PRELOADING ON POSITION-DEPENDENT EMG ACTIVITY IN HUMANS. S.H. Brown and J.D. Cooke. Dept. of Physiology, University of Western Ontario, London, Canada.

It has recently been shown in this laboratory that the EMG activity initiating arm movements depends on the starting position of the limb. Initial agonist burst magnitude increases as starting position becomes more extended. Experiments were performed to determine whether changes in burst magnitude compensate for changes in mechanical advantage with joint angle or whether they are simply due to changes in tonic EMG activity associated with the different initial positions.

Subjects performed 10 deg step-tracking flexion movements about the right elbow. Initial positions ranged from 75 to 125 deg absolute joint angle. Subjects moved promptly and accurately with emphasis placed on maintaining the same speed in all trials. Surface EMGs were recorded from the biceps and the lateral head of triceps. In some trials steady pre-loads were applied. These steady forces shifted the passive equilibrium position of the limb and thus altered the tonic EMG activities.

The initial agonist burst was greater for movements in the extended than in the flexed starting position. The second agonist burst of the triphasic pattern was present in movements made from extended but not in those made from flexed starting positions. Application of a steady force which shifted the passive equilibrium position of the arm into extension (105-115 deg) from its normal position (70-80 deg) did not alter the magnitude or timing of the EMG bursts despite a decrease in tonic antagonist (triceps) activity. Similarly, no significant change in phasic EMG activity was seen when the steady force required an increase in tonic biceps activity at a given joint angle. At the same time, movement trajectories were virtually identical under all conditions.

Position-dependent changes in phasic EMG activity thus are not a result of changes in tonic EMG activities. Both the initial and the later phasic bursts are modified in order to maintain constant movement trajectory in the face of altered mechanical advantages and muscle properties associated with different joint angles.

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101.19 ELASTIC PROPERTIES OF MUSCLES MEASURED AT THE ELBOW IN PARKINSONIAN MAN. R.L. Watts, A.W. Wiegner, and R.R. Young, Clinical Neurophysiology Lab., Massachusetts General Hospital, Boston, MA, 02114.

We recently studied normal passive elastic properties of muscles measured at the elbow in man (Soc Neurosci Abstr 9: 632, 1983) and found spring-like properties in the muscles which (a) return the arm from a displaced position to a neutral elbow angle of 112 ± 10^{o} (SD) and (b) define elbow compliance as a linear relation between displacement and applied torque over a range of $\pm30^{o}$ from neutral position. We have now studied 7 patients with Parkinson's Disease (PD) using the same technique in order to quantitatively determine the compliance and neutral angle of the relaxed right upper extremity (RUE) affected by "cogwheel" rigidity.

Each patient was clinically examined by R.L.W. or R.R.Y. and "tone" in the RUE assessed on a clinical scale of 1+ (mild) to 4+ (severe). Use of L-Dopa (Sinemet) and timing of last dose were noted. Subjects were studied in a chair with RUE supported on a table at shoulder height and the forearm, wrist and hand strapped to the lever arm of a printed motor (AXEM MC19S) mounted below the table. Slow bidirectional ramps in torque were applied to the forearm of each subject, who was instructed to remain relaxed. Triceps EMG was monitored to detect reflex or voluntary activity. Data obtained during rising and falling torque ramps were combined to take into account the effects of limb hysteresis, and 10 trials were averaged for each subject. Most subjects had only mild "cogwheel" rigidity and no RUE tremor. Those who had unrepressible EMG activity were excluded from the study.

We observed: (1) The neutral angle to which the RUE returned after displacement was significantly smaller (98 ± 9^{o}, $P<0.02$, Mann-Whitney test). (2) Curves obtained by plotting displacement vs. applied torque were again linear over a range of $\pm30^{o}$ from neutral position; elbow compliance ranged from 20 to 116^{o}/N-m (mean 52) in PD compared to 35 to 171^{o}/N-m (mean 76) in controls with a negative correlation ($r=-0.75$) between upper arm mass and compliance at the elbow that was less remarkable than in normal subjects.

These results suggest that changes in the passive mechanical properties of the muscles acting about the elbow may have taken place, defining a new set of length-tension curves and therefore a different neutral angle and compliance. The smaller neutral angle could be the result of increased stiffness of the elbow flexors or decreased stiffness of the extensors; we favor the former.

102.1 A SYSTEM FOR RECORDING LIMB MOTION IN A THREE DIMENSIONAL SPACE. J.G. Chubbuck*. and A.P. Georgopoulos. (Spon:J. Massey) Applied Physics Laboratory, and Department of Neuroscience, School of Medicine, The Johns Hopkins University, Baltimore, Maryland.

A tracking system has been developed for recording the motion of a monkey or human subject's arm in a three dimensional (3-D) space during the performance of a reaching task. Arm movements are defined in terms of the motion of two miniature spark gaps - one attached to the subject's elbow and one to the back of the hand. The spark gaps function as sonic transducers which may be fired on command from a computer. The subject is placed within a rectangular 3-D space whose corners are defined by the locations of 8 miniature sonic receivers. Each receiver occupies a 38 mm diameter cylinder and is permanently located so that at least three of them will be activated for any desired arm position and orientation. Sonic propagation time from the location of a spark gap to each of the activated receivers is measured by counting cycles of a crystal controlled oscillator (clock). These propagation times are direct measures of distance from the spark gap at the instant of firing to the receivers. A microcomputer contained within the tracking system control circuitry 1) selects the optimum set of three sonic measurements from which to compute spark gap position, 2) computes the position of the spark gap in cartesian coordinates and 3) outputs this data to the main computer for data recording. Since these measurements are made on-line during neurophysiological experiments (in animals simultaneously with single unit recordings) the spark gaps are shielded by a fine copper mesh to eliminate interference with the recorded neuronal signal. This serves also to prevent accidental touching of the spark gap discharge by the subject. Thus configured, the spark gap assembly measures 12 mm in diameter and 10 mm in height. The spark gaps are specially designed to prevent errors due to erosion of the electrodes. The spatial resolution of the system is better than 1 mm^3. The sampling frequency limit is 200/s to allow for sonic propagation time and the attenuation of reflections from nearby surfaces. (Supported by USPHS Grant NS07226.)

102.2 THE MAKING OF MOVEMENTS IN DIFFERENT DIRECTIONS: A BEHAVIORAL STUDY. A.P. Georgopoulos and J.T. Massey. Department of Neuroscience, The Johns Hopkins Univ., Sch. of Medicine, Baltimore, MD 21205.

How do human subjects produce movements in directions other than going straight to a target? (e.g., "Move at 45 degrees clockwise relative to going straight to the target") We studied this problem in a two-dimensional space by measuring the reaction times (RT) to initiate movements at 0, 45, 90 and 135° of angular deviation within counterclockwise (CCW), clockwise (CW) or either (EI) direction. Ten subjects were asked to move an articulated manipulandum over a planar working surface in a direction dictated by an angular deviation and a directional constraint. All trials started by turning on a He-Ne laser beam back projected onto the center of a frosted plexiglass working surface. The subject had to capture that light with the distal end of the manipulandum and hold there for a variable period of time, after which the beam jumped to a point on a circle of 2 cm radius. The location of this point varied between trials. Each subject performed 20 trials for each angular deviation within each directional constraint. All subjects made accurate movements, within ± 8° of any specified angular deviation. Only correct directional responses were analyzed in the CCW and CW condition.

A salient and consistent, among subjects, finding was that the RTs increased as a function of the angular deviation within any directional constraint. The median RTs for the 0, 45, 90 and 135° angular deviations were, respectively, 455, 820, 948, 1149 ms for the CCW condition; 478, 957, 1002, 1232 ms for the CW condition; and 476, 749, 727, 923 ms for the EI condition. (The RTs were overall lower in the EI condition, that is in the absence of directional constraint.) We hypothesized previously (Georgopoulos et al., J. Neurosci. 2:1527, 1982; Exp. Brain Res. Suppl. 7:327, 1983) that the production of a movement in a desired direction involves the generation of a neural representation of the upcoming movement vector. We interpret the present results to mean that the generation of a movement at an angular deviation from a reference orientation may involve an internal rotation of this vector until the desired angular deviation is reached. It would follow, then, that the larger the angular deviation required the longer the time needed to complete the rotation; that is, the longer the RT will be. (Supported by USPHS grants NS17413 and NS07226.)

102.3 INVARIANT FEATURES OF HAND POSTURAL STIFFNESS. F.A. Mussa-Ivaldi*, N. Hogan* and E. Bizzi. Dept. of Psychol., MIT, Cambridge, MA 02139.

When the hand is displaced from an equilibrium posture by an external disturbance, a force is generated to restore the original position. We developed a new experimental method to measure and represent the field of elastic forces associated with the posture of the hand in the horizontal plane. Subjects were asked to hold the handle of a two-joint planar manipulandum in different positions in the work space. A case was applied to the wrist joint leaving the subject's arm only two degrees of freedom. While the subjects were maintaining a given posture, two servo-controlled torque motors produced small (5-10 mm) displacements of the hand along different directions. The corresponding restoring forces were measured before the onset of voluntary reaction. The stiffness in the vicinity of the equilibrium position was estimated by analysing the force and displacement vectors. The stiffness matrix has two components; one may be represented by an ellipse which characterises the magnitude (the area), the shape (the ratio of the axes) and the orientation (the direction of the major axis) of the elastic force field. The other component is the curl of the force field. This quantity is directly related to that reflex activity which establishes a cross coupling between the stretch produced at one joint and the torque developed at another. Our experimental findings indicated that the curl was small, a fact suggesting that reflex pathways are not altering the spring-like nature of the muscles acting upon the arm. Furthermore, we found that the shape and orientation of the stiffness were invariant over subjects and over time. We also investigated the ability of our subjects to produce voluntary and adaptive changes in the stiffness. Our data indicated that, when a disturbance acting along a fixed direction was imposed, the magnitude of the stiffness was increased, but only minor changes in shape and orientation were observed. (Research supported by NIH grants AM27610 and NS09343. F.A. Mussa-Ivaldi supported by a CNR Fellowship.)

102.4 EFFECT OF TEMPORARY PATH CONSTRAINT DURING PLANAR ARM MOVEMENT. B. McKeon*, N. Hogan* and E. Bizzi (SPON: R. Held). Dept. of Psychol., MIT, Cambridge, MA 02139.

It has been proposed (Polit and Bizzi, J. Neurophysiol., 42:183, 1979) that single movements might be based on "step-like" changes in desired equilibrium position between the length-tension curves of agonist/antagonist muscles. Observed smooth movements were proposed to result from inertial and visco-elastic properties. Subsequent studies (Bizzi et al., Exp. Brain Res., 197:1, 1982) have shown that this was not the case - the observed movements were found to be based on a gradual change in the equilibrium position during movement. These latter studies were based on movements of the elbow joint. This study extends those findings to the two-joint case (elbow and shoulder).

Subjects gripped the handle of a two-link manipulandum while keeping their fore- and upper-arm horizontal. The subject was instructed to move the handle between two visually presented target locations. Activation of a clutch in the manipulandum caused subsequent hand movement to be along an arc with radius equal to the length of one link of the manipulandum. The target locations and the timing of clutch engagement were arranged such that the constrained path took the handle away from the intended path. The clutch could then be released at different times thereby removing the path constraint.

Following clutch release, 17 or 22 initial trials (11 subjects) showed a tendency for the path to return to the intended path rather than proceed directly to the final position. In 11 further experiments (5 subjects), each consisting of 30 to 50 trials, handle force was measured. Movement duration was within the range 600 to 1200 msec. While the clutch was engaged, handle force was found to be always strongly oriented so as to restore the hand to the unconstrained path and not to the end-point of the path.

These findings support the proposal that the nervous system specifies movement trajectories as a series of equilibrium positions defined by activity of limb musculature. The actual path is strongly influenced by inertial and visco-elastic properties of the limb and any load. (Research supported by NIH grants AM27610 and NS09343. B. McKeon supported by the National Health and Medical Research Council of Australia.)

102.5 CHARACTERIZATION OF JOINT-INTERPOLATED ARM MOVEMENTS. J.M. Hollerbach and C.G. Atkeson. Dept. of Psychology, M.I.T., Cambridge, MA 02139.

In terms of possible planning variables for human arm movement, two of the most apparent are joint angles and hand position. To assist in distinguishing between these viewpoints, we have theoretically characterized the set of trajectories derivable from a joint based planning strategy and have compared them to experimental measurements. Despite these differing viewpoints, it is puzzling that similar experimental results have been invoked for support of each. Several laboratories have shown that hand trajectories in point-to-point reaching movements are essentially straight lines, evidently supporting hand variable planning. On the other hand, supporters of the joint-planning hypothesis argue that for certain movements the joint rate ratio tends towards a constant value, compatible with a strategy of joint-space planning.

Our theoretical characterization of trajectory generation by joint-angle planning reconciles these viewpoints.

1. A strategy of constant joint rate ratio of shoulder and elbow joints is formally equivalent to joint interpolation, i.e., straight-line paths in joint space.

2. For a given joint rate ratio, all hand trajectory points lie on a polar coordinate curve called an n-leaved rose whose locus is independent of the exact time profile.

3. These n-leaved roses tend to be strongly curved, so that joint based planning generally yields trajectories readily distinguishable from straight-line hand paths.

4. However, in movements towards the edge of the workspace, the ratio of shoulder angular velocity and elbow angular velocity tends towards a constant value no matter how the edge of the workspace is approached. This is a general property of two-link mechanisms, arising solely from kinematics.

We note that the experimental movements provided as evidence for joint based planning were unidirectionally towards the workspace boundary. Though the hand described a straight-line path, point 4 shows a constant joint rate ratio would be approached. Thus it is inappropriate to suggest a planning strategy in terms of constant joint rate ratios, an artifact of arm kinematics and workspace boundary, so that all data are completely consistent with trajectory planning in hand space. This work was supported by NIH Grant AM 26710, awarded by the National Institute of Arthritis, Metabolism and Digestive Diseases and by an NSF Graduate Fellowship (CGA).

102.6 INVARIANT PEAK VELOCITY/DISPLACEMENT RELATIONSHIP DURING RAPID MULTIJOINT MOVEMENTS. T. Kaminski* & A.M. Gentile Teachers College, Columbia Univ., New York, NY 10027

An invariant relationship between elbow and shoulder joint velocities has been observed during the deceleratory, but not the acceleratory phase of multijoint pointing movements (Soechting and Lacquaniti, 1981). For the acceleratory phase of single joint movements, however, a stable relationship between peak velocity and displacement has been reported (Cooke, 1980). In the present experiment involving multijoint movement, peak velocity and displacement were found to be highly correlated and to have the same ratio at both the shoulder and elbow joints.

Kinematics and kinetics of the shoulder and elbow joints were analyzed for eight subjects who performed rapid pointing movements to a variety of target locations. The subject's right arm was strapped to a manipulandum which permitted shoulder abduction/adduction and elbow flexion/extension in the horizontal plane. In one series of movements, the arm started from the same initial position and moved to three different target locations. In the other series, the arm started from three different starting positions and moved to the same final location.

Regression analyses of individual and group normalized data indicated that 89% of the variability in peak velocity was accounted for by displacement. This relationship was 1) the same at both the elbow and shoulder joints, 2) not affected by changes in initial and final positions, and 3) not influenced by amount of movement in the adjoining joint. In contrast, torque varied with both initial and final positions, with the shoulder producing most of the torque required to drive the limb to the target. Indeed, under some conditions, resultant initial elbow torque was in the flexion direction even though an elbow extension movement was required.

The observed linear relationship between peak velocity and displacement is characteristic of a mass spring model of movement control. A movement strategy based on changes in the length-tension relationship between antagonist muscles could take maximum advantage of interactional torques inherent in all multijoint movements, thereby decreasing the amount of muscle torque generated. Muscle torque would be required only to the extent that interactional torques could not establish the desired length-tension relationship.

102.7 SOME INVARIANT CHARACTERISTICS OF REACHING MOVEMENTS. M. L. Levine. Ashton Graybiel Spatial Orientation Lab. Brandeis Univ., Waltham, Ma. 02254.

Two aspects of reaching movements were investigated using cinematographic techniques: limb projection, the extension of the hand from the body to the object, and hand shaping, the movements of the fingers to conform with the object's shape. Subjects were filmed while they reached to pick up objects at different positions. The three-dimensional trajectory of a point on the hand, the resultant acceleration and velocity, the width of grasp (distance between thumb and index finger) and hand opening and closing velocity and acceleration were analyzed. Several aspects of the reaches did not change across conditions despite diversity in spatial form and constituent joint angular changes. Limb projection was affected primarily by object position whereas hand shaping was also affected by object shape. The absolute time of peak limb acceleration was invariant across conditions. The absolute time of peak limb velocity varied with object position and the proportion of elapsed to total movement time varied unsystematically but was less than the 50% found by others with restrained pointing movements (Abend, W. et al., Brain, 105:331, 1982). The magnitudes of limb acceleration and velocity peaks were linearly related to the amplitude of the reach but were unaffected by object shape or initial hand posture. The magnitude of hand opening acceleration was affected by object shape but was unaffected by object position or initial hand posture. The limb paused and the hand started to close at a relatively constant distance from the object and time from the end of the movement.

These constancies suggest that translation of the endpoint of a multisegment limb is controlled rather than constituent joint angular changes. They further suggest that the neuromuscular events underlying the movements are regulated to limit the variability of the kinematics with some situational changes. The initial positional characteristics of the target are used in adjusting the magnitudes and timing of the endpoint velocity and acceleration peaks, whereas the impact of other situational features is internally minimized. Hand shaping kinematics are affected by features present both at the onset of the reach and as it progresses although some minimization of variation of kinematics is also evident. (Supported by a NSF doctoral dissertation grant BNS 7910641)

102.8 QUANTITATIVE ANALYSIS OF INFANT ARM MOVEMENT L.Fetters* and J. Delatizky. Gait Analysis Laboratory, The Children's Hospital, Boston, MA 02115.

The development of infant arm movements has historically been studied using descriptive techniques. These techniques are informative, but provide little insight into the development of the underlying neural control processes. Few quantitative studies, which can yield more understanding of the motor control process, have been done in infants. In this research we performed quantitative analyses of the kinematics of infant arm movements, and found that patterns similar to those reported for adults are evident by 9 months of age.

Infants were studied between the ages of 5 and 9 months, during the time when they reportedly shift from bilateral to unilateral reaching strategies. We hypothesized that this behavioral change would be accompanied by the emergence of specific patterns in the kinematics of arm movements. Ten infants were each filmed at 5, 7 and 9 months of age. Infants reached for a clear plastic box placed at two standard distances, one within easy reach, the other with the arm fully extended. Distances were scaled to subjects' arm lengths. The base of the index finger of each hand represented the position of the arm in space. Three high speed 16 mm cameras were used for filming. Cameras were placed bilaterally at 45°on each side of the infant's midline; one was directly overhead. The film was analyzed frame by frame using a Vanguard Motion Analyzing System. Computer software enabled any two views to be combined to yield three dimensional points in space using standard photogrammetric algorithms. This method produced data which were highly reliable and accurate.

For each reach we calculated kinematic characteristics. These included trajectory shape, tangential velocity and acceleration, curvature, and torsion. The straightness of the trajectory was measured as the ratio between arc length traversed on the actual trajectory and the straight line distance between the start and end points on the trajectory. In addition, reaches were coded as bilateral or unilateral.

The shift from bilateral to unilateral movement previously reported was confirmed. We noted however that most infants were able to use a variety of arm patterns at the older ages. Trajectories became straighter with age, though there was considerable intersubject variability. Peak deceleration occured close to points of maximum curvature, as reported for adults who were asked to produce a curved trajectory (Abend, Bizzi and Morasso, Brain 105:331-348,1983).

In summary, quantitative data from 5 to 9 month old infants suggests that during this period kinematic patterns emerge that resemble those found in adult reaching data. We propose that as early as nine months of age the neural processes responsible for the control of reaching in adults may be established.

102.9 CONTROL OF BIPEDAL CYCLING UNDER NOVEL INTERLIMB PHASE CONSTRAINTS. M.R. Zomlefer, C.C. Boylls, Jr., L.A. Cohen*, J.H. Onyski*, D.F. Schwandt*, F.E. Zajac III. Rehabilitation R&D Center, VA Medical Center, Palo Alto, CA 94304.

In order to examine the "neural constraints" which may influence the control of movement during cyclic tasks, we studied male subjects (n=7; ages 21-30) performing a number of novel pedaling tasks on a modified bicycle ergometer. This device could be adjusted so that the angle of the cranks relative to each other could be set at any fixed value from 0 to 360 degrees while maintaining a one-to-one gear ratio between the two cranks (Schwandt et al, An apparatus for studying the neural control and biomechanics of bilateral coordination in conventional versus novel pedaling, Proc. SAE Int. Congress, 1984). The subjects were instructed to pedal the ergometer at near maximum velocity for at least ten steady-state cycles against a load of about 7 kp. In addition, the phase angle between the left and right cranks was set at angles ranging from 180 degrees (corresponding to conventional or symmetric cycling) to 0 degrees (in-phase) in increments of 45 degrees.

Electromyograms (EMG's) were recorded bilaterally with surface electrodes from the vastus lateralis (VL), biceps femoris (BF), medial gastrocnemius (MG) and tibialis anterior (TA) muscles. Crank and pedal angles were also measured with potentiometers, with the 0 degree crank angle corresponding to the topmost crank position, and increasing angle in the forward-pedaling direction. The EMG's and pedal angles were recorded on an analog FM tape recorder and digitized at 2 KHz. All of the EMG's were then filtered and rectified using computer algorithms; at least 10 cycles from each trial were then averaged and normalized to a single cycle, as defined by one complete revolution of the right crank.

All of our subjects developed similar EMG patterns during the conventional cycling trials, consistent with previous ergometric studies. As the phase difference between the right and left cranks decreased, we could see little difference in the activation times of each VL relative to its associated crank. Temporal changes, corresponding to less than 5% of the normalized cycle period, could occasionally be observed in the EMG burst patterns for the flexors BF and TA. The ankle extensor, MG, changed its burst onset over a period ranging up to 15% of the cycle, with the greatest change occuring during 90 degree phase differences. These rather continuous adjustments in MG's activity may be due to slight changes in the mechanical loading of the system in the asymmetric cases or to a change in control strategy forced on the subject for other mechanical reasons.

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102.10 NEUROMECHANICAL ORGANIZATION: THE EMERGENCE OF STABLE LIMB OSCILLATIONS DURING PAW-SHAKE RESPONSES. M.G. Hoy, R.E. Zernicke, J.L. Smith and A. Garfinkel. Dept. Kinesiology, UCLA, CA 90024.

The paw-shake response (PSR) in cordotomized cats is characterized by the recruitment of hindlimb flexors and extensors in a regular, mixed-synergy pattern (Koshland & Smith, Neurosci. Abst. 21.4, 1983). Since limb motion during PSR is influenced by mechanical interactions between segments and by muscle forces (Hoy, et al., Neurosci. Abst. 21.3, 1983), hindlimb kinematics may not reflect the regular cyclic behavior of corresponding EMG. We examined the pattern of hindlimb kinematics to understand how limb motion is organized during the 10-12 cycles of the PSR.

PSRs were elicited in 4 adult spinal cats by applying tape to the hindpaw; limb motion was filmed (200 f/s), and hip, knee, and ankle kinematics were obtained. Lateral gastrocnemius, tibialis anterior (TA), and vastus lateralis were chronically implanted with bipolar electrodes; EMG data were synchronized with angular kinematic data.

Phase portraits of PSR showed 3 distinct regions: start-up (SU), steady-state (SS), and slow-down (SD). In SU (5±1 cycles), a TA burst initiated the PSR; knee and hip oscillations did not appear until approximately the third ankle joint cycle. Successive ankle excursions increased, and mean angles increased for all joints. Hip and knee actions were out of phase with the ankle, and ankle joint maxima preceded knee joint maxima by 10-15 ms. In SS (3±1 cycles), stable joint oscillations existed, but phasing between joints shifted so that knee motion led ankle motion by 5-10 ms. In SD (3±1 cycles), ankle excursions decreased, mean joint angles decreased, and knee-ankle phase relationship reverted to the SU pattern. Kinematic and EMG cycle durations increased from SU to SS to SD. Muscle synergies were relatively invariant over the three regions and muscle activity was out of phase with joint displacement during the entire PSR.

Phase relationships between hindlimb joint motions are critical in facilitating and maintaining large, stable oscillations at the distal joint. The three regions of the PSR emerged from an interaction between the neural code and the organizational influence of the segmental mechanics. Supported by NIH grant NS 19864.

102.11 NEUROMECHANICAL ORGANIZATION: THE EMERGENCE OF UNSTABLE LIMB OSCILLATIONS DURING PAW-SHAKE RESPONSES. G.F. Koshland, M.G. Hoy, J.L. Smith and R.E. Zernicke. Dept. Kinesiology, UCLA, CA 90024.

Most paw-shake responses (PSRs) in spinal cats are characterized by stable oscillations at the hip, knee and ankle joints (Hoy, et al. Neurosc. Abst. 1984). Some PSR records, however, show disrupted kinematic patterns in which the steady state is not achieved. Thus in this study, we determined whether atypical EMG patterns were associated with unstable cyclic motions of the hindlimb.

Twenty-eight PSR from five spinal cats were filmed at 200 f/s and hip, knee, and ankle kinematics were obtained. The EMG of gluteus medius, iliospoas , vastus lateralis (VL), anterior biceps femoris, tibialis anterior, and lateral gastrocnemius were also recorded and synchronized with the joint displacements.

There were two categories of disrupted kinematics. In the first group, the ankle and hip maintained the typical in-phase motion while the knee never achieved a stable, out-of-phase motion. Moreover, the knee motion shifted, and at times oscillated once for two ankle cycles. This shift could be explained by a change in the onset latencies of the VL-EMG bursts or by an absence of VL activity. In these records, the knee was decoupled from the hip and ankle. In the second group of disrupted patterns, none of the three joints established stable oscillations and there were irregular thrusts of simultaneous hip, knee and ankle extension and flexion. However, the EMG records were typical of normal PSR (Phillips, et al. Neurosc. Abst. 21.2 1983) suggesting the irregular kinematic pattern could be better explained by altered intersegmental dynamics that influenced the limb motion.

Two conclusions from these preliminary results are suggested. First, the coordinative neural network in the lumbosacral cord permits an uncoupling of the knee joint while oscillations at the hip and ankle are relatively stable. This uncoupling has not been reported for other "automatic" movements of the cat's hindlimb (e.g., locomotion and scratching). Second, the large variability in the kinematic patterns with the relatively little variability in the general EMG pattern support the concept that the limb motion of the PSR is an emergent pattern governed by centrally generated neural code, intersegmental dynamics and segmental feedback. Supported by NIH grant NS 19864.

102.12 ALTERATIONS OF KNEE EXTENSOR ACTIVITY IN PAW-SHAKE RESPONSES FOR PERTURBED LIMB IN SPINAL CATS. E.M. Cox* and J.L. Smith. Dept. Kinesiology, UCLA, CA 90024.

The paw-shake response (PSR) is a rapid cyclic movement of the cat's limb in response to an irritant stimuli on the paw. The PSR has a mixed synergy with vastus lateralis (VL), a knee extensor, coactive with tibialis anterior (TA), an ankle flexor. Kinetic data of the PSR (Hoy, et al, Neurosci. Abst. #21.3,1983), show that at the knee joint the muscle moment opposes the inertial component caused by the accelerating paw. Our study was designed to test the hypothesis that VL activity is regulated by the limb dynamics, and dependent primarily on peripheral feedback, to oppose the forces created by the accelerating paw.

PSRs were evaluated in three spinal cats under five conditions: freely moving limb (FL), weighted paw (28 and 46 gm), casted ankle joint (CA), and casted knee-ankle joint (CAK). PSRs were elicited by wrapping tape around the paw. EMG was recorded from VL, TA, and lateral gastrocnemius (LG). Analyses included burst and cycle duration (CD), and intralimb synergies were determined by onset latencies (OL, expressed as a percent of average CD).

There was no influence on burst duration or number of cycles across conditions. The two weight conditions had no impact on average cycle duration (93 ms), but casting increased CD by 10 ms (CA) and 20 ms (CAK). TA-OL averaged 60% in the FL and decreased slightly for the weighted paw, while casting delayed TA-OL to 65% (CA) and 75% (CAK). The VL-OL showed a similar trend with a decrease in OL for both weights, 59% in FL vs 52% for weights, and a delay in OL with casting (54% CA, 82% CAK). VL activity was inconsistent with missing bursts increasing from 7% in FL to 11% and 15% with 28 or 46 gm on the paw and missed 10% and 30% for CA and CAK, respectively. Double bursting of VL rarely occurred in FL (1%), but increased for all perturbed conditions (7%=28 gm, 9%=46 gm, 9%=CA, and 15%=CAK).

Our results show that VL activity is substantially altered by a weighted paw or with immobilization. These results are consistent with the hypothesis that VL participation in the PSR is regulated primarily by the emergent limb dynamics. Supported by NIH grant NS 19846.

103.1 BIMANUAL POSITIONING MOVEMENTS: THE EFFECT OF AN UNEXPECTED LOAD ON DUAL TASK PERFORMANCE. D.C. Shapiro and D.E. Young*. Motor Control Lab., Dept. of Kinesiology, UCLA, Los Angeles, CA 90024.

Previous evidence (Shapiro, 1984) demonstrated that in a bimanual positioning task, inhibiting the production of one movement, produced slowing and overshooting of the target in the contralateral limb. In the present study, a single limb was unexpectedly loaded and the effects on the loaded limb and on the contralateral limb were examined.

The task required simultaneous horizontal elbow flexion to targets located 30^0 from each start position. Movement time conditions were 200 and 400 ms. Subjects practiced for 150 trials and then performed 150 experimental trials, receiving their movement times after each response. Unexpectedly, on 10% of the experimental trials a weight was added to the left lever. During each trial, EMG activity of the biceps brachii (agonist) and the lateral triceps (antagonist) was recorded with surface electrodes from both limbs and displacements were indicated by potentiometers. In addition, strain gauges attached to the levers provided a measure of relative forces in the horizontal dimension.

In the 200 ms movement condition, the loaded (left) limb performed in a significantly greater movement time than during the experimental trials without the added load. The target was slightly overshot as well. The contralateral limb was not significantly affected by the loaded limb and performed the movement as planned. In the 400 ms movement time condition, the unexpected load significantly slowed down the contralateral limb and minimally affected target accuracy. Again, the limb that was not loaded was uninfluenced by the perturbation in the opposite limb. In both experiments the EMG activity was similar across all conditions. Both the durations and amplitudes of the forces increased with the added load.

The results indicated that adding a load unexpectedly to a single limb in a bimanual task mainly affects the temporal characteristics of the disturbed limb and does not appear to affect the contralateral limb. Therefore, the manipulation of a single limb during dual task performance may not always produce contralateral interference. The results are discussed in terms of motor control models.

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103.2 DYNAMIC MATCHING OF BILATERAL ARM MOVEMENTS IN HUMANS. D. al-Senawi* and J.D. Cooke (SPON: J.J. Seguin). Department of Physiology, Univ. of Western Ontario, London, Canada.

It is well known that humans possess a remarkable ability to match the static positions of the two arms and that this ability is under both visual and proprioceptive control. In addition recent studies have shown close matching of some kinematic features of movements made simultaneously or independently by the arms. In the present study we have investigated the dynamic or moment to moment matching of simultaneous arm movements as well as the influence of varied visual feedback on this matching.

During experiments subjects held handles on the ends of two manipulanda. The manipulanda were pivoted on the other ends and could be rotated horizontally. Subjects performed alternate flexion/extension movements about the elbow. Required movement amplitude was varied from trial to trial and was the same for both arms. Three conditions of visual feedback were used: 1) target and right handle position displayed, 2) target and left handle position displayed and 3) neither target nor handle positions displayed.

In the no-display condition, movement amplitudes, peak velocities and durations were the same for both arms. Arm positions and velocities were matched for the two arms throughout the movements with no phase lags or leads. When the position of one arm was displayed to the subject the amplitude and peak velocities of movements made by the other (non-displayed) arm were consistently greater. Movement durations and times to peak velocity were also greater for the non-displayed than for the displayed arm. In all subjects (n=8) the relation between movement peak velocity and movement amplitude was linear ($r>0.95$) and was the same for both arms. This relation was unaffected by the visual display.

The data support the hypothesis that, during simultaneous movements, the two limbs are treated as a functional unit, perhaps sharing a common motor command. At the same time this basic organization can be modified when the subject's attention is directed primarily to one limb through action of the visual system.

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103.3 WITHDRAWN

103.4 AMENDMENT LATENCIES FOR LIMB MOVEMENTS AS A FUNCTION OF THE ACTIVE STATE OF THE MOTOR SYSTEM. L.G. Carlton* and M.J. Carlton* (SPON: K.M. Newell). Biomechanics and Motor Behavior Lab., University of Illinois at Urbana-Champaign, Urbana, IL 61801.

An important feature of the motor system is that it is capable of generating response amendments when errors occur in response production. When amendments are generated in response to a visual stimulus during the course of movement, the general finding has been that response latencies are shorter when the modification requires an increase in the velocity of the movement as compared to when the modification requires the subject to reverse the movement direction. The present experiment examined the hypothesis that differences in response latencies are related to the physiological state of the motor system when the amendment is required. For example, generating an increase in activity in an already active muscle will require less time than when the activity in an active muscle must be terminated and an antagonistic muscle group must be activated.

The movement examined was horizontal flexion of the right arm. During the movement visual stimuli were presented in one of four phases of the response, 1) during initial agonist activity; 2) during the mid-portion of agonist activity; 3) between the end of agonist and the initiation of antagonist activity; and 4) at the beginning of the antagonist phase. In alternating blocks of trials subjects were instructed to either speed up the response or to reverse the direction of the movement upon presentation of the visual stimulus.

Amendment latencies were evaluated from electromyographic (EMG) patterns of individual trials. The results followed the hypothesised trend. When subjects were required to increase the velocity of the movement the mean EMG latencies ranged from 164 msec when the stimulus was presented at the beginning of the agonist phase, to 209 msec when the stimulus was presented during antagonist activity. Reversal amendment times were longest when the stimulus was presented during the beginning of agonist activity (237 msec) and latencies were shortest when the stimulus coincided with antagonist activity (195 msec). The present findings provide strong support for the notion that amendment latencies are directly related to the active state of the motor system when the modification is required, and the nature of the correction to be generated.

103.5 QUANTITATIVE ANALYSIS OF THE RELATIONSHIP BETWEEN MOVEMENT TIME AND THE DISCHARGE OF PALLIDAL NEURONS. Marjorie E. Anderson. Depts. of Physiology and Biophysics and Rehabilitation Medicine and Regional Primate Research Center, Univ. of Washington, Seattle, Wa 98195.

Lesions of the globus pallidus (Horak and Anderson, J. Neurophysiol., in press) or stimulation in the globus pallidus (Horak and Anderson, J. Neurophysiol., in press) produce changes in the movement time (MT), but not the reaction time, measured for monkeys performing an arm reaching movement in a simple reaction time task. Furthermore, numerous pallidal neurons have phasic changes in discharge prior to or during the movement.

In light of the observed changes in movement time as a consequence of disruption of pallidal activity, the correlations between movement time and several parameters of pallidal discharge have been examined. These included time of initial and peak changes in discharge rate, duration of changes in discharge rate, time from initial to maximum discharge rate, maximum amplitude of change in discharge rate, and total spikes added or subtracted during various epochs of the behavioral task.

M. fascicularis performed the same arm-reaching simple reaction time task that had been used previously for lesion and stimulation studies, but they were not forced to such consistently minimum movement times. Data from trials with similar movement times were grouped into 10 or 20 msec MT bins for analysis.

Many pallidal neurons with significant task-correlated changes in discharge also showed a correlation between movement time and one or more of the discharge parameters examined. In general, these tended to be related to the rate of change of firing, as expressed by a correlation between movement time and the time, relative to movement initiation, of the minimum or maximum discharge rate or the time from the initial to the maximum change in discharge.

In addition, we have begun to examine the tonic firing characteristics and task-related changes in discharge of thalamic neurons whose discharge can be modified by stimulation through electrodes positioned in the globus pallidus.

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103.6 RESPONSES OF MOTOR CORTEX NEURONS TO NATURAL STIMULATION OF PERIPHERAL RECEPTORS ARE NOT ABOLISHED BY COOLING OF SENSORY CORTEX IN CONSCIOUS MONKEYS.

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In conscious monkeys, 75% to 85% of neurons in the precentral gyrus respond at short latency to passive movement of one or more joints. Whether such inputs utilize cortico-cortical relay to the motor cortex from the areas 1 and 2 of the post central gyrus was examined in conscious behaving animals. Recordings were made from single neurons in the motor cortex in two conscious, behaving monkeys during a lever pulling task and while they sat quietly and allowed natural stimulation of the limb. A 6 mm x 10 mm cooling plate was placed subdurally over the arm representation of areas 1 and 2. It could be perfused with ice-cold water to lower the temperature rapidly and to depress neuronal function locally. Peri-response time histograms of the discharges of individual cortical cells during lever pulling were obtained, as well as peri-stimulus time histograms in association with natural activation of the limb by touches or by passive joint movement. Recordings were made from 115 neurons of which 24 were sufficiently stable to allow full analysis before and after cooling for an average of 10.6 min. Cooling for 2 to 7 min produced slowing and clumsiness of movement, and later, ataxia of the contralateral limb. After 10 min the animal seemed unable to use the limb purposefully. The activity of cells in the motor cortex increased during cooling, but the modulation with movement retained the temporal pattern chracteristic of that unit. Short-latency responses to natural peripheral stimuli could consistently be demonstrated before, during and after cooling, even when the animal could no longer move the limb. On rewarming, the motor deficit disappeared in 3 to 5 min and the level of discharge and response patterns returned to control levels. A control experiment showed that the temperature of the motor cortex remained unchanged during cooling of the sensory cortex. Histological examination of the cortex revealed no abnormalities of the sensory cortex under the plate. Thus in the conscious animal, peripheral receptors activated by natural stimulation of the limb reach the motor cortex without first activating areas 1 and 2.

103.7 A MECHANISM FOR CORRECTING MOVEMENTS THAT IS INDEPENDENT OF VISION, STRETCH REFLEXES AND THE INTERMEDIATE-LATERAL CEREBELLUM. J. Hore, T. Vilis and D. Flament*. Dept. of Physiology, Univ. West. Ont., London, Ont., Canada N6A 5C1.

Two disorders associated with lesions of the cerebellum in man and monkeys are oscillations in movements (tremor) and dysmetria (Holmes, 1939; Brooks et al., 1973). However a feature of published records of movements during cerebellar dysfunction is that the arm reaches the region of the aimed target. The objective of the present study was to investigate the mechanism underlying this ability to achieve the aimed position during cerebellar dysfunction.

Five Cebus monkeys were trained to make fast and accurate step-tracking elbow flexions by superimposing a handle cursor on a target displayed on an oscilloscope. Cooling through probes implanted lateral to dentate and through interpositus produced a deflection (oscillation) in movements. In spite of this deflection movements reached the aimed target (width 14°). This occurred irrespective of the amplitude of the aimed movement (40°-60°) or the level of constant force applied to the handle. This ability was not dependent on vision since movements in which the handle cursor was unexpectedly removed still reached the target.

Inspection of the associated EMG records showed that the initial deflection in these flexion movements was produced by an abnormal burst in triceps (the antagonist) and that compensation for the deflection (i.e. continuation of the movement towards flexion) was achieved by a large second agonist burst. A direct relation was found between the degree of deflection of the movement from its expected velocity trajectory and the integrated value of the second agonist burst. This second agonist burst was not simply the tail end of an original step command to the agonist because it was often larger in magnitude than the first agonist burst. It was also not due to stretch reflexes from stretch of biceps. In the majority of movements velocity did not reach zero and biceps was not stretched during the deflection.

These results show that a central mechanism exists which compensates for deflections in movements so that the aimed position is achieved. This mechanism operates independently of the lateral and intermediate cerebellum and does not involve stretch reflexes or vision.

Holmes, G. (1939) Brain 62, 1-30.
Brooks, V.B. et al. (1973) J. Neurophysiol. 36, 974-995.

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103.8 VISUAL TRACKING BY MONKEYS: FEEDBACK CONTROL OR OPEN LOOP? R. M. Wylie. Depart. Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307.

Tracking of visual stimuli has been interpreted in terms of negative feedback control systems (see Stark, Neurological Control Systems, Plenum, NY, 1968). The demonstration that deafferented monkeys can point to stationary targets (Taub, Goldberg & Taub, Exp. Neurol. 46:178-186, 1975; Polit & Bizzi, J. Neurophysiol. 42:183-194, 1979) has challenged feedback theories. Bizzi and his colleagues have proposed that control is fundamentally open loop. The relative levels of co-contraction of the muscles acting at a joint establish an equilibrium position independent of initial position.

I have trained normal monkeys to use forearm rotation to track moving spots. Discrete trials are used with an initial and a final stationary position. The monkeys obtain reinforcements by pointing at the spot from the beginning to the end of a trial. The monkeys successfully track steps, ramps and parabolas.

I have developed a linear, negative feedback model of the visual tracking paradigm. The plant in the model simulates the mechanics of the forearm: a resting equilibrium position between flexor and extensor springs under tension, with viscous and inertial resistances to movement. The flexor or extensor torque required to maintain any angle away from the resting equilibrium position increases in proportion to the angle. A delay simulates reaction time. The controller has derivative, proportional and integral terms. The gains for the three error terms are adjusted to approximate the performance of the monkeys. Setting the feedback of arm position to zero renders the model open loop. The input then becomes the error. Unless the gain for the integral term is small, the integral term grows monotonically and the angle of the joint increases without bounds. With this constraint, the other gains can be adjusted to make the output approximate the input. If the input comes to rest, the output will come to a final position proportional to that of the input. The final position of the output is independent of the initial position of the output and of the trajectories of both the input and the output.

To apply this result to the performance of deafferented animals pointing to stationary targets, we must assume the deafferented system can reduce the neural activity formerly providing feedback to a level equivalent to zero. The neural equivalent for the gain of any integral control must also become the equivalent of zero.

The open loop final position hypothesis is a property of feedback systems operated in an open loop mode. A feedback system can be adjusted to operate as an open loop system.

103.9 ROLE OF CENTRAL AND PERIPHERAL VISION IN THE CONTROL OF DIRECTION AND AMPLITUDE MOVEMENTS. M. Fleury, C. Bard, L. Hay*. Physical Activity Science Lab., Laval Univ., Quebec; Lab. of General Psychophysiology, CNRS, Marseilles.

Visual feedback improves accuracy in a pointing task. In movements aimed at eye-fixated targets, central and peripheral vision are both present. Studies taking into account specific requirements of movement are needed to clarify the situations for which central vision, contributing to homing-in performances, and peripheral vision, allowing directional adjustments, are necessary. The aim of the present experiment was therefore to compare the respective efficiency of central and peripheral vision in the regulation of movements in direction and amplitude. Two tasks were used: a purely directional task (A) and a task involving amplitude only (B). In task A, Ss holding a lever, had to aim at and overshoot one of three targets (0°, 20°, and 40° of eccentricity). Absolute directional errors (ADE, in degrees) and constant directional errors (CDE, in degrees) were recorded at the target plane. In task B, Ss had to point and stop at one of three targets located in a sagittal plane, at distances of 240, 290, and 340 mm. Absolute amplitude errors (AAE, in mm) and constant amplitude errors (CAE, in mm) were recorded. The targets consisted in vertical lines of 10 cm. Eight Ss had to perform both tasks in one open-loop (OL) and two closed-loop conditions with central feedback (CF) or peripheral feedback (PF). Ss were holding a lever, brushing the inferior portion of the targets at termination of movement. In CF, Ss fixated the inferior portion of the targets and their hand; in PF, Ss had vision of the upper part of the target and peripheral vision of their hand. Separate ANOVA were applied to directional and amplitude errors. For task A, there is a significant effect of feedback; both CF and PF conditions improve significantly and similarly the accuracy of movements, as expressed by ADE, as compared to OL. In task B, there is also a significant effect of feedback condition; the greatest improvement was in the CF condition, with PF occupying an intermediate position. In terms of constant errors, results are similar to those obtained in absolute terms except for the fact that in task B, PF and OL have similar effects, with CF still leading to better accuracy. In summary, the results show that the regulation in direction benefits from both types of visual feedback, whereas regulation in distance,though it can be based on peripheral vision,is more specifically concerned with central vision.

103.10 CONTROL AND CORRECTION OF POINTING MOVEMENTS AT DIFFERENT SPEED, WITH DIRECTION OR AMPLITUDE REQUIREMENTS, L. Hay*, D. Beaubaton, C. Bard, and M. Fleury (SPON: J. PAILLARD). Lab. of General Psychophysiology, CNRS, Marseilles; Physical Activity Sciences Lab., Laval Univ., Quebec.

Pointing tasks involve both directional and amplitude requirements. The aim of the present experiments was to investigate programming and visual control of movements in a purely directional task (A), a task involving amplitude only (B), and a task combining both directional and amplitude requirements. In Experiment 1, Ss had to point at one of two luminous lines, which appeared on a vertical panel (providing the rectangular coordinates of the index impact). Ss had to reach any point on the line. In task A the lines were diverging from the starting point (25° apart); absolute directional error (ADE, in degrees) was recorded. In task B the targets were two parallel horizontal lines (100 mm apart); absolute amplitude error (AAE, in mm) was recorded. Four different movement times were assigned from 130 to 300 ms. Twelve adults performed all tasks in all speed conditions, in open (OL) and closed loop (CL). Results show significant main effects of Feedback and Speed on both ADE and AAE. A significant linear component was found on speed effect, accuracy increasing with MT, except in the case of ADE in OL. In experiment 2, Ss holding a lever which reduced the degrees of freedom of movements moved towards targets. In task A, Ss had to aim at an overshoot one of three targets (0°, 20°, and 40° of eccentricity), located 304 mm from them; ADE was recorded at the target plane. In task B, Ss had to point and stop at one of three targets located in the sagittal plane, at distances of 240, 290, and 340 mm; AAE was recorded. In task C, aiming at the same targets as in task A, Ss had to stop at the target; both ADE and AAE were recorded. Eight Ss performed all three tasks in OL and CL, at two different speeds (200 and 400 ms). Moreover, in OL, Ss could correct their initial aiming by a readjustment of their hand position. The results show, for ADE, a significant effect of feedback and speed; no effect of task and correction was found significant. For AAE, there is a significant effect of feedback, speed, task, and correction. Accuracy is better in CL than in OL, at low than at high speed, in task B than C in OL. Correction improves accuracy more in task C than in task B, and at high than at low speed. Amplitude regulation appears to be more time dependent than direction regulation, and it is more sensitive to readjustments, suggesting a dependency on the coding of terminal position.

103.11 THE AGE FACTOR IN THE CONTROL OF MOVEMENTS WITH DIRECTION OR AMPLITUDE REQUIREMENTS. C. Bard, M. Fleury, and L. Hay*, Physical Activity Sciences Lab., Laval Univ., Quebec; Lab. of General Psychophysiology, CNRS, Marseilles.

Reaching ability appears to be acquired early in infants, but is still open to improvement whenever precise direction and distance requirements are introduced. Indeed, developmental patterns for the control of direction and distance do not seem to be identical (Bard & Hay, 1983). The aim of the present experiment was to investigate the role of the initial program and the contribution of visual feedback to the accuracy of performance, according to age, in a purely directional task (A), a task involving amplitude only (B), and a task combining both directional and amplitude requirements (C). In task A, Ss holding a lever, had to aim at and overshoot one of three targets (0°, 20°, 40° of eccentricity), located 304 mm from them. Absolute directional errors (ADE, in degrees) were recorded at the target plane. In task B, Ss had to point and stop at one of three targets located in a sagittal plane, at distances of 190, 240, and 290 mm. Absolute amplitude errors (AAE, in mm) were recorded. In task C, Ss had to aim at the same targets as in task A, and stop their movement at the target. Both ADE and AAE were recorded. Four groups (6,8,10 years, and adults) of eight subjects had to perform all three tasks in open (OL) and closed loop (CL). Moreover, in OL, Ss could correct their initial aiming performance by a readjustment of their terminal hand position. ADE and AEE were analyzed by a three-way analysis of variance: Age (4)x Task (2)xFeedback (2). In CL, ADE decreases significantly over all age groups. No main effect or interaction was found for age and task. For AAE, three main effects are significant: the two youngest groups are less accurate than the 10-year-olds and adults; task B is performed with less accuracy than task C; the performance in CL is more accurate than in OL. Interactions are significant, revealing that 8-year-olds are much less accurate in task B performed in open loop. The analysis of variance (Age 4xTask 2x Error 2) reveals that, in tasks B and C corrections improve accuracy only for amplitude performances in all groups. In summary, the results show that the success in the control of direction and amplitude of movements are not synchroneous. Directional control is established early whereas amplitude control is achieved around 10 years of age, with an important strategical change appearing at 8 years.

103.12 MOTOR CONTROL CHANGES IN A MOVEMENT SEQUENCE TASK AS A FUNCTION OF LEARNING AND FORGETTING. R.G. Marteniuk and S.K.E. Romanow*. Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

Three experiments investigated the changes occurring in the motor control characteristics of the right arm as subjects learned and then forgot a sequential movement involving a series of flexions and extensions of the elbow joint. Of primary interest was to determine if there were qualitative changes in the characteristics of motor control that might suggest the use of more advanced information as a motor skill is acquired and a regression to less advanced information as the skill is forgotten.

In the first experiment, subjects practiced the movement for 800 trials and received knowledge of results after every trial. RMS error indicated subjects became quite proficient at producing the criterion task. Cross-correlation analyses between the movement kinematics (displacement, velocity, and acceleration) of the subject's performance and those of the criterion movement showed the displacement correlations were largest early in acquisition but then those based on velocity and then acceleration became larger as practice continued. Cross-spectral density analyses of the subjects' performance and the criterion movement also indicated a shift in the characteristics of motor control from relatively slow frequency components early in learning to relatively rapid frequencies late in learning.

In the second experiment, subjects who had learned the task were not allowed to practice for three months. For some subjects, forgetting resulted in a decrease in timing ability (tau and its variability increased) as well as a shift from the presence of relatively high component signal frequencies in the movement of the limb to lower frequencies. Other subjects maintained high frequency control but exhibited a constant shift in timing accuracy which explained their performance deterioration.

Experiment three was designed to determine the role that perceptual factors could play in the motor control changes observed as a function of learning. In part, support was found for the fact that motor control acquisition can be facilitated by the form of perceptual information a subject is given about the movement. However, it would appear that advanced motor control can only be achieved after the motor system develops the ability to control relatively high movement component frequencies. Perceptual information about these higher frequencies cannot be used until this ability develops.

103.13 IS PARKINSONIAN AKINESIA A DISTURBANCE IN MOTOR PREPARATORY PROCESS? C.W.Y. Chan and S.J. Sullivan*, School of Physical and Occupational Therapy, McGill Univ., and Dept. of Exercise Science, Concordia Univ., Montreal, PQ, Canada, H3G 1Y5

Single unit recording has shown that movement-related discharges in basal ganglia generally precede those of motor cortical cells, indicating their involvement in the initiation of movement. To test the hypothesis that Parkinsonian akinesia could be due to a disturbance in motor preparation, we measured the extent to which the normal pattern of H reflex excitability prior to a ballistic ankle plantarflexion in a simple reaction time paradigm, is modifiable by akinesia. Nine age-matched normals and eleven Parkinsonians were examined. They were instructed to plantarflex the right ankle as rapidly as possible in response to a visual signal (RS) following a tone burst (WS) at 1000 msec, while EMGs were recorded from the soleus and tibialis anterior (TA) simultaneously with measurement of ankle angle.

Under this "control" condition: (1) the mean soleus EMG and movement onset latencies were significantly longer ($p<.05$) in Parkinsonians than normals. The control values measured before and after the test conditions did not differ significantly ($p>.05$), showing no evidence of fatigue, training or arousal effects. (2) Differences in EMG response patterns were observed, in that the antagonist (TA) activity also occurred later in Parkinsonians than normals.

Next we measured changes in the excitability of the soleus motoneuron pool at six predetermined intervals following the WS and prior to EMG response onset by means of the H reflex. Three findings emerged under these "test" conditions: (1) As before, the mean soleus EMG and movement onset latencies were significantly longer ($p<.001$) in Parkinsonians than normals. (2) H reflex amplitude did not differ significantly between the two subject groups and among the various H reflex sampling intervals (at 300,600, 1000 msec post-WS), indicating similar CNS excitability during the "fore-period" between Parkinsonians and normals. (3) H reflex facilitation was similarly time-locked to EMG response onset for the two groups, with increases in H reflex amplitude commencing around 60 msec, and reaching a mean peak up to 218% of control values at 20 msec, prior to agonist onset. These findings therefore pointed to a delay in the facilitation of the H reflex relative to the RS as a cause of akinesia.

In conclusion, our data supported the notion that akinesia could be attributable to an impairment in the motor preparatory process.

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104.1 NEURONAL NETWORK SUBSERVING THE KINDLING MODEL OF EPILEPSY: THE ROLE OF THE FIMBRIA. R.L. Gellman & J.O. McNamara. Departments of Medicine (Neurology) & Pharmacology, Duke University, Durham, NC 27705.

Elucidation of the neuronal network subserving kindled seizures (sz) is an important first step towards understanding the underlying cellular and molecular mechanisms. Several lines of evidence suggest that the hippocampus (HPC) is involved in this network. 1) HPCal slices from kindled rats exhibit abnormal excitablity that could increase the likelihood of szs (King,GL et.al., this volume). 2) The HPC is a site of biochemical alterations in kindled animals. 3) Disruption of its intrinsic circuitry has been shown to interfere with the development of kindling. We postulated that if the HPC is involved in the generation of a kindled sz, then transecting its major efferent pathway, the fimbria, should impair sz expression. We selected two sites from which to kindle, the lateral entorhinal cortex (LEC) and the amygdala (AMYG).

Rats implanted with bipolar electrodes in the LEC or AMYG were stimulated until sz of consistent duration and threshold were obtained. The durations of the motor component (motor) and total afterdischarge recorded on EEG (limbic) measured response. Anesthetized rats received lesions made with a Scouten stereotaxic knife. Control lesions were restricted to the overlying cortex. After a 3d recovery, response to a threshold stimulation was determined. Electrode placements and lesions were verified histologically with Nissl and AChE stains.

Fimbria transection markedly suppressed sz responses in animals kindled from both LEC and AMYG. The control group had no significant change. The AMYG kindled group exhibited reductions of 66% in limbic and 87% in motor sz duration (mean ± SEM, in seconds: motor, prelesion 39.2 ± 3.5, postlesion 5.25 ± 3.5; limbic, prelesion 90.7 ± 12.2, postlesion 30.4 ± 10.7). These effects were significant ($p<0.001$, student's paired t-test 2-tailed). The LEC group showed a similar pattern of marked attenuation of motor response and shortened limbic sz duration. The suppression could be overcome by increasing the stimulation current 95%.

Our interpretation is that fimbria transections impair sz expression by eliminating transmission of sz information through HPCal efferents residing in the fimbria. The fimbria appears to play a similar role in seizures originating from either the LEC or AMYG. However, this pathway is not necessary for the generation of a sz since the suppression can be overcome.

104.2 MICROINJECTION OF CLONAZEPAM INTO SUBSTANTIA NIGRA SUPPRESSES KINDLED SEIZURES. P.H. King* and J.O. McNamara (Spon: D. Bonhaus). Depts. of Medicine (Neurology) and Pharmacology, Duke Univ. Medical Center, Durham, NC 27710.

Kindling is an animal model of epilepsy whereby repeated electrical stimulation to focal areas of the brain results eventually in clonic motor seizures. IP administration of the benzodiazepine (BZ), clonazepam (CZP), markedly suppresses amygdala-kindled seizures, but the site(s) where the anticonvulsant (AC) effect is exerted remains unknown. We hypothesized that the substantia nigra (SN) mediates the AC effects of systemically administered BZs because: 1) using microinjected muscimol, we previously demonstrated the importance of SN GABA-receptive neurons in the regulation of kindled seizures, 2) the BZs are thought to increase the responsiveness of such neurons to GABA, and 3) the SN contains a high concentration of BZ receptors. To test this hypothesis, we microinjected CZP bilaterally into the SN and measured its effect on kindled seizures.

Rats implanted with an amygdala electrode and guide cannulas bilaterally over SN were then kindled until motor seizures were elicited at a consistent threshold. Afterdischarge threshold (ADT, the lowest current eliciting an afterdischarge at the stimulating electrode), and generalized seizure threshold (GST, the lowest current required to elicit a class 4 or 5 seizure) were then determined. 30 minutes after CZP injection into SN, ADT and GST were again determined. Animals were restimulated in several days to demonstrate reversibility. Electrode and cannula placements were verified histologically.

Microinjection of CZP (500 ng in 0.5 ul vehicle) bilaterally into SN reticulata (SNr) elevated the GST by 75% (N=4; $P<.05$, Student's t test): pre-injection 228 ± 37 (mean ± SEM), post-injection 420 ± 70, reversal 250 ± 42. ADT was not significantly altered: pre 220 ± 36, post 285 ± 29, reversal 210 ± 40. Microinjection of vehicle (N=6) did not affect the GST: pre 243 ± 43, post 243 ± 47; or the ADT: pre 223 ± 33, post 213 ± 35. Animals receiving injections in sites nearby (N=6), but not in SNr bilaterally, demonstrated a 12% increase in the GST: pre 270 ± 39, post 303 ± 43. ADT was unaffected: pre 263 ± 38, post 257 ± 34.

The data indicate that microinjection of CZP into SNr, but not nearby sites, suppresses kindled seizures. We conclude that at least part of the AC effects of systemically administered BZ are mediated at the SN. We are presently investigating whether the systemic AC effects can be explained solely by an action at the SN.

104.3 SYSTEMIC γ-VINYL GABA RETARDS KINDLING DEVELOPMENT AND SUPPRESSES KINDLED SEIZURES. C. Shin, S. Legg* and J.O. McNamara. Departments of Medicine (Neurology) and Pharmacology, Duke Univiversity; Epilepsy Centers, Duke University and VA Medical Centers, Durham, N.C. 27710.

Previous work from our laboratory demonstrated that intranigral microinjection of γ-vinyl GABA (GVG), a GABA transaminase inhibitor, blocks both motor and limbic seizures in fully kindled rats (J. Neurosci. in press). Others have found that systemic GVG was effective in blocking motor seizures but not in blocking limbic seizures (Myslobodsky et al., 1979; Kalichman et al., 1982). Kalichman et al. (1981) also found that systemic GVG did not retard the development of kindling. This paradox between the intranigral and systemic GVG effects led us to examine the effect of systemic GVG on the fully kindled seizure as well as on the development of kindling.

Male Sprague Dawley rats with a bipolar electrode in the right amygdala were administered 1500 mg/kg of GVG intraperitoneally. 16 hours later, animals received kindling stimulations at currents 100 μA above the afterdischarge threshold 7 times a day at 90 minute intervals, until 3 Class IV or V seizures occurred. Control animals received saline IP.

To study the effects of GVG on seizures in previously kindled animals, a separate group of rats was kindled from amygdala and generalized seizure threshold (GST) was determined. Animals were then given 900, 1200 and 1500 mg/kg of GVG IP and received stimulations (at currents 10% over the GST) beginning 8 hours later and at daily intervals thereafter.

Animals receiving IP GVG required 82% more kindling stimulations (34.3 ± 3.7) than control animals (18.8 ± 1.8) ($p < 0.01$, Student's 2-tailed t-test). Systemic GVG suppressed both motor and limbic seizures in previously kindled animals in a dose dependent manner. The effect was maximal at 30 hours and its time course approximated that of GVG mediated increases of nerve terminal GABA (Gale and Iadarola, 1980).

Our results support the idea that pharmacologic enhancement of GABA mediated neurotransmission suppresses development of kindling as well as both motor and limbic seizures in a fully kindled animal. We think that differences in dose and kindling paradigm account for the discrepancies between our results and those of previous investigators.

104.4 EVIDENCE IMPLICATING DENTATE GRANULE CELLS IN WET DOG SHAKES ASSOCIATED WITH LIMBIC SEIZURES. D.P. Frush and J.O. McNamara , Depts. of Medicine and Pharmacology, Duke University Medical Center, Durham, NC 27710.

Violent shaking of the head, neck, and body while standing on all four limbs is a stereotypy commonly observed in normal rats. This behavior has been termed "wet dog shakes" (WDS) because of the similarity to the rapid movements observed in a dog shaking itself after having been wet. An increase in the the frequency of WDS can be induced in a variety of instances, including pharmacologic treatments (eg opiate withdrawl), and in kindled limbic seizures. The brain structures subserving the generation of WDS are unknown. The hippocampal formation (HPF) has been implicated as one of the structures responsible for WDS because: (1) stimulation of the HPF or its afferents increases the frequency of WDS, and (2) destruction of CA3/4 neurons is associated with suppression of WDS. During the course of experiments examining the effects of elimination of dentate granule cells (DGC) on kindling develpment by lateral entorhinal cortex (LEC) stimulation, we serendipitously discovered that elimination of DGC was associated with a striking suppression of WDS.

Male S-D rats were implanted with a bipolar stimulating electrode electrophysiologically localized to the LEC by stimulations evoking a monosynaptic population excitatory post-synaptic potential recorded via a dentate hilar microelectrode. Animals received either intradentate injections of artificial CSF (IDaCSF) or colchicine dissolved in aCSF (IDc). A third group received colchicine injections into the frontal cortex (FCc). Following a two week recovery period, animals were periodically stimulated until kindling was complete. The number of WDS was measured during evolution of kindling by behavioral observations during and for at least one minute following the end of the afterdischarge. Histologic exam revealed extensive DGC destruction in the IDc but not IDaCSF or FCc animals. IDc animals demonstrated a 96% decrease in the average number of WDS elicited during kindling when compared to IDaCSF ($p<.001$). FCc animals did not differ significantly from IDaCSF (Average # of WDS ± SEM, n=, range. IDaCSF: 110.7 ± 10.1, n=6, 71-136; IDc: 4.5 ± 2.5 , n=6, 0-16; FCc: 79.8 ± 44.9, n=5, 27-259).

We suspect that the effect of colchicine is mediated through destruction of DGC. If correct, the data suggest that the presence of DGC is an important and perhaps necessary component of the neural network underlying generation of WDS during LEC kindling.

104.5 EVIDENCE IMPLICATING DENTATE GRANULE CELLS IN LATERAL ENTORHINAL CORTEX KINDLING. J.L.Giacchino, D.P. Frush*, and J.O. McNamara. Depts. of Medicine and Pharmacology, Duke University Medical Center, Durham, NC 27710.

Kindling is a phenomenon in which periodic administration of initially subconvulsive electrical stimuli to a brain structure leads to limbic and clonic motor seizures. Identification of the network of brain structures containing the alterations responsible for kindling would simplify elucidation of underlying mechanisms. We postulated that the hippocampal formation (HPF) constitutes part of the network underlying kindling from lateral entorhinal cortex (LEC) for three reasons: (1) the HPF, in particular the dentate granule cells (DGC), receive a major projection from the LEC, (2) the DGC are the site of at least three molecular alterations in kindling, and (3) the HPF of LEC kindled animals exhibits intrinsic abnormal excitability which could predispose the animal towards a seizure (King, G. et al. this volume). To test this postulate we examined the effect of destruction of DGC (by the quite selective neurotoxin colchicine) on the development of LEC kindled seizures.

Male S-D rats were implanted with a bipolar stimulating electrode electrophysiologically localized to the LEC by stimulations evoking a monosynaptic population excitatory post-synaptic potential recorded via a dentate hilar microelectrode. Animals received intradentate injections of either artificial CSF (IDaCSF) or colchicine dissolved in aCSF (IDc). A third group received injection of colchicine into the frontal cortex (FCc). After a two week recovery period, the afterdischarge threshold (ADT) was determined and periodic stimulations continued until kindling was complete. Histologic examination revealed extensive DGC destruction in IDc but not IDaCSF or FCc animals. IDc animals exhibited a significant elevation in ADT (n=10, 1750 $\pm$ 106 A) when compared to IDaCSF animals (n=12, 1350 $\pm$ 142 A; $p<.012$). IDc animals also required a greater number of stimulations to kindle (28.9 $\pm$ 3.2) when compared to IDaCSF animals (21.4 $\pm$ 1.6; $p<.012$). FCc animals did not differ significantly from IDaCSF animals.

We suspect that the effect of colchicine is mediated through DGC destruction. If correct, the data suggest that: (1) the presence of DGC is an important determinant of ADT in LEC kindling, (2) the integrity of the DGC is an important component of the neural network underlying kindling from the LEC, and (3) since kindling can be established (although less efficiently) in the absence of DGC, these are not necessary for establishment of LEC kindled seizures.

104.6 AGE-RELATED DIFFERENCES IN KINDLED SEIZURE DEVELOPMENT AND DENTATE GYRUS BENZODIAZEPINE RECEPTORS. R. J. Fanelli and J. O. McNamara. Depts. of Med. and Pharm., Duke Univ., and Epilepsy Center, VA Med. Center, Durham, NC 27705.

Previous work from our laboratory demonstrated that kindled seizures cause increased numbers of benzodiazepine (BZ) receptors on granule cells (DGC) in the dentate gyrus (DG) of the hippocampal formation (HF). We postulated that this molecular alteration underlies an endogenous protective mechanism designed to stabilize neuronal excitability and suppress kindling development. Two observations support this hypothesis: 1) removal of DGCs by intradentate colchicine is associated with a reduced rate of entorhinal kindling (see Frush et al. this volume), and 2) there is enhanced inhibition of DGCs in HF slices from kindled animals (see King et al. this volume). To further test this idea, we compared BZ receptors in animals kindling at a normal rate [young-adult (YA), 3 months old] with animals kindling at a slower rate [middle-aged (MA), 12 months old].

Male Sprague-Dawley rats of two age groups (YA and MA) received kindling stimulations twice daily in the right entorhinal cortex. Control animals underwent electrode implantation but were not stimulated. Twenty-four hours after an animal exhibited its third class 5 seizure, it was sacrificed along with an age-matched control, and the brains were prepared for biochemical assay. BZ receptor binding was measured under equilibrium conditions using saturating concentrations of [^{3}H]flunitrazepam with membranes prepared from microdissected HF [DG, regio superior (RS), and regio inferior (RI)].

Establishment of kindling required 30% more stimulations ($p< .05$) in MA (60 $\pm$ 4) than in YA (46 $\pm$ 3) animals. Kindled seizures resulted in an equivalent increase (23% more, $p< .05$) in BZ receptors in the DG of both the YA and MA rats. There was, however, a difference between the unstimulated rats of the YA and MA groups in BZ binding. The MA rats had significantly greater numbers of BZ binding sites (43% more, $p< .01$) in the DG (but not in RS or RI) than did YA rats.

Our results confirm previous observations (Welsh and Gold, 1983) that the rate of kindling diminishes with age. The reduced rate of kindling development in MA animals cannot be accounted for by differences in seizure-induced alterations of BZ receptor numbers in the DG. The increased benzodiazepine receptor binding in the dentate gyrus of the middle-aged animals may be one factor contributing to the reduced rate of kindling development in these animals.

104.7 KINDLING OF SEIZURES IN CAUDATE OR GLOBUS PALLIDUS: COMPARISON TO LIMBIC KINDLING. F. J. Madryga*, J. Plant*, and M. E. Corcoran. Department of Psychology, University of Victoria, Victoria, British Columbia, Canada, V8W 2Y2.

Although there is evidence that repeated stimulation of the caudate nucleus or globus pallidus (GP) can kindle seizures, kindling in these areas has typically involved few electrode placements or has been done to test the effects of other treatments. We therefore decided to reexamine extrapyramidal kindling and to investigate transfer of seizure susceptibility between these areas and amygdala.

Male hooded rats received implantation of chronic electrodes in amygdala and either caudate or GP of the same hemisphere. Half of the rats were kindled first with extrapyramidal stimulation and subsequently, after one week of rest, with amygdaloid stimulation; and the order was reversed for the remaining rats. Histological verification of electrode placements was obtained at the completion of testing.

Thresholds for afterdischarge were quite variable in caudate and GP ($\bar{x}$ = 252 μA; SEM, $\pm$74), but were significantly higher than in amygdala ($\bar{x}$ = 52 μA; SEM, $\pm$24). The seizures that occurred during kindling of the caudate or GP were immediate in onset, of short duration, and tonic-clonic or clonic-tonic-clonic in form, much like seizures induced by stimulation of anterior neocortex, but quite unlike amygdaloid kindled seizures. Extrapyramidal kindling also involved periodic regressions, in which usually effective intensities of stimulation occasionally evoked "larval" seizures or no ictal manifestations whatsoever. Recovery from periods of regression was variable and unpredictable, as in neocortical kindling. In some rats repeated stimulation of extrapyramidal sites also resulted in the later development of "limbic-type" generalized seizures, similar to the late generalization occurring with anterior neocortical kindling.

Preliminary tests of transfer between extrapyramidal sites and amygdala indicated that only partial savings in kindling rate (less than complete transfer) occurred, regardless of which site was kindled first. A possible exception to the partial transfer occurred when amygdala was kindled in rats that had been prekindled in the far anterior caudate. In these rats fully generalized seizures could be triggered by the first amygdaloid stimulation.

It thus appears that the neural substrates of limbic and extrapyramidal kindling are partially independent of each other. (Supported by grants from NSERC and MRC)

104.8 EXCITATORY AND INHIBITORY SYNAPTIC MODULATION AND DENTATE GRANULE CELL EXCITABILITY ASSOCIATED WITH PERFORANT PATH KINDLING. E. Maru* and G.V. Goddard. Department of Psychology, University of Otago, New Zealand.

The purpose of the present experiment was to assess the changes in (i) transmission efficacy at the perforant path-dentate granule cell synapses (ii) granule cell excitability and (iii) hippocampal commissural inhibition of the granule cells that accompany and follow perforant path kindling.

Cross-bred Wistar/Sprague-Dawley albino rats were kindled by 2 sec 100 Hz electrical stimulation once each day for three weeks through an electrode implanted in the perforant path fiber bundle. The dentate field potentials evoked by a series of different strength test pulses of perforant path stimulation were recorded once every 3 days for four weeks before kindling, throughout kindling and one month after kindling.

Seven animals with good stable evoked potential developed bilateral clonic behavioral convulsion within the three weeks of kindling stimulation. The population EPSPs evoked at the perforant path-granule cell synapse, regressed on the stimulus strength values, had a slope of approximately 0.5 before kindling, 0.6 after the first one or two kindling trials, 0.7 at the end of kindling, and 0.8 at two weeks after the last convulsion, decaying to 0.7 by one month after kindling. Equivalent values in six non-kindled control animals remained between 0.5 and 0.4 throughout the three month period. In contrast, the excitability of the dentate granule cells (ratio of population spike to population EPSP at the various stimulus pulse intensities) gradually but strikingly decreased as kindling progressed, and gradually recovered (to 85% of the undepressed level) over the four weeks after cessation of the kindling treatment.

The ability of prior commissural activation to inhibit the granule cell population spike was also monitored throughout the study. It showed progressive potentiation during kindling, and a gradual decrease when kindling ceased.

A single re-kindling trial after the one month rest provoked an immediate seizure and re-established the maximum EPSP and reduced excitability.

Thus, kindling in this area of the brain is associated with a very long lasting increase in EPSP, a less long lasting decrease in excitability, and a temporary elevation of inhibition.

104.9 RECOVERY OF GABA RECURRENT INHIBITION IN DENTATE GYRUS AFTER ENTORHINAL KINDLING IN RATS. T.L. Babb, W.J. Brown, J. Pretorius and W. Kupfer. Dept. of Neurology, Div. of Neuropathology and the Brain Res. Inst., UCLA Sch. of Med., Los Angeles, CA 90024.

The hippocampus is known to have widespread recurrent inhibition that is GABA-dependent and detectable anatomically by glutamic acid decarboxylase (GAD) immunocytochemistry. Several studies have suggested that hippocampal seizures may result from a chronic loss of this GABA-mediated recurrent inhibition. For example, our initial studies demonstrated that either immediately or 24 hours after 19 kindling stimulations to entorhinal cortex, GAD levels in terminals surrounding dentate granule cells were significantly reduced compared to unkindled rats that received 19 massed stimulations (Babb et al., Reduction of GAD-positive cells and terminals after entorhinal-dentate kindling in rats. Neuroscience Abstracts, 1983, p.489).

The present experiments were designed to test the time course of this reduction in GABA synthesis. Rats received 19 stimulations to entorhinal cortex and exhibited Stage 4 or 5 seizures for the last three days. Six rats were sacrificed after 48 hours delay, six rats were sacrificed after a one week delay and each group was compared to non-stimulated controls. There were no differences in GAD-positive terminal staining between the groups as detected by quantitative densitometry of the ipsilateral stratum granulosum, the site of recurrent inhibitory axons. These findings suggest that although daily hippocampal seizures may suppress the rate of GAD synthesis, the GAD enzyme recovers to normal presynaptic levels within 48 hours even though the seizure-sensitivity persists.

104.10 ACCELERATION OF AMYGDALOID KINDLING BY NOREPINEPHRINE DEPLETION USING DSP-4. G.P.Carre and C.W.Harley. Dept. of Psychology, Memorial University of Nfld.,St. John's,Nfld.,Canada A1B 3X9.

Depletion of norepinephrine (NE) in the CNS by 6-hydroxydopamine administration intraventricularly or into the dorsal noradrenergic bundle has been reported to accelerate kindling development in the amygdala and other forebrain structures. In the present study we assess the effects of another noradrenergic neurotoxin on kindling development. DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride) has been reported to be selective on locus coeruleus terminals following pretreatment with a 5-hydroxytryptamine (5-HT) uptake inhibitor.

Twelve adult male Sprague Dawley rats were pretreated with CGP 6085A (2.7 mg/kg ip), a 5-HT uptake inhibitor. Thirty minutes later a fresh preparation of DSP-4 (63 mg/kg ip) or a saline vehicle was injected. Two weeks following drug treatment bipolar kindling electrodes were implanted into the right basolateral amygdala of all rats. Following postoperative recovery afterdischarge (AD) thresholds were determined using a 1 sec 60 Hz sine wave stimulus with the current initially set at 10 uA and raised at one minute intervals in 10 uA increments until an AD was obtained. Kindling proceeded the next day using a 200 uA current every 24 hrs until a stage 5 motor seizure was obtained.

AD thresholds did not differ between DSP-4 treated animals (n=6) and controls (n=6). The number of days required to produce stage 5 motor seizures was significantly fewer in DSP-4 treated animals (mean=3.8) than controls (mean=7.5,$p<.05$). The behavioral duration of the stage 5 seizure was longer for DSP-4 animals than controls due to a significantly longer time spent by DSP-4 animals (mean=29.2 sec) than controls (mean=4 sec,$p<.01$) in preclonus behavior. The extended period of preclonus behavior in DSP-4 animals may be an obligatory feature of the extremely rapid appearance of the stage 5 level, or a consequence of NE depletion beyond forebrain sites (Corcoran,Kindling II,87-104,1981).

Conclusions drawn by earlier studies that NE attenuates kindling development is supported by the facilitatory actions of DSP-4 treatment. The lack of effect on kindling threshold levels suggest again that NE's role is related to the slowing of recruitment or propagation from the AD site.

104.11 HIPPOCAMPAL SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN AMYGDALOID KINDLED AND NON-KINDLED RATS. R.S. Greenwood, J.H. Gilmore,Jr.*and K. Winstead*. Dept. of Neurol and Neurobiology Prg., UNC Sch. of Med., Chapel Hill, NC 27514.

Somatostatin-like (SRIF-L) immunoreactivity is widely distributed in the central nervous system. SRIF may function as a neurotransmitter or neuromodulator. SRIF may also play a role in certain types of seizures. Intracerebroventricular injections of somatostatin can induce generalized seizures and EEG epileptiform activity. Amygdaloid kindling has been shown to be associated with an increase in SRIF content in several brain areas (Kato et al, 1983). Amygdaloid kindled seizures are suppressed by cysteamine, a drug which reduces brain SRIF levels. The hippocampus is prominently involved in the spread of after-discharge in amygdaloid kindled seizures. SRIF-L immunoreactivity has a very well defined and limited distribution in the hippocampus. We therefore, immunocytochemically compared hippocampal somatostatin immunoreactivity in kindled and non-kindled rats.

Rats were implanted with bipolar amygdala stimulating electrodes and were stimulated twice daily with biphasic pulse trains (400 uA peak-to-peak, 60 cps, 1 sec train). Control rats were matched for age and strain and were either unimplanted or were implanted with electrodes but were not kindled. At the completion of kindling, experimental animals were matched with a control animal. Paired sections (30 um thickness) from experimental and control rats were immunocyochemically processed together to reduce technical variations and were compared for hippocampal somatostatin immunoreactivity. Cell counts were made in the dentate, regio inferior, regio superior, and the subiculum.

No differences were noted in distribution of SRIF-L immunoreactive cells or fibers. Cell counts revealed a greater number of dentate SRIF immunoreactive cells in kindled rats than in control rats but this was not statistically significant. In all other regions cell counts were not significantly different in kindled and non-kindled rats.

We conclude that there is a small but insignificant increase in SRIF-L immunoreactive cells in the dentate of kindled rats but no other qualitative or quantitative changes. Therefore, propagation of seizures through the hippocampus is not associated with any immunocytochemical changes in hippocampal SRIF-L immunoreactivity.

104.12 RELATIONSHIP OF AFTERDISCHARGES (AD's) TO CHANGES IN INHIBITION AND LEVELS OF CALCIUM-BINDING PROTEIN (CaBP) IN THE HIPPOCAMPAL FORMATION DURING KINDLING-INDUCED EPILEPSY. M.W. Oliver*, I. Mody, K.G. Baimbridge and J.J. Miller, Dept. of Physiology, University of B.C., Vancouver, B.C., Canada, V6T 1W5.

Previous studies from our laboratory have shown that kindling-induced epilepsy is characterized by specific alterations in the dentate gyrus of the rat. Biochemical changes include the loss of a presumed intraneuronal Ca^{2+}-buffer, CaBP, immunohistochemically localized to granule cells. Electrophysiological alterations consist of an enhancement of a Cl^--independent inhibition as determined by paired-pulse stimulation of the perforant path. Since AD's are evoked in the dentate gyrus without convulsions during the process of kindling, the purpose of the present study was to determine whether the development of these biochemical and electrophysiological changes was related to the number of AD's elicited by kindling stimuli.

Male Wistar rats were kindled (100 μA/60Hz/1 sec-daily) through electrodes implanted in the hippocampal commissures and AD's were monitored from the dentate gyrus. Levels of hippocampal CaBP were determined on animals exhibiting 0, 5, 10, 20+ AD's and full motor seizures using standard RIA. Paired-pulse inhibition in the dentate gyrus was assessed on in vitro slices prepared from hippocampi not used for RIAs. The following table shows the relationship between number of AD's, hippocampal CaBP levels and the % change in amplitude of perforant path evoked granule cell population spike response following a conditioning pulse (80 msec (C-T).

MEAN ± SEM	CON	0	5	10	20+	SEIZ
CaBP(ng/mgTSP)	962±40	936±36	907±29	786±43*	635±31*	653±31*
(# hippocampi)	(8)	(12)	(14)	(11)	(18)	(11)
% cond. resp. @80 msec C-T	137±10	105±8*	71±9*	58±7*	N.A.	67±6*
(# of slices)	(12)	(10)	(12)	(10)		(12)

*-denotes significant change from control (CON)

The results indicate a progressive decline in hippocampal CaBP levels and an enhancement of the Cl^--independent inhibition related to the number of AD's. These changes may reflect a common underlying mechanism dependent on alterations in intraneuronal Ca^{2+} regulation.

104.13 Spontaneous Interictal Spiking in the Aged Kindled Rat. K.A. Welsh, K.A. Stokes, and P.E. Gold. Dept of Psychology, University of Virginia, Charlottesville, Va.

The development of kindled seizures is much slower in aged rats than in their younger counterparts. One index of abnormal neural excitability in the kindled focus is the appearance of spontaneous interictal spikes (SIS). It has been suggested that SIS represents an inhibitory mechanism which reduces the likelihood of seizure occurrence (Engel & Ackermann, 1979). The goal of the present study was to explore the relationship between the frequency of SIS and the development of kindled seizures in young and aged rats.

Bipolar electrodes were implanted into the basolateral amygdalae in both 3- and 12- month old Fischer F-344 rats. One week after surgery, the kindling trials were begun (unilateral, monophasic square waves, 60 Hz, 250 uA, 1 sec, 1 stim/day). Trials were continued for 30 days until each animal had demonstrated at least 3 stage 5 seizures. The frequency of SIS was assessed daily in each animal (Ns=8) during the 60 sec preceding the stimulation trial and during the 2 min immediately following the afterdischarge activity. An SIS was defined as a sharp transient with a total duration of less than 80 msec and an amplitude twice the background.

Our results indicate very different patterns in the frequency of interictal spiking between young and old rats. Older animals demonstrated enhanced SIS during the prestimulation period as well as enhanced firing during the 2nd minute of the poststimulation period. This increase in SIS was confined to the seizure focus; no age-related difference in SIS was seen in the contralateral site. The differences in prestimulation SIS persisted throughout the development of kindling; no relationship to behavioral stage was apparent. In contrast, poststimulation differences were related to the behavioral progress of the kindled seizures. Poststimulation SIS typically increased throughout the progression of kindling in both groups and reached a maximum just prior to the completion of kindling (Stg 3 or 4). Once Stg 5 convulsions had been demonstrated, no age-related differences in poststimulation SIS were apparent.

Based on these results, we suggest that tonic inhibitory processes, represented here by the SIS, are elicited in the seizure focus of aged animals and act to restrict the spread of abnormal excitation to other anatomically related sites.

104.14 CHRONIC EPILEPTOGENESIS INDUCED BY KINDLING IN THE HIPPOCAMPAL FORMATION: THE ROLE OF THE DENTATE GYRUS. C. Harrison*, T. Sutula, O. Steward. (SPON: R. Daly), Depts. of Neurosurgery, Neurology, Physiology, University of Virginia, Charlottesville, VA 22908

The hippocampal formation(HF) is unusually susceptible to seizures. Kindling in the HF has been studied as a model of chronic epileptogenesis, and its cellular mechanism is of interest.

The perforant path from entorhinal cortex(EC) to dentate gyrus(DG) is a major input to the trisynatpic circuit of the HF. As the EC has connections with neocortex, thalamus, limbic system, and midbrain, the DG can be viewed as a "gate" for input to the hippocampus, a critical site for development of epileptogenesis. Increases in synaptic efficacy in the DG might be expected to enhance epileptogenesis.

Long term potentiation(LTP) is an increase in synaptic efficacy, and has been proposed as a possible mechanism of kindling. To better define the relationship of LTP in the DG to kindling in the HF, we investigated the role of DG in a) the development and b) the maintenance of kindling. If events in the DG, such as LTP, are critical for development of kindling, one would predict that ablation of DG prior to stimulation would impede kindling. If the DG was necessary for maintenance of the kindled seizures, ablation of DG after kindling would abolish the seizures.

Electrodes were implanted in the EC of rats, which received daily stimulation to induce kindling. Afterdischarge (AD) in the EC was recorded via the stimulating electrodes. If the DG was destroyed by colchicine prior to stimulation, an average of 15.48 stimuli were required to induce AD, compared to 2.85 stimuli for controls ($p < 0.001$). Once AD was induced, there was no difference in the number of AD's required to induce a class V seizure. Animals in which the DG was ablated after kindling continued to have class V seizures.

The DG appears to facilitate development of AD and kindling in the HF. After ablation of the DG, the development of AD in the EC is delayed. The DG could be a relay for reentrant circuits in the HF, or might synchronize input from the EC into hippocampal neurons which might then relay back to the EC, thereby increasing the likelihood of synchronous pyramidal activity. After kindling is fully developed, the DG is unnecessary for expression of the seizures. Events in the DG have a role in the development, but not the maintenance of chronic epileptogenesis in the HF.

104.15 ALTERED (Na,K) ATPase ACTIVITY IN KINDLED RATS. D.R. Fowler* and T.J. Hoeppner, Dept. of Physiology and Neurology, Rush Medical College, Chicago, Il. 60612.

The enzyme (Na,K) ATPase functions as an electrogenic pump and is in part responsible for stabilizing neuronal membrane potential so that alteration in the enzyme could affect the excitability of neurons. The experiments evaluated (Na,K) ATPase activity in (4) brain areas of interest (caudate, amygdala, hippocampus, frontal cortex) in controls, partially kindled animals, and in fully kindled animals. Animals were sacrificed during the seizure or at 24 hours. Some animals were given a 6 week stimulus free period before the sacrifice to determine the permanence of any enzyme changes. The enzyme activity was analyzed as a partially purified (SDS solubilized) homogenate (15-50 mg) of each brain region. Enzyme activity was measured by a modified Technicon Autoanalyzer designed to measure released Pi. Results: All areas kindled to Stage 5 were found to have significantly decreased enzyme activity in the area stimulated. The amygdala and, to a lesser extent, the hippocampus showed a significant enzyme decrease regardless of site kindled. When the amygdala or hippocampus was kindled, the caudate and cortex remained unchanged. This pattern of decrease in enzyme function was found at immediate sacrifice, 24 hours, 3 weeks, and at 6 weeks post kindling. Interestingly, the partially kindled animals (Stage 3) showed a pattern of increased enzyme activity at each area stimulated if measured by immediate sacrifice. If measured at 24 hours post seizure, the enzyme was again at baseline control.

The pump is dependent upon glucose for energy and its activity accounts for the majority of glucose utilized by neurons. Ouabain, an inhibitor of the pump, causes seizures when injected intraventricularly and a decrease in glucose utilization would be expected on injection. Glucose utilization during ouabain induced seizures was examined with 2 DG. Glucose utilization in tissue surrounding the injection site markedly increased during the seizure. This suggests that pump activation or other metabolic activity subsequent to inhibition is detected by the 2 DG technique and the initial precipitating events are missed.

104.16 FOREBRAIN COMMISSUROTOMY AND DORSAL HIPPOCAMPAL KINDLING IN RATS. Dan C. McIntyre, Gene N. Stuckey*, and Qadeer Ahmad*. Dept. of Psychology, Carleton Univ., Ottawa, Ont., Canada, K1S 5B6.

For over 40 years forebrain commissurotomy has been used in man to ameliorate intractable seizures. Recently the kindling model of complex partial seizures has been utilized to further study seizure genesis in commissuorotmized animals. The amygdaloid complex was chosen exclusively as the focus in those investigations. It was reported that forebrain bisection facilitated the kindling rate at the primary site, and had little effect on the positive transfer to kindling at the secondary site in the contralateral amygdala.

We examined the generality of these amygdala results in the slow kindling dorsal hippocampus of rats. Daily stimulation with 2 s of 100 μA (peak-to-peak) square waves, pulsed at 60 Hz, did not facilitate the primary site kindling rate in rats with total forebrain bisection, compared to sham controls, and corpus callosal or midbrain sectioned groups. Subsequent kindling of the secondary site in the contralateral hippocampus produced strong positive transfer in all groups except the total splits, which showed kindling rates similar to their primary site scores. These data suggest that kindling in intact rats either establishes a strong epileptic predisposition in the contralateral hippocampus during primary site kindling, or that the secondary site has considerable commissural access to the primary site, which is not available to the total split rats. These two hypotheses are discriminable by sectioning animals after primary site kindling. Such a test is currently in progress.

Except for lateralized afterdischarges and motor seizures in the total splits, and only lateralized motor seizure in the callosal splits, all other seizure characteristics (latencies to onset and durations) were similar between the groups. This supports the suggestion that the driving mechanism for kindled seizures is located in the kindled hemisphere and, initially, only passively involves the contralateral hemisphere in commissurally-intact animals.

Since total forebrain commissurotomy did not facilitate primary site kindling, yet completely blocked secondary site transfer, the interhemispheric mechanisms of hippocampal and amygdala kindling may well be very different.

104.17 EFFECT OF RAISING NORADRENERGIC ACTIVITY ON THE FULLY DEVELOPED AMYGDALA-KINDLED SEIZURE. M.M. Okazaki and W.M. Burnham. Dept. of Pharmacology, Duke Univ. Med. Ctr., Durham, N.C. 27710 and Dept. of Pharmacology, Univ. of Toronto, Toronto, Canada M5S 1A8.

The effect of raising noradrenergic activity in amygdala-kindled rats was examined. 22 Male Royal Victoria hooded rats were implanted with bipolar stainless steel electrodes in the right amygdala. Following a one-week, post-surgical recovery period, all rats were "kindled" using a one-second train of biphasic, 60 Hz, 1 msec pulses with peak-to-peak intensity of 400 uA. Stimulation was administered twice daily (minimum time interval between stimulations: 6.5 hrs) until a criterion of 20 Stage "3-5" seizures was reached. When this criterion had been achieved, each subject's threshold was determined on a 24-hour schedule, using the "half-split" method. The subjects were subsequently stimulated at 40% above their generalized seizure (GS) threshold until stable GS responses had been elicited. Drug tests consisted of the i.p. injection of a test drug (or vehicle) 30 minutes prior to administration of the kindling stimulus, on a 48-hour schedule. Four doses of each drug were administered in randomized order. To evaluate drug effects on kindled seizures, two components of the seizures were scored quantally: the generalized motor seizure (Stage "4-5") and the focal electrographic seizure. Five drugs were tested, all of which are reported to elevate noradrenergic activity: d-amphetamine sulfate (0.2-7.5 mg/kg), methylphenidate HCl (1-20 mg/kg), LY139603 (0.5-10 mg/kg), tolazoline HCl (1-40 mg/kg) and yohimbine HCl (0.5-10 mg/kg).

None of the drugs tested suppressed the focal afterdischarge at any of the doses tested. The generalized motor seizure was not suppressed by any of the drug doses tested, with the exception of the highest dose of d-amphetamine, methylphenidate and tolazoline. The generalized seizure was suppressed in only 10% of the subjects at these toxic doses.

The present study utilized five drugs with a common mechanism of action (increasing noradrenergic activity). Since none of the drugs, at any of the doses tested, significantly suppressed the generalized motor seizure or the focal electrographic afterdischarge, it suggests that raising noradrenergic activity does not affect the expression of the fully developed amygdala-kindled seizure. These results do not appear to support the hypothesis that noradrenaline is involved in the persistence of the kindled state.

104.18 REVERSIBLE SUPPRESSION OF AMYGDALOID KINDLED CONVULSION BY THE INJECTION OF GABACULINE INTO SUBSTANTIA INNOMINATA IN RATS AND CATS. M.Okamoto, M.Sato, K.Morita* and T.Ogawa* Dep. of Neuropsychiatry, Okayama University Medical School, Okayama, Japan 700.

Although anticonvulsant action of central GABAergic systems have been indicated by lines of clinical and experimental evidence, their role in amygdaloid(AM) kindling is still obscure. This might be due to regional difference in GABA-mediated neuronal inhibition in various brain areas. In recent reports, it has been suggested that cholinergic neurons in substantia innominata(SI) might have critical role for the development of AM kindled convulsion. Based on this assumption, we examined the effects of intracerebral injection of gabaculine(G), a inhibitors of GABA-transaminase, into SI on AM kindled convulsion.

10 male cats and 5 male hooded rats were used for electroclinical evaluation of anticonvulsive effects. Chronic combined cannula-electrode was implanted into left SI and bipolar or tripolar electrodes were implanted into bilateral AM under pentobarbital anesthesia. All animals were kindled at AM by daily electrical stimulations until a stable generalized convulsion was elicited for more than 5 successive days. Following the completion of kindling, G was injected into left SI through cannula-electrode and left AM was stimulated at varying intervals following the injection. G was dissolved in distilled water and buffered to pH7.2 by sodium bicarbonate. 13.8µl/3µl was used in cats and 19.2µl /3µl was used in rats.

AM kindled generalized convulsion was completely suppressed during 48 to 108 hours after the injection of G in all rats and a half of cats. In another half cats, the latency for seizure generalization was prolonged during this period. In addition, the latency for the onset of forelimb clonus was prolonged prior to the complete disaappearance of motor convulsion in all rats.

These results indicate that SI plays a critical role in gaining access from AM to motor systems, and that the pharmacological enhancement of GABAergic transmission in this brain area inhibit this functional connection.

The result of intra-AM injection of G and measurement of regional GABA concentration after the injection of G into AM and SI will also be presented.

104.19 RAIN REGIONAL LEVELS OF N- ACETYLASPARTYLGLUTAMATE (NAAG):THE EFFECT OF KINDLED SEIZURES J.L. Meyerhoff, K.J. Koller, D.W. Walczak* and J.T. Coyle. Dept. of Medical Neuroscience, Div. of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, D.C. 20307 and Dept. of Psychiatry, Div. of Child Psychiatry, Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205

N-Acetylaspartylglutamate (NAAG), a dipeptide specific to the central nervous system, has been shown to cause prolonged seizure activity when injected into the hippocampus. The seizures resembled those seen following infusion of the glutamate agonist quisqualic acid. NAAG has also exhibited a higher affinity than quisqualic acid for glutamate receptors in brain tissue. In the present study, we have examined the effect of a series of stage 5 amygdalar-kindled seizures on brain regional levels of NAAG.

Male Sprague-Dawley rats were kindled from bipolar depth electrodes implanted in the left amygdala (-2.2 mm AP, +4.7 mm LAT, -8.5mm DOWN, relative to bregma, using the atlas of Paxinos and Cartwright). The stimulus parameters employed were: 200 microamperes base-to-peak, 60 Hz, 1.0 msec pulse duration and 1.0 sec train duration. Stimulation was applied once every 24 hours until a criterion of five successive stage 5 seizures was achieved. Sham-operated rats were implanted, but never stimulated. A control group was subjected to daily handling, but never operated. Rats were sacrificed by decapitation 48 hours after their last kindled seizure. The brains were removed and immediately dissected on ice. The regional samples were weighed, immediately frozen on dry ice and stored at -70° C until assayed. Samples were homogenized, extracted and analyzed by high-pressure liquid chromatography for NAAG and N-acetylaspartate (NAA), as well as glutamic and aspartic acids.

Regional levels of NAAG (nmol/mg protein) in control rats were: entorhinal cortex (3.12 ± 0.37), pyriform cortex (2.67 ± 0.41), hippocampus (4.08 ± 0.10), amygdala(6.01 ± 1.45), substantia nigra (9.08 ± 0.77) and spinal cord (38.36 ± 1.83). In a preliminary study in kindled rats NAAG levels were significantly elevated in entorhinal cortex (4.88 ± 0.45, $p<0.05$), but not in the other regions studied. No changes were seen in levels of NAA, glutamic or aspartic acids. Further studies are in progress to explore the potential involvement of NAAG in the development of kindled seizures.

104.20 DAILY PRETREATMENT WITH A COMBINATION OF ATROPINE AND MECAMYLAMINE INCREASES KINDLING LATENCY MORE EFFECTIVELY THAN PRETREATMENT WITH EITHER DRUG ALONE. D.D. Walczak*, T.J. Lynch*, V.E. BATES , and J.L. Meyerhoff.(SPON: F.J. Manning) Dept. of Medical Neurosciences, Div. of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, D.C., 20307.

Male Sprague-Dawley rats were given one of the following 30 minutes prior to receiving daily kindling stimuli: saline = SAL, Atropine (20 mg/kg) = ATRO, Mecamylamine (10 mg/kg) = MECA, or Atropine + Mecamylamine (20 + 10 mg/kg,) = COMBO. All injections given i.p. Rats were kindled from a bipolar depth electrode implanted in the left amygdala (-2.2 mm AP,+4.7 mm LAT,-8.5 mm DOWN, relative to bregma), using a standard stimulus: 200 µA base-peak, 60 Hz, 1.0 msec pulse and 1.0 sec train duration. Interstimulus interval = 24 hours. Motor convulsions were ranked using the rating scale of Racine. Kindling latency was defined as the number of stimulations required to reach the first of three consecutive stage 5 convulsions. The study ended at day 20, and rats not achieving criteria were assigned a maximum latency score of 20.

ATRO and MECA groups showed lower average seizure severity during kindling, but changes were not significantly different from SAL at $p \lesssim 0.05$. The COMBO treatment was very effective in reducing seizure severity during kindling. Kindling latency was significantly increased in the COMBO group, but not in ATRO or MECA. Mean kindling latencies were (days ± S.E.M.): SAL = 9.2 ± 0.9, ATRO = 11.4 ± 0.9, MECA = 11.9 ± 1.1, and COMBO = 15.9 ± 1.6. Eight out of 13 subjects in the COMBO group failed to exhibit fully generalized seizures at day 20. Increases in kindling latency reported here are significantly different for COMBO groups in the following comparisons: COMBO vs. SAL, $p \lesssim .0001$, COMBO vs. ATRO, $p \lesssim .009$, COMBO vs. MEC, $p \lesssim .019$. No other comparisons were significant at $p \lesssim .05$.

Atropine has been reported to significantly retard kindling in rats when given in higher doses. Muscarinic receptors exhibit transient downregulation following fully-generalized kindled seizures. Data presented here suggest that combined blockade of muscarinic and nicotinic receptors in the CNS is more effective in retarding kindling than blockade of either receptor class alone, and that both nicotinic and muscarinic cholinergic function may be required for seizure generalization during kindling. Further studies are underway to determine the actual kindling latency in COMBO groups, pharmacokinetic characteristics of Atropine and Mecamylamine in our rats,and to determine the effects of COMBO against fully-kindled and other types of seizures.

105.1 DENDRITIC ACTION POTENTIALS AND CALCIUM ACTIVATED POTASSIUM CONDUCTANCE IN NEOSTRIATAL NEURONS. E. Galarraga*, J. Bargas*, J. Aceves* (SPON.: H. Arechiga). Depto. de Fisiología y Biofísica. Centro de Investigación y Estudios Avanzados del IPN. Apartado Postal 14-740 07000 México, D.F. MEXICO.

The presence of a Ca-component in action potentials of neostriatal neurons (see accompanying abstract) prompted us to look for: 1) action potentials of dendritic origin, and 2) a Ca-activated K^+ conductance which could determine the firing pattern of these neurons.

In 300 µm thick striatal slices, action potentials (AP's) could be evoked by direct, orthodromic, or antidromic stimulation. The mean resting membrane potentials of the recorded neurons was 68 ± 8 mV (n=50; mean ± S.D.). Orthodromic stimulation elicited either a single or, by increasing the strength of stimulation, a burst of AP's. Single AP's originated from an EPSP, while the burst originated from a plateau potential into which the EPSP merged. The burst discharge occurred when the plateau reached a critical level of depolarization. The repolarization of the plateau potential was significantly slower than that of the EPSP. Either single or repetitive AP's could be seen arising from underlying fast prepotentials (FPP's). When not readily seen, FPP's could be demonstrated by paired shock stimulation, hyperpolarization, or stimulation with a short train of high frequency stimuli. FPP's were only seen by orthodromic stimulation; antidromic or direct stimulation did not evok FPP.s. Antidromic stimulation only very rarely showed "axon hillock potentials".

After potentials of the neostriatal cells did not hyperpolarized the membrane beyond the resting level. The hyperpolarization was with respect to the triggering level. Two after potentials were observed: a fast one, followed by a slow one. By applying depolarizing currents, the neurons fired repetitively. In this condition, the slow potential showed the characteristics of a pacemaker potential (hyperpolarization gradually returning to the firing level). Superfusion with solutions containing Co^{++} (5 mM) or Mn^{++} (3 mM) inhibited the slow after potential and repetitive firing could not be induced (depolarizing currents fired only one AP).

The present results indicate that, as in many other neurons, AP's probably due to Ca^{++} entry originate in dendrites of neostriatal neurons. The results also indicate that the slow after potential is due to a Ca-activated K^+ conductance, which plays a significant role in controlling the firing pattern of these neurons.

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105.2 EFFECTS OF TETRAETHYLAMMONIUM ON ELECTRICAL PROPERTIES OF NEOSTRIATAL NEURONS. J. Bargas*, E. Galarraga*, J. Aceves*. (SPON.: J. García-Ramos). Depto. Fisiología y Biofísica. Centro de Investigación y Estudios Avanzados del I.P.N. Apartado Postal 14-740 07000-México, D.F. MEXICO.

While using tetraethylammonium (TEA) to block voltage-dependent K^+ channels to make evident a Ca^{++} component in action potentials of neostriatal neurons, a clear effect of TEA on the neuronal input resistance (R_N) was observed. This prompted us to study the effect of TEA on the passive electrical constants of neostriatal neurons. Intracellular records ings of the neurons were done in striatal slices of the rat brain.

TEA (10 mM) reversibly changed the passive electrical constants of neostriatal cells. Input resistance (R_N) increased from 29.2 ± 8.1 MΩ (n=14; mean and standard deviation) to 55.7 ± 9.2 MΩ (n=5; $p<0.001$), and membrane time constant (τ_m) from 5.21 ± 1.15 msecs (n=14) to 11.33 ± 2.39 msecs (n=5; $p<0.001$), after 30 minutes of TEA. Neither the equalizing time constant (τ_1) nor the whole neuron capacitance (C_N) were altered by TEA. The values before and after TEA were: τ_1 = 1.03 ± 0.28 msecs and 1.17 ± 0.25 msecs; C_N = 190.8 ± 67.4 pF and 206.34 ± 47.34 pF. As a result of the increase in τ_m without a change in τ_1, the electrotonic length $L = \pi (\tau_m/\tau_1)^{-1/2}$ decreased from 1.57 ± 0.28 (n=13) to 1.09 ± 0.21 (n=5; $p<0.005$). Apparently, TEA did not affect the resting membrane potential.

TTX- resistant action potentials could be evoked in the presence of TEA. These action potentials were blocked by simultaneously lowering the Ca^{++} concentration and adding Co^{++} or Mn^{++}, and were potentiated in amplitude and duration by Ba^{++}, Sr^{++} and high Ca^{++}. When TEA was intracellularly injected (microelectrodes filled with 3M TEA) the potentials appeared faster than when the compound was extracellularly administered.

The results indicate the presence of TEA-sensitive, K^+ channels active at resting membrane potential (RMP) of the neostriatal neurons. The channels might be located at the dendrites because TEA did not affect the somatic RMP. Also, the present results confirm previous findings (Kita et al., 1983, Neuroscience Meet. Abs. No. 278.2) about the presence of voltage-dependent Ca^{++} channels in the somatodendritic membrane of these neurons.

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105.3 EFFECTS OF BACLOFEN ON THE INTRACELLULAR RESPONSE OF CAUDATE NEURONS TO CORTICAL AND THALAMIC STIMULATION IN CAT. James S. Wilson. Department of Anatomy, Howard University, Washington, D.C. 20059.

Baclofen is a structural analogue to γ-aminobutyric acid, GABA, and is used clinically as a muscle relaxant. Experimental evidence suggests that baclofen depresses the release of excitatory amino acids from presynaptic terminals and/or binds postsynaptically to GABA-b receptors. Therefore, we decided to use baclofen to investigate possible mechanisms underlying the inhibitory response recorded in caudate (Cd) neurons. Cats were anesthetized with barbituates and prepared for intracellular recording. Intracellular responses of caudate neurons were studied following electrical stimulation of precruciate cortex and intralaminar thalamus. Typically, stimulation of either cortex or thalamus at 1 Hz evoked an initial excitatory postsynaptic potential (E) followed by a longer duration hyperpolarization and inhibition (I) of spiking activity. The first effects of (-) baclofen were observed 30 seconds after a systemic injection (5 mg/kg, i.v.) and included a hyperpolarization of the cell's membrane and cessation of all spontaneous activity. By one minute, cortical and thalamic stimulation no longer evoked an E and/or I response. The initial E response began to recover by 6 minutes post-injection. If stimulation rates were less than 0.1 Hz, the initial E was often followed by a large amplitude, long duration depolarization which could reach threshold for spike generation. Unlike the E, the I response and spontaneous spiking activity did not demonstrate any recovery 7 hours post-injection. Systemic injections of (+) baclofen at comparable doses failed to have any noticable effect on Cd's physiology thus suggesting stereoselectivity of the drug's action. Dose/response characteristics were studied by making multiple, 1 mg injections of (±) baclofen every minute and measuring the amplitude of the I response. The first indication of an effect was observed after the animal received a total of 4 mg which resulted in a 30% reduction in I amplitude. Spontaneous activity was also reduced at this dose. After the animal received 8 mg, the I response was not observed. These data suggest that (-) baclofen effects inhibitory mechanisms within the Cd nucleus. We thank Ciba-Geigy for baclofen. Supported by NIH grant 2S06-RR08016-13.

105.4 UNILATERAL DOPAMINE DEPLETIONS ATTENUATE THE RESPONSE OF NEOSTRIATAL NEURONS TO AMPHETAMINE IN BOTH HEMISPHERES. A. E. Basse and G. V. Rebec. Dept. Psychol., Indiana Univ., Bloomington, IN 47405

Amphetamine-induced alterations in neostriatal neuronal activity are thought to be mediated primarily by an increased release of dopamine (DA) from axons originating in the ipsilateral substantia nigra. In fact, unilateral lesions of this pathway have been reported to block the amphetamine-induced depression of firing rate in the ipsilateral neostriatum. An accumulating body of evidence, however, suggests that a unilateral change in DA transmission produces a corresponding change in the opposite hemisphere. It is conceivable, therefore, that a unilateral lesion of the nigro-neostriatal pathway alters the amphetamine response in both hemispheres. To test this hypothesis, neostriatal activity was recorded bilaterally from intact sham lesioned animals and from rats pretreated 10 to 15 days earlier with a unilateral 6-hydroxydopamine (6-OHDA) lesion of the substantia nigra. Following isolation of single-unit discharges, both groups were challenged with intravenous injections of 0.2 mg/kg d-amphetamine administered at 2-min intervals.

In line with previous evidence, predrug baseline firing rates were significantly elevated in the lesioned neostriatum when compared to those recorded in sham-lesioned controls. This increase has previously been attributed to a release from dopaminergic inhibition. However, in the lesioned animals, the intact hemisphere exhibited a similarly increased baseline rate despite a greater than 98% difference in DA levels between the two hemispheres. The response to amphetamine challenge was also similar in both the intact and lesioned sides of 6-OHDA treated animals. Whereas increasing cumulative doses of d-amphetamine produced a progressive inhibition of firing rate in the neostriatum of sham controls, this response was significantly attenuated in both sides of rats that sustained a unilateral 6-OHDA lesion. Interestingly, amphetamine-induced excitations were sometimes recorded from both intact and unilaterally-lesioned rats; again, however, a unilateral lesion reduced the response on both sides. These results confirm and extend previous reports that a functional interaction between hemispheric DA systems may help to maintain a balanced output between each neostriatum.

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105.5 6-OHDA LESIONS OF THE IPSILATERAL MEDIAL FOREBRAIN BUNDLE AND ELECTRICAL STIMULATION OF THE IPSILATERAL SUBSTANTIA NIGRA MODIFY GLUCOSE UTILIZATION IN THE DORSOLATERAL CAUDATE NUCLEUS. H.H. Holcomb, M. Kadekaro, P. Gross*, H. Everist, A. Pert, and G.N. Ko. National Institute of Mental Health, Bethesda, MD, 20205 and *Dept. Neurosurgery, SUNY, Stony Brook, N.Y. 11794

Unilateral electrical stimulation of the substantia nigra,pars compacta and pars reticulata, increases local cerebral glucose utilization (LCGU) in the ipsilateral globus pallidus, entopeduncular and subthalamic nuclei; LCGU is reduced in the corresponding lateral habenula. Using a computer enhanced imaging system we have assessed the metabolic effects of nigral stimulation in animals with and without 6-OHDA lesions of the ipsilateral medial forebrain bundle (MFB). Employing the quantitative autoradiographic [^{14}C] 2-D-deoxyglucose method, we measured caudateputamen LCGU in consecutive, contiguous rat brain sections, 20μ thick, from about 3 millimeters of the rostrocaudal axis (A 9650 μ to A 6280 μ; Konig and Klippel, 1967).

Regional analyses of caudateputamen (CP) LCGU rates indicate that the dorsolateral "shoulder" of the CP has consistently higher LCGU rates than other regions of similar size. 6-OHDA pretreatment (3 weeks) reduced LCGU activity throughout the ipsilateral CP over the entire rostrocaudal axis. In unstimulated rats the dorsolateral CP LCGU rates were identical between the electrode implanted and the control side. Nigral stimulation caused a 10 μmole/100g/minute LCGU elevation in a restricted region of the rostrocaudal axis. This elevation was restricted to the CP's dorsolateral boundary. Spatially restricted and modest in magnitude, this localized elevation in CP LCGU is associated with much larger metabolic changes in nuclei receiving output projections from the CP (globus pallidus and entopeduncular nuclei). These disproportionate changes may reflect the amplification function of the caudateputamen in the basal ganglia.

105.6 PLASTICITY OF SUBSTANTIA NIGRA PROJECTIONS AND THEIR RELATIONSHIP TO BEHAVIOR. J.P. Huston, S. Morgan* and H. Steiner* Inst. of Psychology III, Univ. of Düsseldorf, 4000 Düsseldorf, F.R.G.

In a series of experiments we examined the effect of experimentally-induced turning on crossed efferents from the substantia nigra (sn). Initially turning behavior was induced by a central lesion; (a) hemidetelencephalization, (b) injection of either kainic acid or 6-hydroxydopamine into the sn on one side. About 1 week after any of these lesions we found an increase in crossed projections from the sn contralateral to the turning direction to the contralateral thalamus and caudate nucleus. These connections were demonstrated either by the retrograde transport of horseradish peroxidase or fluorescent tracers. The appearance of these connections coincided, in time, with compensation for the lesion induced turning. Prevention of the turning behavior resulted in a suppression of the appearance of these connections and the behavioral compensation. From this result we concluded that the relationship between the appearance of these connections and the behavioral compensation is not fortuitous.

We then looked at whether turning behavior, in the absence of a central lesion, would result in the appearance of these connections. We induced turning behavior by denervation of the fore- and hind limbs on one side. We found that this resulted in turning behavior which decreased over time. The decrease in turning behavior was associated with an increase in crossed nigro-thalamic projections ipsilateral, but not contralateral (as shown by horseradish peroxidase uptake), to the peripheral lesion.

We then investigated whether asymmetrical behavior, in the absence of any lesion, is related to these connections. We induced turning behavior in normal rats, by amphetamine (1 mg/kg). We then looked at the nigro-caudate projections, utilizing the retrograde transport of HRP. Our results showed that there is a relationship between the direction of amphetamine induced turning and the nigro-caudate projections.

105.7 DOPAMINERGIC INVOLVEMENT IN THE PROCESSING OF SENSORY INFORMATION IN STRIATUM: SINGLE UNIT STUDIES. E. Abercrombie* and B.L. Jacobs (SPON: S. Auerbach). Prog. Neurosci., Princeton Univ., Princeton, NJ.

Disruption of the nigrostriatal dopamine (DA) pathway results in a syndrome characterized by inattention to sensory stimuli. In order to clarify the role of DA in the processing of sensory information within the striatum, we have examined the interaction of DA with striatal unit responses to peripheral sensory stimuli. Extracellular single unit responses of striatal neurons to repetitive sciatic nerve stimulation were recorded in urethane-anesthetized adult male Sprague-Dawley rats. Unit responses were obtained in striatum (A: 8.7-9.2, L: 3.0-4.0 and D: 2.5-6.0) using glass micropipettes filled with 2M NaCl. Peri-stimulus time histograms (PSTH) were obtained for the striatal units using 32 consecutive sciatic nerve stimuli consisting of single pulses (0.5 msec, 1.0-5.0 mA) delivered once every 5 sec. Of the 20 neurons studied, 65% showed an excitatory response to sciatic nerve stimulation and 35% showed inhibitory responses. For each cell, a baseline PSTH and a PSTH obtained after pharmacological manipulation of DA were collected. Response magnitude (RM) was quantified by obtaining the ratio of the mean PSTH bin counts for bins comprising the response to the mean pre-stimulus bin count. The absolute value of the difference of this ratio and 1.0 gave the RM. The following manipulations were studied: 0.25 mg/kg i.v. Amphetamine; 0.5 mg/kg i.v. Haloperidol + 0.25 mg/kg i.v. Amphetamine; Intrastriatal 6-OHDA lesion 7-10 days prior to 0.25 mg/kg i.v. Amphetamine; and Saline. None of these manipulations caused a significant change in baseline firing rates. Administration of amphetamine (n=10) caused a significant (61%) decrease in the magnitude of the evoked sensory response while saline control (n=4) had no effect. The reduction in RM produced by amphetamine did not occur in animals pretreated with haloperidol (n=3) or in 6-OHDA animals whose striatal DA had been depleted by an average of 75% (n=3). These results indicate that the effect observed with amphetamine is mediated by DA located in nerve terminals within the striatum. Preliminary data (n=3) show a qualitatively similar effect of amphetamine on unit responses evoked by photic stimulation. Thus, the evoked signal to noise ratio of striatal units to polysensory inputs seems modulated by the nigrostriatal DA system. These data lend support to the notion of striatal gating of sensory information into motor systems and provide evidence for the role of DA in this process. (Supported by NSF Grant BNS 81-19840)

105.8 BEHAVIORAL MODULATION OF STRIATAL UNIT ACTIVITY IN FREELY MOVING RATS. D.J. Woodward, M.O. West, A.J. Michael, J.K. Chapin, Dept. Cell Bio., Univ.Tx.Hlth.Sci.Ctr., Dallas, TX.

The aim of the present investigation was to characterize patterns of neuronal activity in striatum that might prove useful in more extended studies of striatal function. Many studies have shown correlations between basal ganglia function and motor behavior. In addition, striatal unit activity has been shown to be influenced by behavioral context. We therefore examined striatal unit discharge during a simple motor behavior (treadmill locomotion) which requires minimal training and shows consistency across recording sessions and across animals.

Adult hooded rats were prepared for chronic recording by surgical implantation of a microelectrode drive system on the skull overlying striatum (on bregma, 3.5 mm lateral to midline). Stable unit recordings were obtained from presumed medium spiny neurons using commercially available tungsten microelectrodes (5-10 megohm). For several days prior to recording, animals were exposed for 1-3 hours/day to a 60 second treadmill (TM) cycle (30 sec on/ 30 sec off) controlled by computer. Recordings commenced after animals exhibited markedly different behaviors during TM-on (steady, rhythmic locomotion) and TM-off (quiet resting).

Striatal unit activity was analyzed by constructing peri-event histograms around TM-onset. Three distinct phases of unit activity were revealed: 1) an initial burst following TM-onset (latency 30-100 msec, duration 1-3 sec); 2) a discharge sustained for the duration of TM-on; 3) an anticipatory burst preceding TM-onset by 1-3 sec. The anticipatory burst was observed in the absence of any sensory cues and could be seen even when TM-off periods were as long as 30 sec. These responses appeared to occur independently, but all could be observed in a single striatal neuron.

The three phases of neuronal activity were highly sensitive to changes in the frequency and regularity of TM-onset, following habituation to the 60 sec cycle. It is interesting that the sensitivity of these responses was observed despite apparently identical locomotor behavior across different TM cycles. Therefore, the changes in TM cycle may have produced changes in a learned pattern of timing, rather than a change in the motor task. Our view is that these responses will be useful in further studies investigating the interactions of learning and motor behavior in the basal ganglia. (Supported by DA-02338, AA-3901 and the Biological Humanics Foundation.)

105.9 EFFECTS OF LOCOMOTION AND D-AMPHETAMINE ON SPONTANEOUS AND CORTICALLY EVOKED STRIATAL UNIT ACTIVITY IN FREELY MOVING RATS. A.J. Michael, M.O. West, J.K. Chapin, D.J. Woodward, Dept. Cell Bio., Univ. Tx. Health Sci. Ctr., Dallas, TX.

We conducted the present investigation to extend our previous studies on the efficacy of corticostriate synaptic input during locomotor versus motionless behaviors. Adult Long-Evans rats were prepared for chronic recording with a detachable microdrive positioned over the striatum (on bregma, 3.5 mm lateral to midline), facilitating stable recording of presumed medium spiny neurons. Stimulating electrodes were positioned in ipsilateral cortical white matter, and were most effective in evoking responses when located rostral to the recording site. Post-stimulus histograms (PSH) of striatal neuron responses to cortical stimulation (single pulse, 100 usec duration, at 1 Hz) were recorded during alternating epochs of treadmill locomotion and rest. PSHs for single neurons were collected over a range of stimuli (100-3500 uA) that evoked a wide range of responses (0.1-1.2 spikes/stimulus) yet did not evoke any muscular twitch artifacts.

Input/Output (I/O) functions of cortically evoked striatal unit activity were constructed after quantitative analysis of PSHs. Analysis was limited to the short-latency (4-9 msec) evoked responses since previous studies of conduction velocities suggest that these responses are mediated monosynaptically.

The most consistent finding in the present study was that spontaneous unit activity increased during locomotion, as compared with rest. In contrast, I/O functions showed that the efficacy of the corticostriate synaptic input is the same across these behaviors, thereby yielding a net reduction in the ratio of evoked activity to spontaneous rate (signal to noise) during locomotion.

After establishing that these physiologic measures are appropriate for the study of striatal function, we examined the effects of doses of d-Amphetamine sulphate (d-A) (0.5-1.0 mg/kg) that induce locomotion. Preliminary results show that d-A consistently increased spontaneous activity across behavioral states, while its effect on cortically evoked activity is not yet clear. Ongoing studies are aimed at further investigation of the influences of d-A on the efficacy of the corticostriate projection, and examination of interactions among d-A, motor behavior, and basal ganglia function.

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105.10 BASAL GANGLIA INFLUENCES ON CEREBELLAR SENSORY PROCESSING. T.I. Lidsky and *C. Manetto. VA-MD Regional College of Vet. Med., VPI, Blacksburg, VA. Recent work suggests that one way that the basal ganglia (BG) influence movement is by gating sensory inputs into other motor areas. To investigate this suggestion, we are currently assessing BG influences on sensory processing by other motor areas. This abstract describes modulatory influences exerted by the BG on the cerebellum.

Cats were anesthetized with chloralose. Cutaneous stimulation was delivered by electrically stimulating the perioral area via intradermal electrodes. Units were recorded extracellularly from the cerebellar cortex. In this experiment a conditioning-test paradigm (C-T) was used: the effects of conditioning stimulation of the CN on cerebellar unit responses evoked by facial stimulation were assessed.

Purkinje cells were identified by the occurrence of complex spiking. The following effects were observed in Purkinje cells as well as other cerebellar cortical elements (total sample = 94 units). BG stimulation affected sensory processing in 75% of the total unit sample. Inhibitory effects were most prevalent (69%). Facilitation of sensory processing was seen in the remaining 31%. Inhibition was seen at C-T intervals ranging from 10 to 1000 msec.

A variety of procedures were used to control for the possibility that BG stimulation effects were due either to antidromic activation of cortical inputs to the CN and/or to current spread to the internal capsule. Strength-duration curves showed that the chronaxies of the elements that affected the cerebellum were similar to those of CN cells rather than fibers from the cortex or the internal capsule. Direct stimulation of the internal capsule at points adjacent to the CN did not affect the cerebellum in a way similar to CN stimulation. Elimination of CN output via lesions of the globus pallidus and entopeduncular nucleus precluded CN stimulation effects on the cerebellum. Finally, lesioning around the stimulating electrodes in the CN prevented CN stimulation from affecting the cerebellum.

These data show that the BG can affect sensory processing in other motor areas. In view of the cerebellum's role in providing movement-relevant sensory information to motor cortex during closed loop operation, these findings are relevant to BG operations at both cerebellar and also cortical levels. (Support NINCDS grant NS21418).

105.11 THE EFFECTS OF MOVEMENTS ON THE SENSORY RESPONSIVENESS OF BASAL GANGLIA UNITS. C. Manetto* and T. I. Lidsky. (SPON: D. Price). VA-MD Regional College of Vet. Med., VPI, Blacksburg, VA 24061. Previous work suggests that the processing of sensory information by the basal ganglia (BG) differs qualitatively from that of sensory systems. Processing in classic sensory systems is more or less "hard-wired" while in the BG it appears, to some extent, to be dependent on the animal's behavioral set and the motor requirements of the task at hand. The present study examined the effects of various behavioral situations on sensory processing in the BG. Units in the caudate nucleus (CN) were recorded extracellulary in awake cats restrained in a device designed to allow horizontal rotational head movements. It was possible to elicit specific types of head movements by presenting various stimuli to the animals. For example, cats would track stimuli they found pleasurable and move away from stimuli they found aversive. Sensory responses in CN units were evoked by electrical stimulation of the face. The magnitude of CN unit responsiveness to facial electrical stimulation during a variety of head movements was compared with sensory responsiveness in the absence of movement (baseline). Of the CN units that showed baseline sensory responses the majority were attenuated during all head movements. In a minority of cases, CN units showed inhibition during most movements, and interestingly, increased excitation during one or two types of head movements. Units failing to show baseline sensory responses generally remained unaffected by facial stimulation during head movements. However, in a significant proportion of these units, facial stimulation evoked responses during head movement. Strikingly, sensory responses appeared in CN units when perioral inputs were relevant to the specific movement. For example, when animals were required to track a milk spout that moved horizontally across the face, orofacial input became increasingly more important as the animal tried to align its mouth with the milk tube. Facial stimulation occurring during this task frequently resulted in sensory responsiveness. In contrast, sensory responses would disappear when animals moved to avoid olfactory stimuli. Arousal may be ruled out as the causal factor in these results, at least for units in which changes in sensory responses were observed only for certain movements. In conclusion, sensory processing in the BG appears to be modifiable by the motor and behavioral requirements of the situation. (Support NINCDS grant NS21418).

105.12 ACTIVITY OF NEURONS IN THE PUTAMEN DURING ACTIVE AND PASSIVE MOVEMENTS OF THE WRIST IN THE MONKEY. Samuel L. Liles, Dept. of Physiology, Louisiana State University Medical Center, New Orleans, LA. 70119.

The close anatomical relationship of primary motor and somesthetic cortical areas with the putamen (H. Künzle, Brain Res. 88: 195, 1975; E.G. Jones et al., J. Comp. Neurol. 173: 53, 1977; S.L. Liles and B.V. Updyke, Unpublished observations) strongly supports involvement of this structure with regulation of distal movements of the arm. In order to study this problem directly, two monkeys were trained to place their hand in a wedge-shaped manipulandum with digits extended and to actively extend and flex their wrist about 35° in response to visual cues. Opposing torque loads of 0.0-0.20 N-m were present during active movements. Additionally, they were trained to accept (i) passive step displacements of the wrist by the experimenter, which were comparable in amplitude, duration and velocity to active movements, and (ii) brief rapid displacements generated by a pulse of torque (0.1-0.2 N-m, 50-100 ms) applied to the manipulandum by a motor. An extensive electromyographic (EMG) study was performed prior to unit recording to characterize patterns of muscle activity during active and passive movements. All units (N=82) were isolated on the basis of a phasic burst of spikes associated with active movement of the wrist. Most (80%) of these cells showed directionally-specific responses during active movement, and the onset of unit firing lagged the onset of EMG activity in 91% of the sample. Phasic unit discharge during active movement increased with increasing torque loads in 59% of the sample, although the rate-torque curves were usually curvilinear (a plateau occurred at heavy torque loads). Phasic unit firing rates rarely exceeded 50 impulses/s. A substantial proportion (44%) of units that were related to active movement showed an excitatory response (latencies usually exceeded 100 ms) to step displacements of the wrist in the absence of phasic EMG activity. About one-third of the units that were related to active wrist movement were excited by torque-pulse stimuli (latency 40-100 ms) which rapidly displaced the hand and elicited reflex EMG discharges in forearm muscles that were stretched by the stimulus. However, 49% of the units that were related to active movement did not respond to either type of passive wrist movement. These data suggest a significant role of many putamen neurons in the integration of both somesthetic inputs (perhaps relayed by central structures such as postcentral gyrus) and central inputs related to the execution of distal movements of the arm. Supported by NIH Grant NS-15485

105.13 FUNCTIONAL AND ANATOMICAL RELATIONSHIPS BETWEEN THE STRIATUM AND MUSCLES OF THE NECK AND SHOULDER.
M. D. Kelland*, and D. Asdourian. Dept. of Psychology, Wayne St. Univ., Detroit, MI 48202.

Electrical stimulation of the caudate-putamen (Cd-Pt) in adult male rats (N=35) was used to determine the nature and extent of the control exerted by the Cd-Pt over activity in muscles representative of major muscle groups of the neck and shoulder. Activity in the trapezius, biventer cervicis, rectus capitis, and scalenus dorsalis was recorded electrically.

In the first experiment, electrical stimulation of the Cd-Pt resulted in driven contralateral activity in all of the muscles studied. Only the rectus capitis displayed driven ipsilateral activity, with the ipsilateral activity always being of lower amplitude than the contralateral activity in the same animal. The parameters of the contralateral activity are as follows: maximal response amplitudes of 0.2-1.9 mV; response latencies of 6-15 msec. (the time between the stimulus artifact and the beginning of the response); durations of 2-20 msec.; and recruitment latencies of 0.5-13.9 sec. (the time it takes for muscle activity to appear following the initiation of stimulation). Spontaneous muscle activity was inhibited totally by ipsilateral Cd-Pt stimulation. These results were obtained with optimal stimulation parameters of 15 twin pulses per sec., each pulse having a 1.0 msec. duration with a 1.0 msec. interpulse interval, at 15V (approximately 0.75 ma).

In the second experiment animals received a unilateral lesion of the substantia nigra pars reticulata (SNr) or the globus pallidus (GP) prior to Cd-Pt stimulation in an attempt to determine the degree to which each of these pathways are involved in the control exerted by the Cd-Pt over the periphery. Lesions of the SNr blocked driven activity in the trapezius, biventer cervicis, and scalenus dorsalis. Lesions of the GP blocked driven activity in the trapezius. Thus, the pathway through the SNr seems to be more important for mediating the control exerted by the Cd-Pt over the neck and shoulder.

That the muscle activity driven by Cd-Pt stimulation is primarily contralateral may serve as an explanation for the contraversive motor asymmetry and circling behavior (MAC) seen during Cd-Pt stimulation. The ipsilateral inhibition of muscle activity during Cd-Pt stimulation may also contribute to contraversive MAC.

105.14 THE BASAL GANGLIA AS A CHAIN OF GABAERGIC SYNAPSES.
M. García-Muñoz and J. Segovia*. Center of Research in Cellular Physiology, U.N.A.M., México, D.F. 04510.

Gamma-amini-butyric-acid (GABA) is a neurotransmitter found in a chain of structures which starts in the striatum and continues to the globus pallidus (GP), the entopeduncular nucleus (EP), the substantia nigra (SN), and the ventromedial-parafascicular nuclei. The aim of this work was to test how these gabaergic synapses are altered by a striatal dopamine decrease produced by a 6-hydroxy-dopamine (6-OHDA) lesion in the SN. Under barbiturate anesthesia 6-OHDA (8ug/4ul) was injected into the medial forebrain bundle. Apomorphine-induced turning (0.25mg/kg i.p.) was tested a week after the lesion. Animals turning more than 100 times/30 min were selected. An HPLC determination of striatal dopamine in these animals, 4 weeks after the lesion, showed a decrease of 97% on the lesioned side. After a postoperative period of either 4 or 8 weeks, glutamic acid decarboxylase (GAD) activity was determined in each structure.

Four weeks after the nigral lesions GAD activity increased in GP (54%) and in SN (45%) whereas it decreased in EP (74%). At 8 weeks postoperatively GAD activity decreased in GP (35%) and in SN (36%) whereas it increased in EP (90%). At both intervals, thalamic GAD activity remained low (45% at 4 and 31% at 8 weeks). It could be proposed that a chain of inhibitions and disinhibitions exists in the cascade of striatal gabaergic efferents, responsible for these alternating increases and decreases in GAD activity. The mirror image changes between 4 and 8 weeks could be due to a lesion-induced compensation such as reactive synaptogenesis described for the nigro-striatal system after a 6-OHDA lesion, (1).

1.- Pritzel, M. et al. Neuroscience, 9, 879-888, 1983.

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105.15 ACOUSTICO-MOTOR PATHWAYS IN THE RAT AS DEMONSTRATED BY RETROGRADE HRP INJECTIONS.
U.E. Olazabal[1] and J.K. Moore[2]; Depts. of Psychology[1] and Anatomical Sciences[2], SUNY at Stony Brook, NY, 11794.

The Basal Ganglia(BG) have been long considered to play an important role in voluntary movements. Because lesions of the BG seemingly disrupt head, trunk, and limb movements that are modified by somatosensory feedback(while not affecting movements made in other contexts), its role has been more recently considered as that of a "sensory analyzer" for motor systems. Specifically, the involvement of the BG in sensory-controlled movements may occur through the gating of sensory information into motor systems.

Recent evidence has implicated the BG in orienting behavior. Substantia nigra(SN) neurons were observed to decrease their firing rate in relation to eye movements made in response to cue lights. In order to further understand the role of the BG in orienting behavior, small(0.05 uL) injections of a 30% aqueous HRP solution were made into the inferior colliculus(IC) of rats. Following a 24 hour period, the animals were anesthetized and perfused with 1% paraformaldehyde-1.25% glutaraldehyde in 0.1M phosphate buffer. Tissues including SN and IC were removed, sectioned, and processed for HRP enhancement.

Histological examination showed labelling of neurons in caudal portions of the ipsilateral SN reticulata. Labelled cells were sparse, and restricted to the middle to upper aspects of this structure. Marked clustering of these cells was also observed.

These findings indicate that the BG may also be involved in orientation to auditory stimuli. It remains unclear if this involvement is analogous to that reported for visual orientation through the nigrotectal pathway. However, these data suggest that a possible function may exist whereby salient auditory stimuli are selectively attended so as to result in specific patterns of movement.

106.1 IN SITU DETECTION OF RETROGRADE AXONAL TRANSPORT OF TETANUS TOXIN. J. Rosenfeld, W. H. Habig*, J. W. Griffin, B. G. Gold*, J. T. Massey, D. B. Drachman and D. L. Price. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The majority of studies involving the dynamics of retrograde axonal transport require that an animal be killed at a single time interval in order to analyze the movement of either endogenous constituents or exogenous radiolabeled material. These methods are well suited for obtaining data at a single time point per study. Prolonged continuous analysis and repeated monitoring on different days using the same animal have not been possible with most current methods.

In this study, we used a unique, newly developed gamma radiation detector to investigate the retrograde intraaxonal transport of [^{125}I] Fragment C of tetanus toxin in the sciatic nerves of anesthetized rats. The in situ gamma emission detector utilizes a cadmium telluride crystal embedded in a small steel cylinder with the ability to record radioactivity along a 3-mm segment of nerve. These gamma-sensitive microprobes are positioned above a living nerve and monitor the movement and accumulation of transported radioactive material. Microprobe detectors are interfaced with an Apple II+ computer allowing prolonged continuous recording of gamma emissions; analyses using the same animal can be repeated on different days. Using multiple microprobes, we are able to monitor axonal transport at multiple sites along the same nerve.

Following injection of 0.45 µg of [^{125}I] Fragment C (5 mCi/mg) into interosseous muscles of the foot pad, transported radioactivity was eventually detected distal to a ligation around the sciatic nerve. During 17 hours, radioactivity gradually accumulated at the site of the ligature. The ligated portion of the nerve was removed after 19 hours and was placed in a scintillation counter. The counting efficiency of our in situ detectors was calculated to be 8%. Autoradiograms of the ligated region showed that the radioactive protein was located within axons.

This in situ analysis allows us to examine the spatial and temporal patterns of axonal transport in living nerves of healthy individuals and those with abnormalities of peripheral nerves.

106.2 ANTEROGRADE AXONAL TRANSPORT OF ^{125}I-WHEAT GERM AGGLUTININ BY MOTOR NEURONS INNERVATING THE RAT LATERAL RECTUS MUSCLE. Jennifer H. LaVail. Department of Anatomy and Neuroscience Program, UCSF, San Francisco, CA 94143.

Despite widespread evidence of the affinity of wheat germ agglutinin (WGA) for sensory, preganglionic autonomic and other CNS neuron cell bodies, evidence of the uptake and anterograde transport of WGA by motor neurons innervating skeletal muscle is lacking. In light of the suggestion by Wiley, Talman and Reis (Brain Res. 269:357, 1983) that insufficient ricin-HRP binding to CNS neuronal cell bodies might account for the failure of ricin to be taken up and transported in an anterograde direction, the possibility was raised that an analogous condition for motor neurons might exist, i.e., these cells might lack plasma membrane moieties with N-acetylglucosamine for selective uptake and transport of WGA. To test this, 14 rats were anesthetized and 80 µCi (specific act. 12-16 µCi/µg) of ^{125}I-WGA were injected intracisternally into each animal. Two to 5 days later the rats were reanesthetized and perfused with a mixed aldehyde fixative. After dissection of the brainstems and lateral rectus muscles, the muscles were counted in a gamma counter, and the brains and muscles were prepared for light and EM autoradiography. Examination of the brainstems of the animals revealed intense radioactivity in parenchyma surrounding the IVth ventricle, extending throughout the abducens nuclei. The amount of radioactivity recovered in the lateral rectus muscles was highest in the 5-day survival rats. When light microscopic autoradiograms of the muscles were examined, labeled axons were identified in all animals that survived 3 or more days, but no labeled axons were found in the 3 animals that survived only 2 days. The midbelly portion of the muscle contained the highest density of labeled endplates, and this region was examined in greater detail in EM autoradiograms. Although labeled preterminals were obvious in the 3-and 5-day animals, no evidence of intercellular transfer of label to muscle or Schwann cells was found. Thus, abducens motor neurons are capable of uptake and anterograde axonal transport of ^{125}I-WGA. Furthermore, evidence was obtained that at these concentrations, the lectin can also be picked up and transported in a retrograde direction by some neurons whose axons are exposed to the WGA following an intracisternal injection. Supported in part by PHS NIH grants NS 13533 and RR 01508.

106.3 WHEAT GERM AGGLUTININ-RICIN A-CHAIN (WGA-SS-RTA) CONJUGATE: A NEW SEMISYNTHETIC SUICIDE TRANSPORT AGENT. T.N.Oeltmann* and R.G.Wiley (SPON: W.D.Dettbarn) Vanderbilt U.Medical School and Nashville VAMC, Nashville, TN, 37203.

Retrograde axonal transport of ricin, the toxic lectin from castor beans, reliably and selectively kills peripheral neurons (sensory and motor) after intraneural injection. However, ricin is not retrogradely transported by CNS interneurons, is systemically toxic at doses which produce complete lesions of large nerves (i.e. sciatic) and kills all types of peripheral neurons projecting axons through the injected nerve. One solution to these problems is to couple ricin A-chain (RTA), the ribosome inactivating subunit, to a carrier molecule that would mediate uptake and transport of RTA by neurons. As a first step in developing such hybrid toxins of neurobiologic interest, we sought to determine if wheat germ agglutinin (WGA), which is extremely well transported by most neurons, conjugated to RTA would be an effective suicide transport agent. WGA dissolved in PBS with 20 mM N-acetyl glucosamine (GluNAc) was prepared for coupling by reaction with succinimidyl 3-(2-pyridyldithio) propionate. Derivatized WGA was dialyzed and mixed with freshly reduced and desalted RTA. After formation of the disulfide conjugate (WGA-SS-RTA), free RTA was removed by affinity chromatography on a GluNAc column. SDS-PAGE of this partially purified material revealed several high molecular weight bands. Reduction with B-mercaptoethanol prior to SDS-PAGE resulted in 3 discrete bands of lower molecular weight identical to reduced WGA and the 2 reduced isozymes of RTA. Injection of 23-64 ug of WGA-SS-RTA, but not of similar amounts of free WGA and free RTA, into cervical vagus nerves of rats produced cytotoxic changes in some nodose ganglion neurons after 3-4 days. This effect initially consists of loss of all Nissl substance followed by progressive degeneration of the neurons. Indirect immunoperoxidase staining of nodose ganglia after vagal injection of WGA-SS-RTA demonstrated strong granular cytoplasmic staining with anti-WGA but minimal diffuse stain with anti-RTA. No systemic toxicity was ever observed. These results indicate that the prototype hybrid toxin, WGA-SS-RTA, has some activity as a suicide transport agent in vivo. Based on the immunohistochemical results, contamination with unconjugated WGA or premature dissociation of RTA from WGA are possible explanations for the observed low potency and efficiency of the present WGA-SS-RTA preparation. Experiments are in progress to further purify WGA-SS-RTA and assess its CNS suicide transport activity.

106.4 ULTRASTRUCTURAL LOCALIZATION OF SLOW RETROGRADE AXONAL TRANSPORT. AN AUTORADIOGRAPHIC STUDY. M.Mata* and D.J.Fink* (SPON: K. Casey). Neurology Research Laboratory, Univ. of Michigan and VA Medical Center, Ann Arbor, MI 48105.

We have previously used ^{3}H N-succinimidyl propionate (^{3}H N-SP) to covalently label endogenous intra-axonal proteins within the nerve in order to study their subsequent bidirectional transport. At the time of injection virtually all the labeled proteins are found at the injection site. At later times specific patterns of labeled proteins are found within the nerve both proximal to and distal from the injection site, as a result of retrograde and anterograde axonal transport respectively. A unique finding was the existence of a slow retrograde transport, made up predominantly of a 68 K protein which is similar to serum albumin. We undertook the current study to determine the ultrastructural distribution of the ^{3}H N-SP labeled transported proteins in the nerve.

One microliter containing 40 microCuries of ^{3}H N-SP (s.a. 50 Ci/mmole) was injected supepineurially in the sciatic nerve of 350 gram Sprague Dawley rats. Five days after injection the animals were perfused and the sciatic nerves processed for light and electron microscopic autoradiography. In parallel samples the labeled proteins were separated by SDS gradient gel electrophoresis and fluorographed. At the injection site the labeled proteins are predominantly myelin proteins. Distally a pattern similar to that described for slow anterograde transport is seen. Proximal to the injection site there is predominantly the 68 K protein.

Light microscopic autoradiography of the corresponding sections shows diffuse labeling both in axons and myelin at the injection site, with predominant axonal labeling distant from the injection site. Electron microscopic autoradiography of segments distal to the injection site show silver grains which are randomly distributed within the axoplasm without apparent relationship either to organelles or axolemma. In contrast, segments proximal to the injection site show silver grains which are consistently related to membrane bound organelles; predominantly mitochondria but also vesicles and multivesicular bodies.

These results suggest that slow retrograde transport has a unique subcellular distribution which is distinct from that of slow anterograde transport.

106.5 LACK OF EVIDENCE FOR SLOW RETROGRADE TRANSPORT IN PERIPHERAL NERVE USING THE BOLTON-HUNTER REAGENT. B. G. Gold*, J. W. Griffin, B. D. Trapp*, J. T. Massey, G. D. Bailey*, D. B. Drachman and D. L. Price. Neuromuscular Laboratory, Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The present investigation was designed to reevaluate studies suggesting slow retrograde axonal transport of serum albumin [Fink, D. and Gainer, H., J. Cell Biol. 85:175, 1980). To address this issue, we employed the Bolton-Hunter reagent, a [^{125}I] phenyl analog of [^{3}H] N-succinimidyl propionate used by Fink and Gainer. The reagent (1 mCi in 2-10 µl) was injected into the endoneurium of the sciatic or peroneal nerves of rats. Levels of radioactivity and changes with time were monitored using a newly developed microprobe device consisting of cadmium telluride crystals which are sensitive enough to detect low level gamma emissions and small enough for in vivo work. The system consists of six independently mobile microprobes which can be positioned at multiple levels along the length of the nerve, enabling the detection of movement of radiolabeled materials within a single animal at multiple time points. At the conclusion of the recording sessions, nerves were removed, cut into 3-mm segments, and counted in a gamma well counter. Segments were examined by SDS-PAGE with gel autoradiography (ARG). In some experiments, nerves were fixed (5% glutaraldehyde) in situ and prepared for tissue ARG. These studies showed a rapid decrease in the level of radioactivity at the injection site with <0.1% moving in retrograde or anterograde directions. A small fraction of this material was rapidly transported within axons. However, at both early (hours) and late (days) time intervals, the moving material was predominantly composed of a 68-kilodalton protein which was not present within axons; it passed sites of axotomy and moved in the distal stumps of previously transected nerves in which no axons remained. ARG showed endoneurial, not intraaxonal, silver grains. Immunocytochemical staining with antibodies directed against albumin disclosed abundant endoneurial albumin and no staining within axons. These studies support the finding that the Bolton-Hunter reagent labels albumin. However, we find no evidence of slow retrograde transport within axons and suggest that radiolabeled albumin is moving within the extracellular space of the peripheral nerve.

106.6 THE NEURAL CELL ADHESION MOLECULE (NCAM) IS RAPIDLY TRANSPORTED IN CHICK OPTIC AXONS. J.A. Garner, M. Watanabe*, and U. Rutishauser. Dept. of Developmental Genetics and Anatomy, CWRU School of Medicine, Cleveland, Ohio, 44106.

Materials necessary for the maintenance and function of axons and terminals are transported to those regions by the specialized form of intracellular transport, axonal transport. The slow components of axonal transport are thought to convey the cytoplasmic matrix and cytoskeletal proteins while the fast component consists of primarily membrane-associated elements. Most of the fast component proteins appear to be glycoproteins; however, few individual fast component proteins have been well characterized. The neural cell adhesion molecule (NCAM) is a cell surface glycoprotein involved in interactions between neurons and other neurons or target tissues. We have demonstrated that NCAM is rapidly transported in axons of chick retinal ganglion cells.

^{35}S-methionine was injected into the right eyes of 3 day old chicks. Proteins radiolabeled in the retinal ganglion cell bodies were transported down axons through the right optic nerve, chiasm and left optic tract to the left (contralateral) tectum. The presence of radiolabeled NCAM in the left tectum was detected by specific immunoprecipitation followed by gel electrophoresis and fluorography. The transported NCAM consisted of major bands of approximately 200Kd, 150 Kd, and 120Kd, and several additional minor bands. These results suggest that there is heterogeneity in the size of NCAM produced and transported by a single class of retinal neurons. The NCAM reaches the tectum as early as 2 hours after injection, and continues to accumulate at a rate that suggests it is conveyed with the fast component proteins. In order to examine the actual kinetics of transport of this fast component protein, the optic nerve, chiasm, and tract were cut into 1mm segments at 1,3, and 5 hours after injection of label. Sequential segments were immunoprecipitated, electrophoresed, and fluorographed. This analysis revealed a peak of radiolabeled NCAM within the optic pathway at 3 hours after injection of label.

106.7 PROPERTIES OF AXONALLY TRANSPORTED ANTIBODIES TO RAT BRAIN MEMBRANE FRACTIONS. R.H. Fabian*, T.C. Ritchie, and J.D. Coulter. Marine Biomedical Institute, Depts. of Neurology, Physiol. & Biophysics, and Psychiat. & Behav. Sci., Univ. of Texas Med. Branch, Galveston, TX 77550.

Antibodies against various rat brain membrane fractions and against membrane glycoproteins obtained by affinity chromatography on wheat germ agglutinin (WGA) columns were found to be retrogradely transported by motoneurons following injections of antibody into muscle (see preceding abstract). To further define properties of the antisera, axonal transport in CNS neurons was examined and initial characterization of antisera was performed using immunoblots and WGA staining of brain membrane proteins separated by SDS PAGE.

Following spinal cord injections of antisera, neurons stained for the transported antisera were localized in brainstem nuclei known to project to the spinal cord. Similarly, stained cells were seen in sites known to give rise to cerebellar afferents following cerebellar cortical injections. No evidence for anterograde transport was seen except for fibers that may have been damaged by the injections.

Antisera were used to stain SDS-solubilized brain membrane proteins and glycoproteins (obtained by WGA affinity chromatography) absorbed to nitrocellulose following separation on SDS PAGE. Nitrocellulose adsorbed proteins were renatured with 0.5% Triton X-100 and binding of each rabbit antisera was determined using indirect immunostaining with a goat anti-rabbit-HRP conjugate. Preimmune rabbit sera were used as controls. Antisera which were axonally transported were found to stain numerous protein bands from the gels. However, antisera most heavily stained certain WGA binding glycoproteins, especially those co-migrating at 195,000, 150,000, 110,000, 69,000, 50,000, and 31,000 Daltons.

Antibodies made against various brain membrane fractions or against solubilized membrane glycoproteins appear to be transported retrogradely but not anterogradely in the neuronal systems tested to date. As expected, antisera generated against different membrane fractions are immunoreactive with a large number of proteins, but may be directed especially to larger glycoproteins known to be important constituents of the cell surface. Supported by NS12481, NS07185 and NS11255.

106.8 SELECTIVE OCCURRENCE OF ANTEROGRADE TRANSSYNAPTIC TRANSPORT OF WHEAT GERM AGGLUTININ-HORSERADISH PEROXIDASE IN THE MAMMALIAN CENTRAL NERVOUS SYSTEM. S.K. Itaya, C.L. Barnes and G.W. Van Hoesen, Depts. of Anatomy and Neurology, Univ. of Iowa, Iowa City, IA 52242.

Several recent reports indicate that wheat germ agglutinin (or its conjugate with horseradish peroxidase, WGA-HRP) undergoes anterograde transsynaptic transport. Defined as the movement of molecules between synaptically connected neurons in a pre- to postsynaptic direction, such transport does not involve neuroglia, nonsynaptically coupled neurons, nor a post- to presynaptic direction. Using this definition as a working hypothesis, we have examined several appropriate two-neuron pathways. After injecting WGA-HRP in the vicinity of the first neuronal cell body, we looked for WGA-HRP reaction product in the neuronal soma and/or axon terminals of the second neuron as an indication of anterograde transsynaptic transport. In the primary visual pathways of rats and monkeys anterograde transsynaptic transport was observed in the retino-geniculo-striate pathway. In the rat, anterograde transsynaptic transport was found also in the retino-geniculo-reticular, retino-pretecto-oculomotor, and retino-tecto-parabigeminal pathways, but not in the retino-medial terminal nucleus pathway. We injected the entorhinal cortex in monkeys and rats with WGA-HRP and observed labeling in the perforant pathway and within cell bodies of the dentate granule cells, suggestive of anterograde transsynaptic transport. Descending corticofugal pathways were studied in the rat by injecting into the precentral (motor) and sensorimotor cortices. No evidence of anterograde transsynaptic transport was found in the cortico-pontine pathway, however, a few neuronal cell bodies were labeled with WGA-HRP in the contralateral main sensory nucleus of V, suggestive of anterograde transsynaptic transport in the corticotrigeminal pathway. Injections of WGA-HRP into the nasal cavity labeled the vomeronasal nerve fibers and their terminals but not the postsynaptic mitral cells. In summary, we have observed evidence for anterograde transsynaptic transport in 6 pathways, and negative evidence for such transport in 3 pathways. From these preliminary findings, it appears that the capability of anterograde transsynaptic transport resides within selective pathways. In addition to illustrating a new mechanism of interneuronal communication and transfer, anterograde transsynaptic transport may also provide a new characteristic to differentiate pathways or synapses. Supported in part by grant NS 14944 to G.W.V.H.

106.9 EFFECT OF IMIPRAMINE AND CHLORIMIPRAMINE ON FAST ORTHOGRADE AND RETROGRADE AXONAL TRANSPORT. P.-A. Lavoie and M. Tiberi* Département de pharmacologie, Université de Montréal, Montréal, Canada H3C 3J7.

The effect of imipramine and chlorimipramine on fast orthograde transport of [^{3}H]leucine-labeled proteins and on the retrograde transport of acetylcholinesterase (AChE) was studied in vitro. The biological material used consisted of the 8th and 9th dorsal root ganglia of the bullfrog *Rana catesbeiana* and of their associated spinal nerves and sciatic nerve. In the retrograde transport experiments, the experimental spinal nerves were preincubated for 5 h with 0.2 mM imipramine or chlorimipramine, a ligature was then tied on the nerve at 6 mm from the dorsal root ganglion, and the nerves were incubated with the drug for an additional 17-18 h; contralateral control nerves were treated similarly but maintained in drug-free medium throughout. The accumulation of AChE activity (assayed by a radiometric method) in the 2 mm nerve segment distal to the ligature served to evaluate the status of retrograde transport: it was reduced by 53% ($p < 0.05$) and by 69% ($p < 0.05$) by imipramine and chlorimipramine respectively. For the orthograde transport experiments, a 1 h pulse-labeling of ganglia with [^{3}H]leucine was done; the control spinal nerves were then incubated for 16-17 h in physiological solution whereas the experimental spinal nerves were incubated for the same period of time in medium containing 0.2 mM imipramine or chlorimipramine. The accumulation of [^{3}H]proteins at a ligature in the distal portion of the spinal nerve was reduced by 70% ($p < 0.05$) and 92% ($p < 0.05$) respectively by imipramine and chlorimipramine, as compared to the accumulation in contralateral control nerves. To determine whether the magnitude of the transport inhibition caused by chlorimipramine was truly greater than that caused by imipramine, nerves from one side of the animal were incubated with 0.2 mM imipramine and nerves from the contralateral side were incubated with 0.2 mM chlorimipramine; the accumulation of [^{3}H]proteins at the ligature was significantly less ($p < 0.05$) in the nerves exposed to chlorimipramine. The transport inhibition by imipramine and chlorimipramine could be related to the anti-calmodulin activity of the two drugs; the results may thus provide evidence for the participation of calmodulin in the mechanism of axonal transport. In keeping with this hypothesis, chlorimipramine is both a more potent inhibitor of axonal transport and a more potent anti-calmodulin agent than imipramine.

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106.10 OBSERVATIONS ON INTRANEURONAL TRANSPORT: I. EPILEPTIC DISCHARGE AND SELECTIVE LABELLING VIA AXONAL TRANSPORT. II. SOMATOPETAL DENDRITIC TRANSPORT. I. Divac[1], B. Petrovic-Minić[2]*, S. Marinković[3]* and J. Mogensen[1]*, (1) Inst. of Neurophysiology, Univ. of Copenhagen, (2) Inst. of Pathophysiology, Univ. of Beograd, (3) Inst. of Anatomy, Univ. of Beograd.

Intracortical implants of polyacrylamide gel containing horseradish peroxidase labelled cortical efferents and perikarya in some cortical areas and a number of subcortical formations. When epileptogenic penicillin was added to the gel, no labelling was seen in the efferents and cell bodies of the cortex, thalamus and claustrum, whereas the magnocellular nuclei of the basal forebrain, raphe nuclei and locus coeruleus did contain the label. The unlabelled regions differ from the labelled ones by being bidirectionally connected with the cortex and having neurons with unidentified transmitters.

In the cerebellar folia covered by propidium iodide the perikarya and dendrites of Purkinje neurons were intensely labelled. This was found also in the folia in which the granular neurons contained no label. The tracer was not seen in the white matter and the nuclei of the cerebellum, nor in the floor of the forth ventricle. We conclude that dendrites of Purkinje neurons take up the tracer from the surface and transport it to the cell body. Simultaneous labelling of a large number of perikarya and the simple technique may facilitate studies of the neurobiology of somatopetal dendritic transport.

106.11 CALCIUM MODULATION OF ACTIN-SPECTRIN CYTOSKELETON ASSOCIATED WITH BULK-PACKAGED AND -TRANSPORTED CYTOMEMBRANES IN GROWING AXONS. E. Koenig, Edmonds, B.*, Kinsman, S.*, and Repasky, E.* Division of Neurobiology, SUNY/Buffalo, Buffalo, NY 14214.

Goldfish retinal explants produce an elaborate outgrowth of naked retinal ganglion cell axons that contain motile varicosities and intervening phase-dense inclusions. Axial movements of these mobile structures are bidirectional, and their average saltation velocities are ~0.2 um/sec for varicosites and ~ 3 um/sec for dense inclusions. Varicosities contain a convoluted, anastomosing tubular smooth endoplasmic reticulum that is embedded in an amorphous matrix. Dense inclusions shuttle between varicosities and growth cones in the distal axon during elongation or retraction, presumably carrying cytomembranes for insertion or for bulk-repackaging, respectively. Rhodamine-conjugated wheat germ agglutinin injected intraocularly 6 or 24 hrs before explantation results in labeling of some varicosities in explant axons, indicating that the cytomembrane inclusions contain glycoconjugates. Immunofluorescence studies indicate that actin, alpha-spectrin and calmodulin are preferentially distributed in varicosities and dense inclusions. The localization of alpha-spectrin appears to associated with inclusions of the varicosity, which is a departure from its typical subplasmalemmal localization. Movements are arrested by calcium antagonists, such as cadmium, cobalt, barium, but not by strontium. Movements are not halted in the absence of external calcium with 1 mM EGTA present, but will cease if A23187 is present, indicating that transport is calcium-dependent and that intracellular stores are sufficient. In the presence of A23187, calcium, at low concentrations, causes contraction of varicosities (i.e. increased gelation), while, at higher concentrations, calcium causes ballooning (i.e., solation). Calmodulin antagonists, such as calmidazolium or trifluoperizine, also cause solation. A sodium-free medium lowers the threshold for solation induced by calcium or by calmodulin antagonists, and causes a partial gel-sol transition by agents that normally do not do so (e.g., 30 mM K). We infer that an actin-spectrin system is involved in the "packaging" and transport of cytomembranes in these axons, and that the packaging and movement are modulated by intracellular calcium.

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106.12 EFFECT OF FORSKOLIN ON NEURONAL PROTEIN SYNTHESIS AND TRANSPORT IN FROG. Marino De Leon and Richard Carlsen, Dept. of Human Physiology, Univ. of Calif., Davis, CA 95616.

The cell body of peripheral neurons undergoes a complex series of changes in response to axon injury. While these changes include "disruptive" events, a number are geared to the regeneration of a new axon. The molecular processes responsible for the induction of cell body changes following injury are unknown. We are investigating the nature of these signals by attempting to manipulate the appearance of the cell body response (Brain Res. 279:9), and by altering the rate of axon regeneration. Recently, we were able to stimulate the rate of sensory nerve regeneration by 40% in the frog using forskolin, a robust activator of adenylate cyclase (Nature 307:455). We have continued this investigation by determining if forskolin might also affect the synthesis and axonal transport of proteins and glycoproteins in normal and injured frog nerves. Initially, we have compared the effect of different concentrations of forskolin on synthesis and transport in normal lumbar DRG 9 & 10 of the bull frog. Experiments were performed in vitro using the technique of Dravid & Hammerschlag (J. Neurochem. 24:711). DRG 9 & 10 and their spinal nerves were removed from both sides. The ganglia were incubated in vitro in a separate chamber containing either forskolin plus precursor isotopes, or precursors in saline (control). Comparisons of synthesis and transport were made between paired DR 9 and DR 10 ganglia and spinal nerves. Incubation continued for times up to 1 hour and the subsequent transport interval was 18-20 hours. Transported material was collected at a ligature placed 20-25 mm distal to the ganglia. A concentration of 10^{-6} M forskolin for 1 hour increased the synthesis (as recovered TCA-insoluble material) of protein by 25% compared to control. Transported protein was increased by a similar amount (17%). In contrast, a concentration of 10^{-5} M forskolin decreased synthesis of both protein and glycoprotein by 25% and decreased transport by an even greater amount (50%). Forskolin at 10^{-4} M for 30 minutes also had an inhibitory effect. One unexpected result was the discovery that 10^{-5} M forskolin also had a major effect on the time to initiation of transport (time between application of precursor and the appearance of labeled product in the nerve) and transport rate. Time to initiation was decreased from >2 hours to 14 minutes and transport rate was decreased from 137 mm/day to 95 mm/day. These findings suggest that cyclic AMP may play a significant role in both protein metabolism and fast axonal transport in peripheral neurons.

106.13 ANTISERA AGAINST TUBULIN, BUT NOT AGAINST NEUROFILAMENT PROTEIN OR ACTIN, INHIBIT RETROGRADE AXONAL TRANSPORT "IN VIVO". K.M. Johnston, D. van der Kooy and J. Connolly.* Neurobiology Research Group. Dept. of Anatomy, Univ. of Toronto, Toronto CANADA M5S 1A8

Based largely on drug studies and morphological observations, cytoskeletal elements are believed to play a role in the intraaxonal transport of endogenous and exogenous substances. We used antibodies directed against the proteins of the major cytoskeletal elements, tubulin (microtubules), neurofilament triplet proteins (neurofilaments) and actin (microfilaments) as in vivo probes to further investigate their role in retrograde axonal transport. Our test system employed the transport of Fast Blue from the caudate to the substantia nigra pars compacta in adult rats. Undiluted antiserum (0.9 ul) was injected into multiple sites within a small area of caudate to ensure maximal access of antibodies to the neuronal cytoplasm. The serum injection was followed immediately by injection of 0.05 ul of Fast Blue into the same area. Pre-immune serum was used as a control in the contralateral caudate. 24 hrs later, animals were fixed by perfusion and Fast Blue labelled cell bodies counted in consecutive sections of substantia nigra. An FITC-labelled second antibody was used to determine distribution of the primary antibodies.

An antiserum against electrophoretically purified brain tubulin produced a dramatic decrease (39-78% in different rats) in the number of retrogradely labelled nigra cell bodies compared with the control pre-immune side. A similar inhibition of retrograde transport was seen in the centromedian parafascicular complex of the thalamus which also projects to the caudate. When WGA-HRP was used as the axonal tracer, this anti-tubulin serum again produced a severe inhibition (59%) of retrograde transport. In addition, a substantial inhibition of anterograde transport from the caudate to the substantianigra pars reticulata could be observed with WGA-HRP. Two other antisera and one monoclonal antibody directed against purified brain tubulin had consistent but smaller (23-29% decrease) inhibiting effects on retrograde axonal transport in the same in vivo system. Antisera directed against either actin or neurofilament proteins produced no consistent effects on retrograde axonal transport in our system. These results suggest that, by interfering with microtubule function in vivo, antibodies to tubulin cause a profound decrease in the amount of exogenous substance retrogradely transported.

106.14 SLOWING OF NEUROFILAMENT TRANSPORT AS A MECHANISM FOR RADIAL GROWTH OF THE AXON. P. N. Hoffman, J. W. Griffin and D. L. Price. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Recent evidence suggests that neurofilaments, which are composed of three polypeptides conveyed by slow axonal transport (Hoffman, P.N. and Lasek R.J., J. Cell Biol., 66:351-366, 1975), play a major role in the control of axonal caliber (Hoffman, P.N. et al., J. Cell Biol., in press). In order to test this hypothesis, the transport of neurofilament proteins was examined in axons undergoing radial growth during postnatal development. In both 3- and 12-week old rats, lumbar motor neurons were labeled by the intraspinal administration of [^{35}S] methionine. Using SDS-PAGE, gel fluorography, and liquid scintillation spectroscopy, the distribution of transported cytoskeletal proteins within the sciatic nerve was analyzed at various times after injection of isotope. In both age groups, the velocity of neurofilament transport progressively declined with increasing distance along the nerve. Reduction in velocity per unit distance along the nerve and velocity at every point along the nerve were greater in the 3- than in the 12-week old animals. Correlative morphometric studies demonstrated that cross-sectional areas of these axons increased linearly between 3 and 18 weeks of age. At every age examined, axonal calibers were the same in proximal and distal regions of the L5 ventral root. Thus, radial growth occurred simultaneously at the same rate along these roots (i.e., there was no proximal-to-distal tapering of these axons over distances of at least 30 mm). Electron microscopy revealed that neurofilament density was identical in fibers of all calibers and at all ages examined, indicating that radial growth correlated with a proportional increase in the neurofilament content of these axons.

Since there appears to be negligable turnover of neurofilaments along axons, axonal transport is the primary mechanism by which neurofilaments can enter or leave any region of the axon. Therefore, local neurofilament content should increase when influx exceeds egress. We propose that progressive slowing of neurofilament transport along nerve fibers should allow neurofilaments to enter every region faster than they leave, resulting in increases in neurofilament content and simultaneous radial growth along the length of the axon.

106.15 THE RAPID PHASE OF ANTEROGRADE AXOPLASMIC TRANSPORT IN THE CENTRAL NERVOUS SYSTEM. W.J. Crossland, Department of Anatomy, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

Past investigations of the rapid phase of anterograde axoplasmic transport have demonstrated a rate of 410 +/-60 mm/day for the peripheral nervous system but a rate of 250 mm/day or less for the central nervous system. The discrepancy may be due to technical difficulties in measuring the rate in the central nervous system or may reflect a fundamental metabolic difference between central and peripheral neurons.

In the present study 10 µl of concentrated tritiated proline was injected adjacent to the nasal retina of hatchling chickens or into the central portion of the vitreous body of two-month-old rats. The animals were sacrificed at short intervals between one and two hours after injection, fixed with formalin, embedded in paraffin, and prepared for autoradiography. The position of the transported protein was determined from the autoradiographs, and a transport distance interpolated from photographs and previous measurements made on the fixed brains before embedding. The rates were calculated by regression analysis of the survival time and transport distance.

Post-hatch chicks revealed transport rates of 367 mm/day. Transport of tritiated material in the rat visual pathway was similar, approximately 341 mm/day. If one allows as little as 10% linear shrinkage in the formalin fixed material, the transport rate in the chicken and rat could be as high as 404 and 377 mm/day, respectively.

Allowing for modest shrinkage during fixation the rate of the rapid phase of anterograde axoplasmic transport in the visual pathways of these two classes of vertebrates is within the range reported for axons of the peripheral nervous system (350-470 mm/day). The similarity in rate between the central and peripheral nervous system is consistent with the idea that a common mechanism of transport is at work in both locations.

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106.16 AXONAL TRANSPORT OF ANTIBODIES TO MEMBRANE FRACTIONS OF RAT BRAIN. T.C. Ritchie, R.F. Fabian*, P.J. McKinney* and J.D. Coulter. Marine Biomedical Institute, Depts. Physiol. & Biophysics, Psychiat. & Behav. Sci. and Neurology, Univ. of Texas Med. Branch, Galveston, TX 77550.

Axoplasmic transport of certain macromolecules, including nerve growth factor, cholera and tetanus toxins, various plant lectins, and antibodies to several neurotransmitter enzymes, is considered to involve binding to specific receptors on cell surface membranes. In an initial step towards identifying classes of endogenous brain proteins which are involved in receptor mediated endocytosis and transport, we investigated axonal transport of antibodies made against brain fractions isolated by differential centrifugation (Gurd et al., 1974) and by affinity chromatography on wheat germ agglutinin (WGA) columns (Gombos and Zanetta, 1977). Antibodies were generated in rabbits to the following preparations: Group 1, (N=1) synaptosomes; Group 2, (N=2) microsomes; Group 3, (N=2) SDS solubilized "crude" microsomes; Group 4, (N=2) Nonidet P40 solubilized microsomes, and Group 5, (N=2) SDS solubilized, WGA-binding glycoproteins. To test for axonal transport, whole antisera or purified IgG fraction were injected in rats into the vitreal chamber of the eye (10µl) and the mystatial vibrissal skin and underlying musculature (50µl). After survival times of 1-4 days, animals were perfused with 4% paraformaldehyde. Axonally transported rabbit immunoglobulins were detected in frozen sections of brainstem with PAP immunocytochemistry with incubation in "link" antiserum (goat anti-rabbit IgG) followed by rabbit PAP and reaction with DAB. Controls consisted of injecting preimmune sera into the vitreal chamber and vibrissal skin and musculature on the contralateral side. Rabbits of all groups produced antibodies which were retrogradely transported by motoneurons of the facial motor nucleus innervating the musculature underlying the vibrissae. Labeled neurons exhibited punctate reaction product in proximal dendrites and in the cell cytoplasm, exclusive of the nucleus. No transport was seen following injections of preimmune serum. To date, no anterograde transport in retinal ganglion cells or in trigeminal afferents has been observed with intravitreal or vibrissal injections of antisera. These results confirm and extend those of Wenthold et al. (1983) and indicate that antibodies against specific neural membrane constituents may ultimately prove useful for characterizing proteins and other macromolecules involved in axonal transport. Supported by NS12481, NS07185 and NS11255.

106.17 LABELING OF AXONALLY TRANSPORTED POLYPEPTIDES BY INTRAVITREAL INJECTION OF 3H-PALMITIC ACID. C. Baitinger* and M. Willard. Dept. of Anatomy and Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Recent experiments in other laboratories have shown that certain membrane proteins are modified posttranslationally by the covalent addition of fatty acid. To identify polypeptides of the nervous system that undergo this post-translational modification, we determined which polypeptides of the rabbit visual system are labelled when ^{3}H-palmitate is injected into the vitreous. Fourteen hours after ^{3}H-palmitate was injected, fifteen polypeptides that were associated with a particulate fraction of the retina were radioactively labeled; the most intensely labeled polypeptide had a molecular weight of 25K. Two of the labeled polypeptides in the retina (mw=197K, 60-70K) had the same electrophoretic mobility on two-dimensional polyacrylamide gels as polypeptides that have previously been shown to be transported rapidly (group I of axonal transport) into the axons of the retinal ganglion cells. Furthermore, polypeptides of the same electrophoretic mobility were among five polypeptides (mw=197K,60-70K,54K,30K,25K) that were labeled with tritium in the optic nerve, optic tract, and superior colliculus (which contain axons and synaptic terminals of retinal ganglion cells) at 14 hours after labeling the retina with ^{3}H-palmitate. At longer periods (8 and 20 days), when more slowly transported proteins have reached the optic nerve and optic tract, at least two of the same polypeptides, as well as several additional ones, were labeled in tissues containing the axons and synaptic terminals of retinal ganglion cells. More than 95% of the ^{3}H-radioactivity that was associated with the proteins in the retina, optic nerve and optic tract, and superior colliculus at early times, and greater than 70% of that associated with the proteins at late times, was removed when the polypeptides were incubated with 1 M NH_2OH or with 0.2 M KOH in methanol. Because fatty acyl ester bonds, but not peptide bonds, are labile to these treatments, this lability indicates that the labeling of the proteins was not the result of the conversion of ^{3}H-palmitate to amino acids. We conclude that a small subset of the proteins synthesized in the retina are posttranslationally modified by the covalent addition of fatty acid, and that two of these correspond to polypeptides that are rapidly transported in the retinal ganglion cells.

106.18 AXOPLASMIC TRANSPORT IN THE SAPONIZED AXON: ORGANELLES AS TRACKS AND FORCE GENERATING MECHANISM. H. Gotoh, T. Takenaka, and M. Sato. Dept. of Physiol., Iwate Medical Univ., Morioka, Iwate 020 Japan and Dept. of Physiol., Yokohama City Univ., Sch. of Med., Urafunechou, Minamiku, Yokohama 232 Japan

We permeabilized single fin nerves of the squid with saponin in order to conduct intracellular pharmacological experiments. Membranous organelles and filamentous networks such as microtubules and intermediate filaments were preserved after treatment with 1 % saponin for 1 hr. Ruthenium Red staining of the present model exhibited a microtrabecular network preserved in the axoplasm. The composition of the artificial intracellular fluid used for such morphological experiments was 300 mM KCl, 10 mM $MgCl_2$, 10 mM NaH_2PO_4, 5 mM ATP, and 0.2 mM EGTA (pH 7.4). To investigate the reactivation of axoplasmic transport, a reduced permeabilization was accomplished using 0.01 % saponin for 2 min. The composition of the fluid was also modified: 400 mM K-Glutamate, 150 mM Glycine, 10 mM $MgCl_2$, 5 mM ATP, 1 mM EGTA, and 20 mM HEPES (pH 7.2). A Nikon dark-field microscope was used for the observation of the particle movement. The movement was reactivated specifically by ATP (5 mM), not by GTP nor by ITP. Depolymerizers of microtubules, colchicine (1 mM) and vinblastine (0.1 mM), blocked directional movement. Local oscillatory movement, however, continued after the disruption of the microtubules. Actin depolymerizers, cytochalasin B and 88K protein/actin complex isolated from porcine brain, also blocked the directional particle movement. Actinogelin, a gelating protein of actin filaments appeared to hinder the smooth movement of particles and decreased their velocities. The present results suggest that microtubules are tracks along which the particles travel, and actin filaments play a structural role in the particle transport rather than a role in transport force generation. The force seems to be generated from a dynein-like ATPase.

106.19 AXONAL TRANSPORT OF MITOCHONDRIA IN GIANT MOTOR AXONS OF THE LOBSTER. D.S. Forman[1], R.S. Smith[2] and K.J. Lynch[1] [1]Department of Anatomy, Uniformed Services University of the Health Sciences, Bethesda, MD 20814 and [2]Department of Surgery, University of Alberta, Edmonton, Alberta T6G 2G3, Canada.

The axonal transport of mitochondria in living axons can be visualized by light microscopy. Mitochondria in isolated axons from the walking legs of lobsters were observed using differential interference contrast optics and video microscopy. Most of the mitochondria in the axon were stationary, but mitochondria were also seen to move in a saltatory fashion. The mean velocity of transport in the retrograde direction (1.33 + 0.65 [S.D.] µm/sec) was greater than the mean velocity in the anterograde direction (0.72 + 0.26 µm/sec), as is also true of vesicular organelles (particles) in the same axons. The mitochondria studied ranged in length from 1 to 25 µm. No correlation was found between the lengths of the mitochondria and their velocities. The details of the motion of the mitochondria were analyzed by the methods of Koles et al. (1982) J. Physiol. 328: 469. Mitochondria moved in a way that could be described as the sum of a constant velocity plus a low frequency variable velocity component. This variable component of velocity had an average frequency of 0.10 - 0.15 Hz. A similar low frequency component was evident in the longitudinal oscillations of mitochondria that showed no net displacement. Retrogradely moving mitochondria showed a greater variability in the magnitude of instantaneous velocities than anterogradely moving mitochondria. The detailed characteristics of the movements of mitochondria and particles were similar, suggesting that the same mechanism may be involved.

A large fraction of the axonal mitochondria were localized immediately below the plasma membrane. The density of mitochondria within 0.3 µm of the plasma membrane was similar in different axons and averaged 0.82 + .19 (SD) mitochondria per µm of axon perimeter. These mitochondria adhered to the plasma membrane when axoplasmic structure was disrupted. In electron micrographs, fine bridges were found connecting some of the mitochondria to the plasma membrane. Attachment of mitochondria to the plasma membrane may assure a constant supply of ATP for the membrane ion pumps.

(Supported by USPHS Grant NS 19676 and by grants from the Medical Research Council of Canada.)

106.20 AXONAL TRANSPORT AND PRESYNAPTIC LOCATION OF α_2-ADRENORECEPTORS IN LOCUS COERULEUS NEURONS. B.E. Levin. VA Medical Center, E. Orange, NJ 07019, and NJ Med Sch, Newark, NJ 07103.

To investigate the possibility that α-adrenoreceptors might be axonally transported in the ascending neurons of the locus coeruleus, unilateral injections of 6-hydroxydopamine (6-OHDA; 2 µl; 4 µg/ µl) were made in the left ascending pathway and the accumulation of binding sites for various α-adrenoreceptor ligands measured as compared to the uninjected side. Binding for the α_2-ligand, {^{3}H} clonidine (0.75 nM) increased linearly over a 3d period following 6-OHDA injections to a maximum of 190% of the uninjected side. This accumulation of binding appeared to be due to the anterograde transport of high affinity binding sites for α_2-receptors since it occurred for {^{3}H}p-aminoclonidine (0.3-1.0nM) with an increase in the maximal number of sites bound (right side: 41.3+0.5; left side: 53.8 1.8 fmol/mg protein; $p<0.05$) without a change in Kd (right side: 0.56+0.06; left side 0.68+0.04 nM). This accumulation could be completely blocked by a more proximal injection of 6-OHDA made into the pathway at the same time as the forebrain injection was made. There was no comparable accumulation of low affinity α_2-binding sites labelled with {^{3}H} rauwolscine (1.0-6.0nM; Bmax: 132+10 fmol/mg protein; Kd: 3.80+0.43nM) or for α_1-adrenoreceptors labelled with {^{3}H} WB-4101 (0.5-2.0nM; Bmax: 151+5 fmol/mg protein; Kd: 0.11nM). These results suggest that high affinity binding sites for α_2-adrenoreceptors are located presynaptically on, and undergo anterograde axonal transport in the ascending neurons of the locus coeruleus.

107.1 POLY(A)$^+$MESSENGER RNA FOR RAT OLFACTORY MARKER PROTEIN IS EXTREMELY LARGE. K.E.Rogers*, M.Grillo*, W.Sydor*, U.Gubler*†, F.Margolis. RIMB and †Dept. Molec. Genetics, Roche Res. Ctr., Nutley, NJ 07110.

Olfactory marker protein (OMP) is a developmentally regulated 18 kilodalton cytoplasmic protein. It is localized exclusively in olfactory chemoreceptor cells and is synthesized only by mature neurons and not by their progenitor cells. The biochemical properties of OMP have been extensively studied but its function remains unknown. With the ultimate goal of isolating the gene for this protein we have begun to characterize its mRNA . We have shown that rat olfactory mucosa poly(A)$^+$mRNA directs the synthesis of OMP using a rabbit reticulocyte lysate translation system for *in vitro* biosynthesis. OMP was immunoprecipitated from total translation products with specific goat or rabbit anti-OMP antiserum. The identity of the immunoprecipitated product was confirmed by co-migration with authentic OMP on SDS-polyacrylamide gel electrophoresis and by competition with unlabeled OMP. In addition, the immunoprecipitated material co-eluted with authentic OMP using reverse phase-HPLC. *In vitro* synthesized OMP was found to represent 0.25-0.35% of the total translated products which is consistent with the level of this protein observed in 100,000 x g supernatant extracts of olfactory mucosa. Denaturing methylmercury-agarose gels were used to size fractionate total poly(A)$^+$mRNA. The OMP synthesizing mRNA was located by direct translation and immunoprecipitation of sequential agarose gel slices. Greater than 60% of the OMP mRNA was approximately 3.0 kilobases in size and migrated slightly ahead of the 28S ribosomal RNA marker. Exceptionally large mRNA is characteristic of brain and an mRNA of this size would contain sufficient information to code for a protein 4-5 times larger than OMP. Since the rabbit reticulocyte lysate translation system is thought to generate only primary translation products, the synthesis of authentic 18 kilodalton OMP argues against the presence of an OMP precursor protein but does not preclude this possibility. This implies that the majority of OMP mRNA consists of untranslated sequences. Currently, we are cloning the OMP messenger RNA to provide answers to these questions.

107.2 ACTIVITY DEPENDENT CHANGES IN PROTEINS EXPRESSED BY PC12 CELLS IN CULTURE. L. Baizer and M.C. Fishman. SPON: (Neil A. Busis). Howard Hughes Medical Institute, Dev. Biol. Lab., M.G.H. and Harvard Medical School, Boston, MA 02214

Neuronal development and patterns of connectivity manifest exquisite sensitivity to electrical activity and to components of the microenviornment. The molecular basis of this sensitivity remains unknown. We have utilized the NGF-responsive clonal adrenergic cell line PC12 in order to search for specific proteins regulated by activity and differentiation. Cultures were incubated for 3 days in either control medium or in depolarizing medium containing 20mM KCl. Cellular proteins were then labelled by a 2 hour incubation in medium containing ^{35}S-methionine, resolved by two dimensional (2-D) electrophoresis, and visualized by fluorography. Of the approximately 200 abundant cellular proteins thus visualized, the level of expression of most was unaffected by potassium depolarization. However, the levels of a very small subset of proteins were reproducibly affected by the treatment. NGF-induced differentiation led to dramatic alteration in cellular morphology and changes in the levels of only a very few proteins. NGF did not appear to affect the depolarization-induced changes.

The limited number of proteins whose levels are altered under these conditions suggests that their regulation is related to neuronal activity and to the state of differentiation.

107.3 NEW RNA POPULATIONS IN PC-12 CELLS TREATED WITH NERVE GROWTH FACTOR. S. Huttner§ K. Morrison-Graham, N. Tillakaratne§ P. O'Lague, and A. Tobin. Dept Biology, Jerry Lewis Neuromuscular Research Center, and Molecular Biology Institute, UCLA, Los Angeles, CA 90024.

PC-12 cells, originally cloned from a rat chromaffin tumor, respond in culture to NGF by expressing a number of neuron-like properties, including among other things the outgrowth of neurites and the appearance of action potential and neurotransmitter mechanisms. The molecular mechanisms by which these effects are produced are not understood. To study possible molecular effects of this neuronal trophic factor we have compared mRNA populations of PC-12 cells grown with and without 7S NGF for various periods of time. Total RNA, isolated by the guanidine thiocyanate-cesium chloride method, and polysomal RNA was prepared from NGF-treated and untreated cells. Polyadenylated (A^+) RNA was separated by oligo-d(T) column chromatography and fractionated by electrophoresis in 1% agarose (5mM methylmercury hydroxide). Two major bands, approximately 3200-4000 and 1500 nucleotides, were detected after ethidium bromide staining of mRNA of NGF-treated cells. They were present after 2 hr exposures to NGF as well as after 14 days of treatment (the longest time tested). The bands represented abundant classes of RNA and could be enriched in the A^+ fraction. ^{32}P-phosphate incorporation into these bands occurred in cells grown with NGF for ten days, indicating that active synthesis continued during prolonged exposure to the factor. The same two bands were also detected in the RNA of PC-12 cells grown without NGF under conditions of phosphate deprivation.

The bands were not degradation products of eukaryotic ribosomal RNAs (28S and 18S rRNAs) judged by blot hybridizations with nick-translated DNA probes coding for rRNAs. They also were not bacterial rRNAs judged by blot hybridizations with probes coding for 23s and 16s rRNAs. The NGF-treated cultures were found to be uncontaminated by mycoplasma by the Mycotect (BRL) cell viability assay.

The identities of the proteins for which these mRNA species may code currently are being investigated using both a cell-free in vitro translation system and a cDNA expression library.

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107.4 MYOGENIC CONVERSION OF AN UNCOMMITTED EXCITABLE CELL FROM A PERIPHERAL NEUROTUMOR RT4. Yasuko Tomozawa, Department of Neurology, Baylor College of Medicine, Houston, TX 77030.

The striated muscle elements have been observed in both cephalic neural crest and neuroectoderm. These phenomena suggest that during development, a population of uncommitted cells in the neural crest may differentiate into neural cells and myoblasts. The neurotumor RT4 system, which includes a stem cell, RT4-AC, an uncommitted excitable cell, RT4-B, and a glial cell, RT4-D, provides a suitable model to study multipotentiality and commitment of neural crest cells (Tomozawa and Sueoka, Proc. Natl. Acad. Sci. USA, 75, 6305, 1978).

These RT4 cells were treated for 24 hours with 10 μM 5-azacytidine, which can activate certain genes by hypomethylation of DNA (Groudine et al., Nature, 292, 311, 1981). By ten days after treatment, an uncommitted excitable cell, RT4-B8, developed myotube-like multinucleation. This new cell line, RT4-B8-AzM, retained the chromosomal marker, $4q^+$, unique for the neurotumor RT4. (Haag, Soukup and Sueoka, Develop. Biol., in press). The RT4-B8-AzM cells showed myotube formation, syntheses of muscle-type myosin and acetylcholine receptor, and showed muscle-like differentiation of excitable membrane properties as expressed by a transition from action potential comprised of Na and Ca components to action potential comprised of only a Na component. The parental cell, RT4-B8, did not express any of these myogenic properties. Moreover, the stem cell, RT4-AC, and glial cell, RT4-D, did not express any of these properties by treatment of 5-azacytidine. Molecular hybridization studies with cDNA probes showed that such non-myogenic genes as β2-casein, albumin and tyrosine hydroxylase were not activated by 5-azacytidine in either RT4-B8-AzM or the parental cell, RT4-B8. 5-azacytidine, therefore, seemed to cause specific activation of genes related to muscle phenotypes in an uncommitted excitable cell. These observations support the myogenic potentiality of mammalian neural crest cells. In view of the action of 5-azacytidine, methylation-demethylation of DNA may be involved in the myogenic conversion. (Supported by NIH-NS15304, ACS-CD1 to Dr. Noboru Sueoka, Dept. Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO.)

107.5 PRODUCTS OF MUSCLE CELLS mRNA TRANSLATION IN XENOPUS OOCYTES ENHANCE DIFFERENTIATION OF NEURONS IN CULTURES. J. Koenig[1-2], Dj Ambroise[1], M. Vigny[3] et D. Schmid[4]. 1 INSERM U. 153 Paris-France, 2 University P. et M. Curie Paris-France, 3 ENS Paris-France, 4 Laboratoire Max Planck de Neurochimie Arcachon-France.

Extracellular matrix components are essential for maintenance and function of neuromuscular synapses. Heparan Sulfate Proteoglycane (HSP) has been shown to effect neurite outgrowth of sympathetic neurons in vitro.

We used Rat primary muscle cells cultivated either in normal medium or in a spinal cord cells conditioned medium. In both cases, mRNA were extracted and injected in Xenopus oocytes. The oocytes are able to post-translationnaly modify and export proteins. We analysed the presence of extracellular matrix components in the oocyte incubation media. Immunochemical detection revealed the presence of collagens, fibronectin and HSP. The synthesis of fibronectin and HSP was enhanced in oocytes microinjected with mRNA from conditioned muscle cells. Furthermore immunofluorescence staining clearly showed an increase of these two components on the surface of muscle cells grown in the conditioned medium. We therefore investigated the possible effect of incubation media of oocytes injected with each of the two types muscle cells mRNA or H_2O on the neuritic elongation of spinal cord cells in culture. Only the incubation medium from oocytes injected with conditioned medium induced a neuritic elongation. Purified HSP from basement membrane triggered the same effect.

Therefore neuronal cells could increase the biosynthesis of HSP like molecules by muscle cells which by a feed-back mechanism would be responsible for the neuritic elongation.

107.6 PLASTICITY OF TYROSINE HYDROXYLASE GENE EXPRESSION IN CULTURED NEURONS. M.C. Fishman, E. Grabczyk*, L. Baizer* E. Summerhill* Howard Hughes Medical Institute and Section on Neurobiology, Dev. Biol. Lab., MGH and Harvard Med. School., Boston, MA 02114.

As part of our investigations into the developmental control of neuronal genes, we are investigating tryosine hydroxylase (TH) mRNA expression in homogeneous cultured neuronal cells. Neurons of the sympathetic nervous system express TH in a highly regulated manner. The amount of neuronal activity, soluble and matrix components, and the age of the animal all influence TH. We have begun investigation of TH mRNA levels utilizing a cDNA probe in cultured neurons of the superior cervical ganglion (SCG) and neuronal cell lines. Dissociated neurons of the neonatal SCG were cultured for periods of up to one month in the absence of background cells and compared to SCG from developing rats at different ages. Neuroblastoma cells of the adrenergic N1E115 line were induced to differentiate using either Bt_2 cAMP or DMSO. Depolarization of cultures was achieved with 20mM KCl. Total cellular RNA was isolated using GTC and CsCl centrifugation and, in some instances, mRNA was isolated on oligo-dT cellulose. After gel electrophoresis RNA was transferred to NC filters and hybridized at 65°C with a labelled insert from a plasmid containing a TH cDNA (Lewis et al, J.B.C., 258, 14632, 1983). TH protein was identified by Western blotting using a highly specific TH antibody.

TH mRNA was identified as approximately 1800 nucleotides in length in cultured SCG, N1E115, and PC12 cells, and in SCG of developing animals. TH mRNA levels increased with time in culture despite little change or a diminution in the number of neurons. Induction of differentation of N1E115 cells in vitro also enhanced TH mRNA levels. On the other hand NGF caused extensive neurite outgrowth from PC12 cells, but did not affect their TH mRNA levels. Activity affected TH levels to a lesser degree. The size of the TH protein remained unchanged at 60K throughout these modulations.

107.7 IN SITU HYBRIDIZATION USING A 3' TERMINAL TRANSFERASE-LABELED SYNTHETIC OLIGONUCLEOTIDE PROBE COMPLEMENTARY TO THE α-MSH CODING REGION OF PROOPIOMELANOCORTIN mRNA. M.E.Lewis,S.Burke*,T.G.Sherman*,R.Arentzen*+, and S.J.Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109 and +E.I.Dupont de Nemours.

In situ hybridization histochemistry can be used to identify cellular sites of peptide biosynthesis as well as study the regulation of gene expression at the single cell level. At present, hybridization probes are typically prepared from cDNA fragments synthesized from mRNA and cloned in bacteria, involving procedures inacessible to most neuroscientists. As an alternative to biosynthesized cDNA probes, we have used chemically synthsized oligonucleotide probes which are complementary to an mRNA of interest (see also Sherman et al, this meeting). We report here that a chemically synthesized 24-mer complementary to the α-MSH coding region of proopiomelanocortin mRNA can be readily labeled and used for in situ hybridization histochemistry. The oligonucleotide was given varying lengths of dC tails by incubating for different time periods (1-30 min) with terminal deoxynucleotidyl transferase and α-labeled (with [^{32}P], [^{35}S], or [^{3}H]) dCTP. Probes with different tail lengths were separated by electrophoresis through a 20% urea gel. The probes were localized by autoradiography or fluorography of the gel with X-ray film, and the bands were cut out of the gel and eluted overnight in buffer containing 500 mM ammonium acetate, 10 mM magnesium acetate, 0.1% SDS and 1 mM EDTA. After ethanol precipitation, solvent removal and resuspension, probes were used for in situ hybridization (see Gee at al, Nature 306:374, 1983) to cryostat-cut sections of pituitary from formaldehyde-perfused rats. As reported by Gee et al, who used 550 base pair probes complementary to most of the proopiomelanocortin mRNA coding region, all intermediate lobe cells were heavily labeled by the $d(C)_n$-tailed α-MSH oligonucleotide probes. This localization indicates that the specificity of the probes was not markedly altered by the addition of a short (n=1-8) dC tail to the 3' end of the oligonucleotide. We are presently attempting to determine the optimum dC tail length to maximize the signal-to-noise ratio, as well as characterize the hybridization of the probes to brain and pituitary mRNA by T_m and Northern blot analyses. We are also using these probes to measure changes in gene expression following exposure to chonic stress or haloperidol treatment. (Supported by NIMH grant MH 15794.)

107.8 THE CO-EXPRESSION OF DYNORPHIN AND VASOPRESSIN: AN IN SITU HYBRIDIZATION AND DOT-BLOT ANALYSIS OF mRNAs DURING STIMULATION. T.G. Sherman*, S.J. Watson, E. Herbert*°, and H. Akil. Mental Health Research Inst., Univ. Michigan, Ann Arbor, MI 48109 and °Dept. Biochem., Univ. Oregon, Eugene, OR 97403.

Previously we have shown that dynorphin and vasopressin (AVP) share a common localization within magnocellular neurons of the rat hypothalamus (Watson et al, Science, 216:85, 1982). The questions remain, however, as to whether or not these two peptide hormones share the same physiological signals for secretion and/or those signals necessary to regulate their biosynthesis. We have used molecular biological tools to answer these questions, and are using both dot-blots for specific mRNA quantitations and in situ hybridization analyses for the localization of specific mRNAs to single magnocellular perikarya. Using this approach, we have examined the relative changes in specific mRNA content and/or distribution for AVP and dynorphin within the paraventricular (PVN) and supraoptic (SON) nuclei while using the physiological manipulation of salt-loading. We had shown previously using poly(A)RNA isolated from single-punched hypothalamic PVN or SON nuclei that a 15-day salt-loading course increases nearly 10-fold the amount of AVP or oxytocin mRNA (Sherman et al, Soc. Neurosci. Abstr. 9: 622, 1983). This manipulation is also effective as both a secretory and biosynthetic stimulus for dynorphin. Using RIAs specific for dyn(1-8) and dyn(1-17), we observed that the neural lobe contents for each of these peptides was depleted by nearly 80% with 4 days of salt-loading. This is correlated with a 3-4 fold increase in dynorphin mRNA as determined by hybridization of a porcine dynorphin probe to total RNA isolated from paired PVN or SON punches in a dot-blot paradigm.

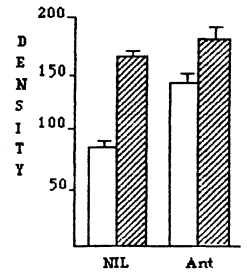
In situ hybridization with [32]P and [35]S labelled oligonucleotide probes has allowed a closer examination of salt-loading at the cellular level. Using a synthetic AVP probe complementary to a 20 base mRNA sequence coding for the C-terminal glycopeptide of the AVP precursor, we can demonstrate autoradiographic labelling of cell bodies limited to the PVN and SON. These cell bodies can be shown to be AVP cells by same section or serial section PAP immunohistochemistry with antisera specific for this same C-terminal glycopeptide. Under the conditions of salt-loading, increased labelling is observed at both the X-ray and NTB2 autoradiographic levels. Attempts to correlate these increases with quantitative dot-blots of punched nuclei mRNAs using this same probe are continuing. This work is supported in part by NIMH grant #MH15794.

107.9 CHANGES IN PITUITARY POMC mRNA LEVELS. J.E. Kelsey, S.J. Watson, and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

A major focus of this laboratory over the past several years has been to study the control of synthesis and release of proopiomelanocortin (POMC) derived peptides. With the advent of recombinant DNA technology it is now possible to study POMC regulation at the level of mRNA. We report here that repeated foot shock stress which induces analgesia raises both NIL POMC mRNA and anterior lobe POMC mRNA content as determined with the "dot blot" method of quantitation.

Male Sprague-Dawley rats weighing 300 grams were subjected to 30 minutes of 1.5 mA shock of 1 sec duration, 1 every 5 sec for 14 days, allowed to rest one day, and sacrificed by decapitation. The pituitaries were rapidly removed, dissected and stored at -70°C. Control animals were unhandled. Total nucleic acids were extracted, spotted on nitrocellulose paper, and probed with 144 base mouse POMC cDNA probe given us by Dr. James Roberts of Columbia Univ. Either 5 or 10% of NIL RNA and 15% of anterior lobe RNA was spotted in duplicate. The blots were then exposed to X-ray film and the density of the spots determined with a densitometer. The results from one such experiment are shown below. Control animals are the hollow bars, stressed animals the stipled bars.

Chronic stress produces a 50-100% increase in NIL POMC mRNA (across two experiments) and a 25-30% increase in anterior lobe content. These increases are accompanied by increases in POMC peptide content in both lobes and biosynthesis changes as indicated by amino acid pulse chase experiments (Shiomi et al., Life Sci., 31:2185, 1982). These increases could be due to either increased rates of transcription, increased stability of POMC mRNA in the cytoplasm, or both. In vitro studies are currently underway to detrmine the role of stability in increased levels of POMC mRNA.



107.10 IN SITU HYBRIDIZATION OF NEUROPEPTIDE mRNA WITHIN THE RAT HYPOTHALAMUS WITH RADIOLABELED SYNTHETIC OLIGONUCLEOTIDE PROBES. L.G. Davis, B. Wolfson*, R. Arentzen*, R.W. Manning*, G.A. Higgins*, Y. Lin* and F. Baldino, Jr. Central Research Dept., E.I. du Pont & Co., Wilmington, DE 19898.

The recent determination of the nucleic acid sequences for rat somatostatin (SS) and vasopressin (VP) mRNA has allowed the synthesis of radiolabeled oligonucleotide probes which can be used to localize these mRNA's in the CNS, in situ. A 46-nucleotide probe complementary to the 3' end of somatostatin mRNA was constructed by ligation of two shorter sequences with T4 DNA ligase. A 48-nucleotide probe, complementary to part of the unique glycoprotein region of vasopressin mRNA, was constructed in a similar fashion. The probes were 5' end labeled (10^8 cpm/µg) with ^{32}P using T4 polynucleotide kinase and γ-^{32}P-ATP, and purified by polyacrylamide gel electrophoresis. For in situ hybridizations, tissue blocks containing the hypothalamus from formalin-fixed, cryoprotected rat brains were serially sectioned, in a transverse plane, beginning at the level of the preoptic area and ending at the level of the paraventricular nucleus. Every other section was slide mounted, deproteinated, delipidated and incubated overnight at room temperature with the radiolabeled probes diluted in hybridization buffer. The following day, these sections were rinsed under stringent conditions, air dried and applied to X-ray film, with and without enhancing screens, for 5-7 days. Alternate sections were processed for peptide immunoreactivity using anti-somatostatin or anti-vasopressin with the avidin-biotin method.

The results demonstrate that the periventricular portions of the preoptic and paraventricular hypothalamus are particularly rich in both SS-immunoreactive neurons and SS mRNA, while vasopressin immunoreactive neurons and VP mRNA are concentrated within the supraoptic and suprachiasmatic nuclei and the magnocellular portion of the paraventricular nucleus. More importantly, however, the results demonstrate the feasibility of rapidly identifying specific neuropeptide mRNA in discrete brain regions in situ, autoradiographically. The regulation of gene transcription and neuropeptide expression in the CNS can now be studied at the molecular level.

107.11 ISOLATION OF A CLONE CODING FOR THE ALPHA SUBUNIT OF A MOUSE ACETYLCHOLINE RECEPTOR. J. Boulter*, K. Evans*, M. Ballivet*, D. Goldman*, W. Luyten, G. Martin*, P. Mason*, S. Stengelin*, S. Ueno*, S. Heinemann, and J. Patrick, Molecular Neurobiology Laboratory, The Salk Institute, San Diego, CA 92138.

BC_3HI is a non-fusing, mouse muscle cell line that differentiates in culture. In the differentiated state the cells synthesize an acetylcholine receptor with the pharmacological properties of a muscle nicotinic receptor. We have purified RNA from this cell line and used size fractionated poly A^+ selected RNA as template for synthesis of cDNA. First strand synthesis was primed with oligo-dT and second strand synthesis was achieved by treatment of the heteroduplex with the endonuclease RNase H followed by DNA polymerase I. The double stranded cDNA was tailed with deoxycytosine residues and annealed with PBR322 cut with Pst I and tailed with deoxyguanosine residues. The plasmid was used to transform DH-1 and a library of approximately 100,000 clones obtained. The library was screened with a fragment of a genomic clone containing sequences coding for the putative acetylcholine binding site of the alpha subunit of chicken acetylcholine receptor. We obtained a plasmid with a 1600 bp insert which contains a nucleotide sequence coding for the mature alpha subunit of BC_3HI acetylcholine receptor. The amino acid sequence deduced from the nucleotide sequence of this clone reveals substantial homology with the alpha subunit found in Torpedo electric organ and human and calf skeletal muscle. The clone has been made radioactive by nick translation and used to identify RNA species of about 2 Kb in poly A^+ selected RNA obtained from innervated diaphragm, denervated diaphragm, log phase BC_3HI, differentiated BC_3HI and both mouse and rat brain.

107.12 EFFECT OF MUSCLE DENERVATION ON LEVELS OF mRNA CODING FOR ALPHA SUBUNIT OF ACETYLCHOLINE RECEPTOR. D. Goldman*, J. Boulter*, J. Patrick and S. Heinemann. Molecular Neurobiology Laboratory, The Salk Institute, San Diego, CA 92138.

Study of the acetylcholine receptor at the vertebrate neuromuscular junction allows one to analyze the influence of nerve on the properties and distribution of a neuroreceptor. In the adult innervated muscle acetylcholine receptor is localized at the neuromuscular junction. Denervation of adult muscle results in a dramatic increase in the amount of extrajunctional receptor. Inhibition of RNA and protein synthesis prevent this increase in extrajunctional receptors (1). These data imply the motorneuron influences the expression of the gene coding for the acetylcholine receptor. Alternatively some other molecule involved in generating functional receptor may be regulated upon muscle innervation.

We have investigated the effect of denervation on the levels of mRNA coding for the acetylcholine receptor. The left hemidiaphragm of adult rats was denervated by sectioning the phrenic nerve. Five days after denervation left and right hemidiaphragms were isolated. Poly A+ RNA was purified and size fractionated on denaturing formaldehyde agarose gels and transferred to nitrocellulose membrane. RNA corresponding to acetylcholine receptor was identified by hybridizing with nick translated mouse alpha subunit cDNA. This cDNA clone was obtained from the mouse muscle cell line BC3HI and contains the coding sequences for the alpha subunit of the acetylcholine receptor. A band of poly A+ RNA, about 2 kilobases in length, hybridized with the cDNA probe. Denervation of hemidiaphragms results in approximately a seven-fold increase in this RNA species.

(1) Fambrough, D.M. (1969) Science 168:372-373.

107.13 MOLECULAR CLONING OF BRAIN-SPECIFIC MESSENGER RNAs.
T.L. Wood*, D.L. Kaufman*, and A.J. Tobin (SPON: M. Wexler). Department of Biology and Molecular Biology Institute, University of California, Los Angeles, CA 90024.

We have prepared intact high molecular weight messenger RNA from specific regions of human, cat, and mouse brain. From these mRNA populations we have constructed recombinant cDNA libraries in the plasmid pBR322 and in the expression vector lambda gt-11.

The pBR-based libraries of cat and human cerebellar mRNA sequences consist of 10^4 - 10^5 individual recombinants. We are screening these libraries with cDNAs copied from the mRNAs of individual brain regions and of non-neural tissue. These experiments will make it possible to determine which sequences are preferentially expressed in the brain, and which are differentially expressed in the cerebellum, striatum, and occipital cortex.

Each lambda gt-11 "expression library" consists of more than $2x10^6$ recombinants and should contain essentially all the mRNA sequences expressed in that region. These libraries are being used for the isolation of specific genes of neurobiological interest by immunological screening with appropriate serum antibodies and ^{125}I-protein A. We are presently screening libraries derived from the mRNAs of cat occipital cortex and human neostriatum with serum antibodies for glutamic acid decarboxylase and S-100, as well as with autoantibodies from patients with chronic hepatitis.

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107.14 IN SITU mRNA HYBRIDIZATION FOR DETECTION OF REGION-SPECIFIC GENE EXPRESSION IN BRAIN.
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Recent estimates of brain poly A(+) RNA sequence complexity suggest that it contains sufficient information to encode 30,000 or more polypeptides. Many of these molecules may be shared by discrete subpopulations of neurons as a consequence of developmental interactions, neurotransmitter choice, common functional attributes, or anatomical connectivity. In an effort to identify such proteins, investigators have raised monoclonal antibodies against brain homogenates and screened for cellular and/or regional specificity with immunohistochemical methods. However, a potentially more powerful approach to the identification of cell- or region-specific proteins is through the use of recombinant DNA hybridization methodology.

Previously, we have screened brain cDNA against non-neural tissues such as kidney and liver, then used the purified brain-specific probes for in situ hybridization of mRNAs expressed by neuronal cell types within the hippocampus, cerebellum, and other regions of the mouse central nervous system (Branks and Wilson, in press). Our present efforts have been directed towards identifying limbic-specific mRNA species through the use of cDNA subtractive hybridization methods. Single-stranded cDNA generated from amygdaloid and hippocampal mRNA are first hybridized to an excess of cerebellar and cerebral mRNA. Hydroxyapatite chromatography is then used to isolate cDNA sequences which do not hybridize to cerebellar and cerebral mRNA, and thus represent mRNAs which are not expressed in, or are present in low abundance in these non-limbic regions. These selected, limbic-enriched cDNA sequences are then cloned and used in in situ hybridization experiments to localize and map the distribution of limbic mRNAs in mouse and rat brain. In situ hybridization probes consist of nick translated cDNAs, or SP6 polymerase RNA transcripts from template DNA, which are applied to deproteinized and defatted cryostat sections for hybridization at 37°C. ^{32}P-labeled probes are combined with x-ray film for rapid analysis of limbic mRNA distribution, while ^{35}S and ^{3}H permit finer localization at the single cell level. It is anticipated that isolation and characterization of cell-, region- and system-specific mRNA transcripts may provide insight into the development and coordination of related neuronal subpopulations within mammalian brain.

107.15 ENHANCED SENSITIVITY OF NUCLEIC ACID HYBRIDIZATION; STUDIES ON BETA NERVE GROWTH FACTOR. L.F. Reichardt and D.L. Shelton (Spon: Y.N. Jan). Div. Neurosci., UCSF, San Francisco, CA. 94143

Hybridization of a labelled nucleic acid probe to nucleic acid bound to a solid phase is one of the most important and commonly used techniques in modern molecular biology. In attempting to measure the extremely low levels of mRNA encoding beta Nerve Growth Factor (NGFmRNA) which are present in physiologically relevant tissues (Shelton and Reichardt, these abstracts), we have developed methods which substantially improve the sensitivity of detecting both RNA and DNA which have been transferred to nitrocellulose after separation by gel electrophoresis. We have found that by optimizing many parameters of the electrophoresis, transfer, hybridization, and washing conditions, we can easily detect ten femtograms of a specific, hybridizing sequence. This has allowed us to detect the NGFmRNA in a single rat iris.

Some of the variables we found to be important in increasing sensitivity were: 1)We altered the geometry of the electrophoresis gels so that the area on the nitrocellulose to which the nucleic acid is bound is minimized, ie. thick gels with narrow lanes. 2)There was a large improvement in sensitivity when a probe labelled with ^{32}P by nick translation was replaced by the single-stranded probe made by primed synthesis on sequences cloned into M13 vectors. These probes take no more than four hours to make and give at least a 15 to 20 fold fold increase in sensitivity. 3)For RNA blots we found that formaldehyde denaturing gels transferred without pre-soaking gave much better results than glyoxal gels or gels which had been equilibrated with transfer buffer. 4)Comparing conditions for RNA transfers showed that in 50% formamide, 5X SSPE, hybridization at 50°C gave much higher signals than than 42°C, and that washing in 0.1X SSC at 60°C reduced background compared to washing at 50°C.

Using these methods, in a twenty-four hour exposure, we can detect an mRNA which is as little as 2 parts in 10^9 by weight of total polyA$^+$-RNA. When one microgram of total genomic DNA from human, mouse, or many other mammals is digested with a restriction enzyme, electrophoresed, transferred, and hybridized as described, we obtain intense autoradiographic bands corresponding to the single copy of the NGF gene after several hours of exposure. The techniques which afford this increase in sensitivity should find wide utility.

107.16 REGIONAL DISTRIBUTION OF POLY A+ RNA IN RAT BRAIN. H.-T. Kao*, S.I.Walaas, and W.C.Wallace. The Rockefeller University, New York, NY 10021.

We are investigating the diversity of gene expression within the brain. A complementary DNA (cDNA) library has therefore been made by obtaining poly A+ RNA from the whole adult rat brain and utilizing the λgt10 molecular cloning vector. In order to determine the regional distribution of the poly A+ RNA which this cDNA library represents, a random selection of clones were probed with brain regional RNA. Adult rat brains were microdissected into 14 distinct regions according to Walaas et al. (J. Neurosci., 3:291, 1983). Total RNA was prepared from each region and converted into ^{32}P-cDNA probes. Similarly, cDNA probes were made from RNA of other tissues. The clones were then analyzed by hybridization with each of these brain region and peripheral tissue probes for +/- screening. We found that approximately 20% of the brain clones hybridized to the adrenal probe (including most, but not all, of the most common poly A+ RNA). Furthermore, the brain clones were found to hybridize differentially to the various brain region probes: the majority of the clones hybridized to some, but not all, of the regional probes, and some of these clones showed a regional specificity, hybridizing to only one regional probe and to none of the others. These results suggest that some poly A+ RNA is regionally distributed within the brain. We are presently using these clones as probes in Northern analyses of RNA from the various regions of the brain to definitively establish a regional specificity. In addition, we have characterized regional poly A+ RNA distribution by Rot analysis. Although this technique probably does not represent the entire mRNA population, our results suggest a greater complexity of total brain poly A+ RNA compared to poly A+ RNA isolated from various brain regions. This observation is consistent with a regional distribution of poly A+ RNA within the brain.

107.17 LOCALIZATION OF BRAIN-SPECIFIC mRNAs IN MOUSE CEREBELLUM BY IN SITU HYBRIDIZATION. C.W. Wuenschell*, D.L. Wandres*, and A.J. Tobin, Department of Biology and Molecular Biology Institute, University of California, Los Angeles, CA 90024

In order to determine whether individual types of neurons in the cerebellum express different sets of genes, we have used in situ hybridization to determine the cellular distribution of individual messenger RNAs. We have examined the distribution of alpha-tubulin mRNA as well as two cloned mRNA sequences found in the library prepared by Sutcliffe, et al. (Cell 33, 671, 1983). One of the latter two sequences, p1B236, is thought to encode a neuropeptide whose cellular distibution has been determined by immunocytochemistry.

In situ hybridization was performed on 10 um frozen parasaggital sections of the cerebellum of female DBA mice. Sections were post fixed in 3:1 ethanol:acetic acid, and pretreated with 0.2N HCl and 1 ug/ml proteinase K. Hybridization probes were labeled with ^{125}I dCTP by nick translation to a specific activity of approximately 10^8 cpm per microgram. Hybridizations were performed overnight at room temperature in 50% formamide, 600mM NaCl and 10% dextran sulfate. Silver grains per unit area were counted for the molecular layer, Purkinje cell layer, granular layer, and the underlying white matter.

Our preliminary data indicate that there is some differential distribution of these sequences. The alpha-tubulin mRNA sequence and the mRNA sequence represented by clone p1B236 have the highest concentration in the Purkinje cell layer, whereas the other brain-specific mRNA appears to be equally concentrated in Purkinje and granular layers.

We are now preparing single stranded RNA probes for these sequences using the newly developed plasmid vectors containing a promoter sequence for the RNA polymerase of the Salmonella phage sp6. We anticipate that the use of these RNA probes, in combination with ribonuclease digestion to reduce background binding, will substantially improve the sensitivity and precision of our studies.

This work was supported by grants to AJT from the NIH (#NS10356) and from the Dystonia Medical Research Foundation. CWW is supported by a USPHS Training Grant in Genetic Mechanisms (#GM7104).

UPTAKE STORAGE AND SECRETION

108.1 DIFFERENTIAL EFFECTS OF NEUROLEPTIC AGENTS ON DOPAMINE RELEASE IN STRIATUM AND NUCLEUS ACCUMBENS IN VIVO. C.D. Blaha* and R.F. Lane, Institute of Neuroscience, University of Oregon, Eugene OR 97403.

It has been hypothesized that extrapyramidal side effects (EPSE) of neuroleptic agents originate from impairment of nigrostriatal dopamine (DA) transmission, whereas their antipsychotic properties may be based on similar actions in the limbic system. Although some studies support this idea by demonstrating biochemical regional selectivity in the limbic system, other studies have failed to confirm this regional selectivity. We have previously shown that in vivo voltammetric techniques can directly monitor changes in DA release in rat striatum in response to typical neuroleptics such as haloperidol (HAL) and chlorpromazine (CPZ) (Eur. J. Pharm., 98:113, 1984). The present study was conducted to examine the effects of a wide range of neuroleptics on DA release in nucleus accumbens and striatum, sites of limbic and nigrostriatal DA neuronal terminals, respectively.

Experiments were conducted with recording electrodes chronically implanted in both the accumbens and the striatum of conscious, freely moving male Sprague Dawley rats. Modified graphite recording electrodes (ca. 150 µm) selective for catecholamines (Blaha and Lane, 1983) were employed throughout the study. Serial 1 sec chronoamperometric measurements were obtained at 1 min intervals using an applied potential of 0.25V. Administration of HAL, CPZ, (+)-butaclamol or l-sulpiride produced dose-dependent increases in the release of DA in both regions; l-sulpiride elicited a more pronounced effect in accumbens. These effects appeared selective for neuroleptics since neither promethazine, d-sulpiride nor (-)-butaclamol produced any changes in either area. In contrast, clozapine or thioridazine administration caused dose-dependent increases in DA release in accumbens but failed to release DA in striatum. Interestingly, metoclopramide treatment stimulated DA release in striatum in a dose-related manner but was without effect in accumbens. Our results provide direct in vivo evidence that the ability of neuroleptics to elicit EPSE is mediated, at least in part, by their effect on DA activity in the striatum. The paradoxical effects of metoclopramide on DA release may be related to its high incidence of EPSE and its apparent lack of antipsychotic action. Results of experiments testing the effects of chronic neuroleptic treatments on regional DA release will also be presented. Supported by USPHS Grants NS 13556 and MH 17148.

108.2 IN VIVO ANALYSIS BY PUSH-PULL PERFUSION OF HYPOTHALAMIC MONOAMINE RELEASE EVOKED BY NEUROACTIVE SUBSTANCES. Amir H. Rezvani*, P. Huttonen* and R.D. Myers, Departments of Psychiatry and Pharmacology and Center for Alcohol Studies, University of North Carolina School of Medicine, Chapel Hill, N. C. 27514.

To examine the characteristics of the release of putative monoamine neurotransmitters, push-pull guide cannulae were implanted stereotaxically above the diencephalon of the rat. At the intended site of perfusion within the hypothalamus, two of three radioactive monoamines, dopamine (DA), norepinephrine (NE) or serotonin (5-HT), were microinjected in order to double-label presynaptic stores of the respective amine. During a sequence of repeated push-pull perfusions of the site with an artificial CSF at 25 µl/min, a benzodiazepine receptor antagonist, opioid receptor agonist, ß-endorphin, enkephalin, ethanol or acetaldehyde was added to the CSF perfusate. To verify the reactivity of the site in terms of any change in the evoked release of amine, 25 mM excess K^+ ions also was added at the end of a series of perfusions. The results show that selected neuroactive factors can selectively inhibit or enhance the release of a monoamine from the hypothalamus in the freely moving rat. However, the nature of the release is dependent not only on the concentration of the test substance but also on the anatomical site of hypothalamic perfusion. To illustrate, ethanol incorporated in the CSF perfusate augments the rat's skin temperature, lowers body temperature at the same time that the hypothalamic release of both NE and 5-HT is attenuated. A systemic control injection of ethanol produces virtually the same effects on monoamine efflux. The first metabolic intermediary of ethanol, acetaldehyde, perfused at the same site exerts the opposite pharmacological effect. ß-carboline releases both NE and 5-HT, whereas morphine can augment the release of either NE or 5-HT or both as a change in the body temperature of the rat occurs. Naltrexone perfusion ordinarily blocks these opioid-induced responses. An enkephalin exerts an effect similar to that of morphine in terms of concurrent NE and 5-HT release and body temperature change. Overall, our findings demonstrate that the in vivo activity of this class of neurotransmitter reflects the mediation of functional responses, as demonstrated by their concomitant and localized release, produced by specific factors acting on the hypothalamus.

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108.3 SIMULTANEOUS RELEASE OF ENDOGENOUS AND [^{3}H]DOPAMINE. Jill B. Becker. Department of Psychology and Neuroscience Laboratory Building, The University of Michigan, Ann Arbor, Michigan 48109.

To study the release of dopamine (DA) from brain tissue, most investigators take advantage of the high affinity uptake system to preload [^{3}H]DA into DA terminals. The implicit assumption is made that the release of [^{3}H]DA is proportional to the release of endogenous DA. This assumption is tested in the experiments reported here, in which the release of endogenous DA from striatal tissue is directly compared with the release of preloaded [^{3}H]DA or [^{3}H]DA newly synthesized from [^{3}H]tyrosine (tyr).

Striatal tissue was incubated for 30 min with either [^{3}H]DA or [^{3}H]tyr. The tissue was washed and placed into superfusion chambers (flow rate 100 µl/min). Effluent samples were collected over 5 min intervals. After a 30 min baseline period, DA release was stimulated with either 10 µM amphetamine (AMPH) or 60 mM KCl (K+). At the conclusion of the superfusion, tissue and effluent samples were assayed for DA content by HPLC-EC. [^{3}H]DA was determined by collecting the DA peak as it eluted, radioactivity was measured by liquid scintillation counting. Total radioactivity was also determined for each sample. Release of endogenous DA, [^{3}H]DA and total radioactivity were normalized for the amount of tissue in the chamber and expressed as a per cent of their respective total pools.

[^{3}H]DA PRELOADING. In striatal tissue preloaded with [^{3}H]DA, the AMPH-stimulated increase in endogenous DA release was significantly greater than the increase in [^{3}H]DA release ($p<0.025$) or the increase in total radioactivity ($p<0.007$). However, there were no significant differences between endogenous DA release and [^{3}H]DA release following K+ stimulation. In addition, the ratio of [^{3}H]DA/endogenous DA decreased significantly over the time period of the superfusion ($p<0.02$).

[^{3}H]tyr PRELOADING. In a preliminary experiment, the AMPH-stimulated increase in the release of endogenous DA and [^{3}H]DA newly synthesized from [^{3}H]tyr were proportional. Further experiments are in progress.

These experiments suggest that the release of preloaded [^{3}H]DA may not be the best index of endogenous DA release since, the ratio of [^{3}H]DA/endogenous DA varies with time and across pharmacological manipulations. The use of [^{3}H]DA newly synthesized from [^{3}H]tyr may be a more accurate index of endogenous striatal DA release.

108.4 RELEASE OF ^{3}H-DOPAMINE FROM THE CAROTID BODY BY INCREASING K^+ IN THE SUPERFUSION MEDIA. L.Almaraz, A. Obeso* and C. Gonzalez. Dept. de Fisiología y Bioquímica. Facultad de Medicina. Univ. de Valladolid. Spain.

The carotid body (c.b.) is a secondary receptor in which type I cells are presynaptic to the sensory nerve endings of the carotid sinus nerve (c.s.n.). These cells contain dopamine (DA) and probably also some noradrenaline. Natural stimulation of the c.b., i.e. decrease of O_2 tension in its environment, induces release of DA in a dose-dependent fashion only if Ca^{++} is present. Nothing is known about the mechanism by which hypoxia induces the entry of Ca^{++} and then the release of DA. In this study we have explored the effect of increasing extracellular K^+ (K_e^+) in the media upon the release of DA and the interaction between high K_e^+ and hypoxia.

The c.bs. were incubated with ^{3}H-tyrosine for 3 h at 37°C and later mounted in a superfusion system which allows recording of c.s.n. activity and collection of the superfusates for ulterior analysis on ^{3}H-DA or ^{3}H-metabolites present in them.

The release of ^{3}H-DA by high K_e^+ has its threshold at about 20 mM and plateaus at 60-80 mM, as a whole the relation K_e^+ to relase has a sigmoid shape. The release is Ca^{++} dependent and does not occur when sucrose is the substitute for NaCl. When the threshold concentration of K_e^+ is used in low pO_2 media the release of ^{3}H-DA is greatly enhanced.

These results suggest that the general scheme of stimulus-secretion coupling is applicable to the c.b. when K_e^+ is the stimulus and that hypoxia may also induce the release via a depolarization of the type I cells. This interpretation is in agreement with ultrastructural studies in which is has been shown an increase of exocytotic profiles when the c.b. is incubated in high K_e^+ media but is in disagreement with other reports in which the membrane potential of type I cells has been found to be independent of K_e^+ and that hypoxia does not induce consistently depolarization of type I cells.

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108.5 EVIDENCE FOR AN EXCHANGE-DIFFUSION MODEL OF NOREPINEPHRINE (NE) RELEASE USING IN VIVO VOLTAMMETRY. S. Howard-Butcher, C.D. Blaha* and R.F. Lane. Dept. of Pharmacology and MRRC, UCLA, Los Angeles, CA 90024 and Institute of Neuroscience and Depart. of Chemistry, University of Oregon, Eugene, OR 97403

Xylamine (Xyl) is a nitrogen mustard which selectively inhibits the release of NE, while having no effect on dopamine (DA) release (Howard-Butcher et al, Neurosci. Abst., 1983). Xyl appears to exert its effect by irreversibly binding with the NE carrier (Dudley et al, JPET 217:834, 1981). The inhibition of NE release in the cortex reaches a maximum within 2 hours after Xyl injection (12.5 mg/kg i.p.) at which time the levels of NE in the cortex are only reduced by 24% of control. This inhibition of release produced by Xyl was examined using in vivo voltammetry.

Male Sprague-Dawley rats were injected with chloral hydrate (400 mg/kg i.p.) and placed in a stereotaxic instrument. Surface modified electrodes, selective for catecholamines were inserted into the cortex.

Chronoamperomatric measurements were made by applying a potential of +0.25V v. Ag/Agcl for 1 sec. with an interval of 1 min. between measurements.

Baseline values of NE release in the cortex were obtained. Animals were then injected with Xyl (12.5 mg/kg i.p.). Two hrs after Xyl treatment, animals were injected with amphetamine (Amph) (1 mg/kg i.v.) or given a local infusion of KCL (30 mM). For local injection of KCl the working electrode was attached to a microsyringe and this assembly inserted into the cortex (tip separation = 0.5 mm).

In control animals Amph produced a 25% increase in NE release from cortex. There was no effect on NE release in Xyl treated animals. In contrast, KCl (1 µl in 30 sec) depolarization produced a rapid increase in NE release.

The effect of a central adrenergic antagonist, yohimbine (1 mg/kg i.v.) was also examined. The effect of yohimbine was similar to that of KCl, in that it produced a rapid increase in the release of NE.

Xyl pretreatment does not block depolarization-induced release of NE and does not appear to be working through an alpha receptor. The results obtained in this study are consistant with the notion that the release mediated by Amph is the result of an exchange-diffusion mechanism involving the NE uptake system.

This work was supported by USPHS Grants HD05615, NS13556, and MH17148.

108.6 ACUTE EFFECTS OF 6-HYDROXYDOPAMINE IN RAT HIPPOCAMPUS IN VITRO. Robert P. Yasuda*, Nancy R. Zahniser and Thomas V. Dunwiddie. (Spon: K.A. Heidenreich) Dept. Pharmacology, Univ. Colo. Health Sciences Center, Denver, CO 80262.

The selective neurotoxicity of 6-hydroxydopamine (6OHDA) has been well-characterized in the peripheral and central nervous systems. However, the short-term effects of this neurotoxin in the CNS are relatively unknown. We have compared the acute effects of in vitro treatment with 6OHDA on the rat hippocampal slice preparation to those observed following 6OHDA lesions in the intact animal. The effects of 6OHDA were assessed electrophysiologically, by high affinity [^{3}H]norepinephrine ([^{3}H]NE) accumulation, and by HPLC determination of NE levels in the hippocampus.

Hippocampal slices were treated for 10 min with 6OHDA (100 uM) and allowed to recover for 1 hr, at which time testing was begun. This treatment alone had no effect on evoked potentials in the CA1 region of the in vitro hippocampal slice. However, subsequent perfusion with 0.5 uM NE produced a maximal increase in the population spike amplitude in 6OHDA treated slices, but had no effect on controls. In contrast, acute 6OHDA lesions did not increase responsiveness to a subthreshold concentration of isoproterenol, a beta-adrenergic receptor agonist that is not a substrate for neuronal reuptake. These data suggest that the increased responsiveness to NE was due to the loss of reuptake rather than to post-synaptic supersensitivity. To directly assess the effect of 6OHDA on NE transport, hippocampal slices treated acutely with 6OHDA were examined for changes in high affinity [^{3}H]NE accumulation. One hour following treatment with 6OHDA, uptake of [^{3}H]NE was decreased by 86%, and there was a concomitant 70% decrease in NE levels compared to untreated slices. These electrophysiological and biochemical effects were qualitatively similar to those observed 2 weeks following intraventricular infusion of 6OHDA or systemic administration of the selective noradrenergic nerve toxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4). The potentiation of the electrophysiological effects of subthreshold concentrations of NE, the decrease in [^{3}H]NE accumulation, and the decrease in NE levels in hippocampal slices treated acutely with 6OHDA suggest that this neurotoxin can rapidly disrupt normal function in noradrenergic nerve terminals.

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108.7 AUTORADIOGRAPHIC LOCALIZATION OF ^{3}H-NOMIFENSINE IN THE RAT AND HUMAN BRAIN. B. Scatton, A. Dubois*, A. Camus*, N.R. Zahniser and M.L. Dubocovich. Synthélabo-LERS, Bagneux 92220 (France), Dept. Pharmacol. Univ. Colorado Med. Sch. Denver, CO 80262 and Northwestern Univ. Med. Sch., Chicago IL 60611.

^{3}H-Nomifensine, a potent dopamine uptake inhibitor, has been used successfully to label a site associated with the neuronal uptake of dopamine in striatal membranes (Dubocovich and Zahniser, Neurosci. Abst. 164.2, 1983). In the present study, we have used the method of quantitative autoradiography to localize and quantify ^{3}H-nomifensine binding sites in the rat and human brain. Slide-mounted brain sections (16 µm thick) were incubated for 1 hr at 0°C in a Tris/HCl buffer solution (pH 7.4) containing 120 mM NaCl, 5 mM KCl and 3H-nomifensine (S.A. 44 Ci/mmol, NEN, 2.5 - 100 nM). Non specific binding was defined by 100 µM benztropine. Sections were rinsed for 1 min and then for 2.5 min in fresh buffer. Tissue sections were exposed to tritium-sensitive film for 4 weeks at 0°C. Saturation studies revealed a single ^{3}H-nomifensine binding site in rat and human caudate nucleus with a Kd of 50 nM. Specific binding accounted for 60% of the total binding in the presence of 10 nM ^{3}H-nomifensine. Displacement of sodium-dependent ^{3}H-nomifensine binding in rat striatal slices by different monoamine uptake inhibitors correlated well with their capacity to inhibit dopamine uptake in striatal synaptosomes. Regional distribution of ^{3}H-nomifensine in rat brain coronal sections revealed highest binding in the striatum, nucleus accumbens and olfactory tubercle, intermediate binding in the septum, hippocampus, frontal and cingulate cortex and lower binding in various thalamic nuclei and occipital cortex. Only in the latter two areas was ^{3}H-nomifensine binding displaced by desipramine (10 µM). A marked reduction of ^{3}H-nomifensine binding was observed in the striatum, olfactory tubercle, nucleus accumbens and cingulate cortex but not in parietal cortex 3 weeks after 6-hydroxydopamine-induced lesion of the ascending dopaminergic bundles. Heavy ^{3}H-nomifensine binding was also found in normal human caudate nucleus and putamen sections. The ^{3}H-ligand binding density was diminished in patients suffering from Parkinson's disease and supranuclear palsy.
These results support the view that ^{3}H-nomifensine is a suitable ligand to label the dopamine transporter complex (and consequently dopaminergic terminals) in rat and human postmortem brain regions.

108.8 BINDING OF XYLAMINE, A CATECHOLAMINE UPTAKE INHIBITOR, WITH SYNAPTIC MEMBRANE PROTEINS. S.D. Cushing*, L.A. Waggaman*, E.A. Mulliez* and A.K. Cho* (SPON: L. Kruger). Dept. of Pharmacol. and Brain Res. Inst., UCLA Sch. of Med., Los Angeles, CA 90024.

Xylamine [(XYL), N-2-chloroethyl-N-ethyl-2-methylbenzylamine], an irreversible inhibitor of norepinephrine (NE) uptake, has been shown to inhibit the uptake of dopamine (DA) into rat striatal synaptosomes (P_2) with an I_{50} of 0.5 µM. Because the striatum is much richer in DA terminals than the cortex or hypothalamus is in NE terminals, the striatum would be the tissue of choice for the isolation and characterization of proteins involved in catecholamine uptake. We have protected against ^{3}H-XYL (0.2 µM, S.A. = 19 Ci/mmol) binding by coincubating cortical, striatal and hypothalamic P_2 with various reversible uptake inhibitors. Following ^{3}H-XYL exposure, the P_2 was lysed and synaptic plasma membranes (SPMs) were isolated by differential centrifugation. In all three tissues, the reversible uptake inhibitors, DMI, amphetamine, cocaine, bretylium and benztropine, protected against ^{3}H-XYL binding to the SPMs by 50 to 80%. The proteins in these preparations were analyzed by SDS polyacrylamide gel electrophoresis (SDS PAGE) followed by fluorometric detection of radioactivity. The fluorographs showed only two major bands of radioactivity in all three tissues; these bands corresponded to molecular weights of about 50K and 30K daltons. Coincubation with the uptake inhibitors resulted in a reduced intensity of these bands.

We have also exposed SPMs to ^{3}H-XYL alone or in the presence of reversible uptake inhibitors. Bretylium (10 µM) inhibited the binding of 0.01 µM ^{3}H-XYL to cortical and striatal SPMs by 15% when coincubated in buffer with 140 meq K^+ instead of Na^+. Benztropine (10 µM) inhibited ^{3}H-XYL binding to striatal SPMs by 50%. Fluorographs of cortical, striatal and hypothalamic SPMs exposed to 0.5 µM ^{3}H-XYL showed only the 50K band. These results show that at pharmacological concentrations XYL is selectively alkylating SPM proteins. These proteins may be relevant to neuronal catecholamine uptake. Supported by USPHS grant MH23839.

108.9 XYLAMINE-INDUCED ENHANCEMENT OF NORADRENERGIC NEUROTRANSMITTER FUNCTION IN THE RAT PINEAL GLAND. K.A. Haak*, R.L. Terry, T. Swink*, P.L. Garvey*, D.M. Bronstein*, and L.D. Lytle. Laboratory of Psychopharmacology, Department of Psychology, University of California, Santa Barbara, CA 93106.

Xylamine (N-2'-chloroethyl-N-ethyl-2-methylbenzylamine) appears to be a specific and irreversible inhibitor of neuronal norepinephrine uptake [J.B. Fischer and A.K. Cho, J. Neural Transmission 220: 117 (1982)]. Since little is known about the possible functional consequences of xylamine-induced alterations in noradrenergic neurotransmission, the present experiments were undertaken to determine whether xylamine might influence noradrenergic control mechanisms important for the synthesis of the pineal gland hormone, melatonin. Norepinephrine release from postganglionic, sympathetic neurons innervating the pineal gland increases the activity of the enzyme, N-acetyltransferase (NAT), which appears to rate-limit pineal gland melatonin synthesis under some conditions, via stimulation of a β-noradrenoceptor linked, cyclic AMP dependent mechanism. Hence, xylamine might increase pineal gland enzyme activity by increasing synaptic concentrations of norepinephrine as a result of its nerve terminal neurotransmitter uptake inhibitory properties.

Different groups of male albino rats, exposed to a 12:12 hr light:dark cycle, were killed during the middle of the light phase. Pineal glands were removed and incubated for a 4-hr period with different concentrations of xylamine (10^{-5}, 5 x 10^{-5}, or 10^{-4} M) and then assayed for possible changes in NAT activity as previously described [A. Altar, T.P. Motroni, and L.D. Lytle, J. Neural Transmission 58: 231 (1983)]. In other experiments, pineal glands obtained from normal intact rats or ones previously subjected to cauterization of the postganglionic fibers innervating the gland were incubated with xylamine, or were coincubated with xylamine (10^{-4} M) and the β-noradrenoceptor antagonist drug, propranolol (10^{-4} M). Xylamine produced concentration dependent increases in pineal gland NAT activity in neurologically intact animals, but these drug-induced changes in enzyme activity were absent in animals cotreated with propranolol, or in ones whose pineal glands had previously been denervated.

Taken together, these data are consistent with the notion that xylamine acutely increases noradrenergic neurotransmitter mechanisms by elevating synaptic concentrations of the neurotransmitter via an uptake inhibiting action exerted on noradrenergic presynaptic nerve terminals. (Supported by NIMH grant MH-31134).

108.10 LABELING OF PROTEINS IN PC12 BY XYLAMINE (N-2-CHLOROMETHYL-N-ETHYL-2-METHYLBENZAMINE), A PUTATIVE AFFINITY REAGENT FOR THE CATECHOLAMINE CARRIER. M. Koide* A.K. CHO* and B.D. Howard (SPON: S. Roberts). Depts. of Biol. Chem. and Pharmacol. and Brain Res. Inst., Sch. of Med., Univ. of California, Los Angeles, CA 90024.

Xylamine (XYL) is a nitrogen mustard that covalently binds to nucleophilic groups and that blocks catecholamine (CA) uptake into neurons (Mol. Pharmacol. 21;380, 1982). We have used XYL to characterize CA transport into PC12 cells, which take up CA by a Na^+-dependent, cocaine-sensitive system. XYL inhibited norepinephrine (NE) uptake by PC12 (IC_{50} = 10 µM). The inhibition was irreversible and maximum inhibition occurred after a 30 min treatment. Pretreatment with 10 µM XYL did not cause inhibition of NE uptake if 10 µM cocaine or 100 µM NE were also present during the pretreatment period, or if the XYL-containing buffer lacked Na^+. These results indicate that XYL must interact with the NE carrier in order to inhibit NE uptake. PC12 accumulated [^{3}H]XYL. This uptake had Na^+-dependent and Na^+-independent components. The Na^+-dependent uptake was a saturable process with a Km of 13 µM and Vmax of 36.8 pmol per min per mg protein and was inhibited by cocaine (IC_{50} = 0.6 µM) and by NE (IC_{50} = 1 µM). Several membrane-associated polypeptides became prominently labeled when intact PC12 cells were incubated with [^{3}H]XYL; these polypeptides have molecular weights of 17K, 29K, 31K, 53,K and 84K daltons, respectively. A M_r 41K polypeptide was also often heavily labeled. In addition, several other polypeptides were labeled less prominently. The labeling of all the polypeptides was markedly decreased when the incubation with [^{3}H] XYL occurred in the presence of 10 µM cocaine, Na^+-free incubation buffer, gramicidin D (which collapses Na^+ gradients), or at 0°C. These results indicate that XYL must be transported into the cells in order for covalent binding to cellular proteins to occur. When the incubation with [^{3}H]XYL occurred with a cell homogenate rather than with intact cells the M_r 31K and 53K polypeptides were still prominently labeled, but the M_r 17K, 29K and 84K polypeptide were labeled less well. With cell homogenates, the XYL-labeling of the M_r 17K and M_r 29K polypeptides was blocked by 100 µM bretylium, which is a CA uptake inhibitor with a structure similar to that of XYL. Thus, these two polypeptides may be components of the CA carrier system. Supported by NIH grants MH38633 and MH23839.

108.11 PC12 VARIANTS ALTERED IN CATECHOLAMINE TRANSPORT. C.M. Bitler*, M-B. Zhang*, and B.D. Howard. Dept. of Biol. Chem. and Brain Res. Inst., Sch. of Med., Univ. of California, Los Angeles, CA 90024.

PC12 is a pheochromocytoma cell line that takes up catecholamines by a Na^+-dependent, carrier mediated process. We have isolated variants altered in the ability to take up dopamine. One such varient was selected by virtue of being resistant to the antihypertensive drug guanethidine. Guanethidine produces several effects on wild type PC12. Pretreatment with 10 μM guanethidine for 16 hr caused a depletion of dopamine stores, inhibition of dopamine uptake, and inhibition of K^+-evoked release of dopamine. Inhibition of dopamine uptake, but not of release, also occurred with acute simultaneous exposure to 10 μM guanethidine. Guanethidine treatment resulted in the extension of much longer processes upon exposure to nerve growth factor. Almost all cells were killed by a 30 day treatment with 100 μM guanethidine. Survivors were more resistant than wild type cells to the long term effects of guanethidine, and they accumulated dopamine less well (decreased Vmax; unchanged Km for dopamine). A second variant was selected by resistance to N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (NMPTP), a compound that causes parkinsonism. These cells could not accumulate dopamine, or store or secrete dopamine or acetylcholine. Wild type and variant cells were compared with respect to ease of labeling of cell proteins with [^{3}H]xylamine which binds covalently to certain proteins apparently only after entering PC12 via the catecholamine carrier. When intact variant cells were used, there was markedly reduced labeling of these proteins by [^{3}H]xylamine. Almost all of these proteins did become labeled when cell-free homogenates were exposed to [^{3}H]xylamine. However, autoradiographs of xylamine-labeled proteins from NMPTP-resistant cells were missing several bands corresponding to proteins labeled in homogenates of wild type PC12 and the guanethidine resistant variants. Thus, the NMPTP-resistant variant offers the opportunity to identify several heretofore unrecognized proteins involved in neurotransmitter uptake, storage, and secretion. Supported by NIH grant MH38633.

108.12 [^{3}H]-DIHYDROTETRABENAZINE BINDING TO BOVINE STRIATAL SYNAPTIC VESICLE CATECHOLAMINE/SEROTONIN TRANSPORTER. J.A. Near* (SPON: R.S. Gurd). Dept. of Chemistry, Indiana University, Bloomington, IN 47405.

Catecholamines and serotonin are accumulated in synaptic vesicles by an ATP-dependent, reserpine- and uncoupler-sensitive process. Previous work has shown that [^{3}H]-reserpine binds to synaptic vesicles with apparent Kd^{app} = 1-2 nM, similar to its K_i for inhibition of catecholamine transport (1). Binding is completely dependent on the presence of ATP, is inhibited by uncouplers, and is not reversed by subsequent permeabilization of the vesicle membrane or incubation with high concentrations of unlabeled reserpine. Transport substrates such as dopamine exhibit inhibitory potencies similar to their K_m's for transport (1-10 μM). Dihydrotetrabenazine (DH-TBZ) is another potent inhibitor of the same system. [^{3}H]-DH-TBZ binds reversibly to bovine striatal vesicles with Kd^{app} of 2.1 nM, and binding is unaffected by ATP. Tetrabenazine and unlabeled DH-TBZ exhibit similar potencies as inhibitors of this binding and of ATP-dependent dopamine transport. However, in the absence of ATP, reserpine inhibits [^{3}H]-DH-TBZ binding with K_i of 100-200 nM, and transport substrates inhibit binding at concentrations in the millimolar range. When synaptic vesicles are pretreated with various concentrations of reserpine in the absence of ATP prior to addition of [^{3}H]-DH-TBZ, a smooth monophasic log-inhibition curve is obtained (K_i = 140 nM). When ATP is present during the preincubation a biphasic curve is observed, with a fraction of the sites inhibited by low reserpine concentrations (K_i = 3 nM) and the remainder by much higher concentrations (K_i = 130 nM). These results suggest that reserpine and DH-TBZ interact with equivalent binding sites, but that a sub-population of these is localized on fragmented or leaky vesicles unable to maintain the ATP-generated proton electrochemical gradient required for high-affinity reserpine binding. It is concluded that reserpine and DH-TBZ represent two classes of transport inhibitors whose binding properties differ with respect to reversibility and ATP-dependence. (Supported by Research Grant NS08309 from NIH)

1. Near, J.A. & Mahler, H.R. (1983) *FEBS Lett.* 158, 31-33.

108.13 Photoaffinity labeling of 45 kd and 56 kd forms of serotonin binding protein (SBP): evidence that 45 kd SBP is stored in synaptic vesicles. H. Tamir, K.P. Liu*, S.H. Hsuing*, R. Gabizon*, and M.D. Gershon. N.Y.S. Psychiatric Inst.; Depts. of Mol. Biol. Hadassah Med. Sch. and Anat. and Cell Biol., Columbia Univ. P&S, Jerusalem, Israel and New York, N.Y. 10032.

Serotonin binding protein (SBP) is a soluble constituent of the synaptic vesicles of central and peripheral (enteric) serotonergic neurons that forms an *in situ* complex with serotonin (5-HT) and Fe^{+2}. Two forms of SBP that differ in molecular weight and charge (45 kd, isoelectric point 6.4; 56 kd, isoelectric points 5.6 and 5.9), but which share antigenic determinants, have been identified. The current study was undertaken in order to define the relationship of the two forms of SBP to each other and to their function. Rat brain was fractionated to obtain crude synaptosomes. These were osmotically disrupted and the released synaptic vesicles were isolated by differential centrifugation in K^+ containing media. SBP was liberated from the isolated vesicles by freezing and thawing and compared with SBP obtained from the supernatants of whole brain homogenates and of disrupted synaptosomes. The SBP in each fraction was photoaffinity labeled with ^{3}H-4-azido, 3-nitrophenylazo-5-HT (^{3}H-ANPA-5-HT). After illumination, the covalently labeled SBP was subjected to SDS-PAGE. Both 45 kd and 56 kd SBP were labeled by ^{3}H-ANPA-5-HT and this labeling was antagonized by 5-HT. The material derived from synaptic vesicles was found to be enriched in 45 kd SBP while the 56 kd SBP was the predominantly labeled form in the supernatant fractions of whole brain homogenates and synaptosomes. The SBP found in the 3 fractions was incubated with ATP-gamma-S. It is known that 56 kd, but not 45 kd SBP, can be phosphorylated, and that this phosphorylation inhibits binding of 5-HT. In contrast to the whole brain supernatant SBP (inhibited > 80%), incubation of the vesicular SBP with ATP-gamma-S only slightly (< 15%) inhibited the ability of the protein to bind ^{3}H-5-HT. These observations suggest that 45 kd SBP is the major form of the protein in synaptic vesicles while the 56 kd form may be dominant in perikarya and/or preterminal axons. These results are consistent with prior observations that newly taken up ^{3}H-5-HT is located mainly in axon terminals and preferentially labels 45 kd SBP *in situ*. We propose that 56 kd SBP is converted to 45 kd SBP during the transport or packaging SBP in synaptic vesicles. Supported by grants NIMH 37595 and NS12969.

108.14 ISOLATION AND CHARACTERIZATION OF PARANEURONAL SEROTONIN STORAGE VESICLES FROM THE SHEEP THYROID. J. Barasch*, M.D. Gershon, E.A. Nunez and H. Tamir. (SPON. by C. Noback) Dept. of Anat. and Cell Biol., Columbia Univ. Coll. of P&S and N.Y.S. Psychiatric Institute, New York, N.Y. 10032.

Parafollicular cells of the thyroid gland are neural crest derivatives that, because of their resemblance to neurons, have been classified as paraneurons. In sheep, these cells co-store serotonin (5-HT) and calcitonin. In common with other neuroectodermal derivatives that store 5-HT, such as central and peripheral serotonergic neurons, parafollicular cells contain the neuronal type of serotonin binding protein. This protein is not found in other 5-HT-storing cells, such as platelets, mast cells, and enterochromaffin cells, that are derived from mesoderm or endoderm. We have investigated 5-HT storage organelles from parafollicular cells in order to gain insight into mechanisms of transport and maintenance of 5-HT within vesicles that may be analogous to the synaptic vesicles of serotonergic neurons. Parafollicular vesicles were isolated by combinations of differential and density gradient centrifugation through either 50% isotonic Percoll or Metrizamide. Metrizamide proved superior to Percoll. The optimum procedure was to layer a 19,400 gt post-nuclear pellet over a 4 step discontinuous gradient of 12-24% Metrizamide and to centrifuge at 58,200 gt. Material sedimenting at the 12-16% interface was collected and then centrifuged on a 2 step 18-28% Metrizamide gradient at 11.5 x 10^6 gt. Greater than 62% of the particulate volume of material collected at the interface consisted of parafollicular granules. This fraction was enriched with 5-HT 60-fold over the initial homogenate; however, there was no enrichment in lysosomes or mitochondria determined morphologically or by measurements of beta glucuronidase or succinic dehydrogenase respectively. Properties shown by the isolated granules included a temperature-dependent uptake of ^{3}H-5-HT that was stimulated by Mg^{+2}-ATP and inhibited by Na^+ and reserpine (10 uM). Confirmation that ^{3}H-5-HT entered granules and not other structures was obtained by quantitative electron microscopic radioautography of the isolated fractions. It is concluded that parafollicular granules specifically take up and store 5-HT and can be used as models of serotonergic synaptic vesicles.

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108.15 EFFECT OF LITHIUM (Li^+) ON PLATELET SEROTONIN (5-HT) RELEASE. Aruna Panini* and Robert Hitzemann, Departments of Psychiatry and Pharmacology and Cell Biophysics, University of Cincinnati, Cincinnati, Ohio 45267-0559.

Recent studies have focused on Li^+s effect on inositol phosphate (IP) metabolism, noting that Li^+ in therapeutic concentrations, blocks IP metabolism. Elevating intracellular IP has been suggested to affect Ca^{++} mobilization. We have examined the effect of Li^+ on a process which requires the mobilization of internal Ca^{++} stores, namely the thrombin-induced release of platelet 5-HT. Platelets, isolated from 20 subjects, were resuspended in either normal Krebs buffer or buffer in which the NaCl was replaced by LiCl. The platelets were incubated for 1 hour at 37°C and then reharvested. This Li^+ loading procedure yielded an internal [Li^+] of 5 mM. Platelets were then loaded with [^{3}H]5-HT ($2x10^{-8}$M), washed, reharvested and resuspended on either normal Krebs buffer or Krebs buffer plus 1 mM Li^+. Release of [^{3}H]5-HT was measured using a centrifugation assay; the reaction was stopped at 2 minutes after adding platelets. The results obtained are shown in the table. The analysis of variance revealed significant differences among treatment groups ($F=11.8$ ($df=7,133$), $p<.001$)) with the critical difference at the $p<0.05$ level equaling 4.78%. As expected, thrombin significantly increased release in a dose- related fashion. 1mM Li^+ + Li^+ load but not 1 mM Li^+ significantly increased (+104%) the effect of .01 unit thrombin on [^{3}H]5-HT release. Overall these results show that intracellular Li^+ can have a marked effect on a process which requires the mobilization of internal Ca^{++}. Studies are currently underway to determine the dose-response for internal Li^+ loading.

Group	Percent Released ± S.E
Basal	9.95±0.95
+1mM Li^+	9.38±0.74
+1mM Li^+ + Li^+ Load	8.41±0.84
Thrombin 0.01 Units	15.88±1.56*
Thrombin 0.03 Units	22.60±1.60*
Thrombin 0.10 Units	28.80±1.73*
Thrombin (.01)+1mM Li^+	15.56±1.59*
Thrombin (.01)+1mM Li^++Li^+ Load	21.43±1.45*†

* - Significantly different from basal, $p<0.05$
† - Significantly different from thrombin (.01) only, $p<0.05$.

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108.16 CHARACTERIZATION OF TRANSMITTER RELEASE FROM THE IN VITRO MOUSE STRIATAL SLICE. R. Tintner, Dept. of Neurology, Southwestern Med. Sch., UTHSCD, Dallas, Tx. 75235.

In vitro superfusion of brain slices has proven a valuable preparation to examine neurotransmitter release from the striatum. A multitude of studies have been performed using neostriatal slices from rabbits, cats, guinea pigs and rats. These animals offer the advantage of relatively large brain size. There exist a number of inbred mouse strains with characteristic heritable parameters of neurotransmitter systems. We wished to use strain as an independent variable in later pharmacologic and lesion experiments and monitor in vitro transmitter release as a dependent variable. As a result, equipment and procedures were developed and characterized using mouse neostriatum.

An inexpensive multi-chamber superfusion device will be detailed. The chambers are 400 µl, plexiglas cylinders. Teflon screw caps allow inlet and outlet ports and silver wire electrodes. One pair of striata (ca. 20mg tissue) are sliced into 400 µm sections and placed in each chamber. Dopamine (DA) and acetycholine (ACh) release are monitored by pre-incubating with low (<1 µM) concentrations of the ^{3}H- and ^{14}C-labeled, respectively, transmitters. A balanced salt solution is superfused at 1 ml/min using a multiple cassette peristaltic pump. Five minute fractions are collected using a microprocessor controlled multiple fraction collector. Voltage-dependent transmitter release is induced by increasing [K+] or electrical field stimulation. The effects of altering stimulus parameters ionic conditions will be described for both DA and ACh release. The effects of number of neuroactive compounds on release will also be described. These results will be compared and contrasted with results from other species.

108.17 DIFFERENT CHARACTERISTICS OF ^{3}H-COCAINE BINDING SITES IN RAT STRIATUM AND NUCLEUS ACCUMBENS. C.Missale, M.Memo, L.Castelletti†, S.Govoni, M.Trabucchi, P.F.Spano and I.Hanbauer.
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Cocaine is known as a potent inhibitor of the active dopamine uptake. Recent data show that saturable binding sites for ^{3}H-cocaine (defined by nomifensine) are present in mouse and rat brain; in particular, two different populations of binding sites labelled by ^{3}H-cocaine have been detected in rat striatum, according to their sensitivity to Na^+ions. The Na^+-dependent component is preferentially located on dopaminergic terminals and is functionally related to the dopamine uptake, a mechanism involved in the regulation of dopamine turn over. In order to investigate the physiological relevance of the Na^+-dependent cocaine binding, we focussed on the function of the dopamine uptake in nucleus accumbens, where the dopamine turnover apparently responds to various stimuli in a different direction, compared to striatum.

A single cocaine administration (20mg/kg, i.p.) inhibits dopamine uptake in corpus striatum (-35%), but increases it in nucleus accumbens (+40%); similarly, after a subchronic cocaine treatment (20mg/kg daily for 21 days, i.p.) tolerance to the effect develops in corpus striatum, but not in nucleus accumbens. In vitro experiments show that dopaminergic terminals in the two areas present a different sensitivity to the inhibitory action of cocaine (IC_{50}= 10 µM in striatum and IC_{50}= 100 µM in nucleus accumbens) and have different populations of ^{3}H-cocaine binding sites; in fact, although a saturable binding for ^{3}H-cocaine is present in nucleus accumbens as well, the Na^+-dependent component, linked to the dopamine uptake system, is not detectable in this area. On this line, the effect of cocaine on dopamine uptake in striatum may be due to a direct action on specific receptors located on dopaminergic terminals; otherwise, the effect observed in nucleus accumbens may be mediated by the possible interaction of cocaine with other neuronal structures influencing the dopaminergic synaptic function.

108.18 [^{3}H]COCAINE BINDING IN VARIOUS BRAIN REGIONS. M.E.A. Reith*, B.E. Meisler*, H. Sershen* and A. Lajtha. Center for Neurochemistry, Ward's Island, New York, NY 10035.

The pharmacology of [^{3}H]cocaine binding indicates an association with serotonergic uptake systems in the cerebral cortex (Mol. Pharmacol. 23:606, 1983) and with dopaminergic uptake systems in the striatum (J. Neurochem. 41:172, 1983). Other brain regions also possess binding sites for [^{3}H]cocaine, and the present studies are aimed at characterizing these sites. As a first step we examined the conditions under which the binding of [^{3}H]cocaine can be measured in an optimal manner. Binding to P_2-membranes from cerebral cortex of male BALB/cBy mice was dramatically lower with 50 mM Tris than with 25 mM HEPES or 5, 25, and 50 mM sodium phosphate, pH 7.7. Scatchard analysis revealed that the higher binding with 25 mM sodium phosphate as compared with 50 mM Tris was due to an increase in affinity (K_d of 150 and 706 nM, respectively) and not in B_{max}, suggesting that Tris is a competitive inhibitor of [^{3}H]cocaine binding. Tris also inhibited the binding of [^{3}H]cocaine to striatal membranes. It would be prudent therefore to avoid Tris in future studies on [^{3}H]cocaine binding. We recommend the use of 25 mM sodium phosphate, pH 7.7. The second series of experiments was aimed at assessing the effect of Na^+ on the binding of [^{3}H]cocaine in various brain regions. Because we needed a buffer that contains no Na^+ for these assays, we used a dilute Tris buffer (5 mM) which is only marginally inhibitory. There were distinct differences between various brain regions in the response to Na^+. Only the striatum showed Na^+-stimulated binding: a maximal four-fold increase was observed with 25 mM Na^+. Concentrations of Na^+ up to 50 mM were without effect in olfactory tubercle, cortex, and midbrain; they inhibited the binding somewhat (<20%) in hippocampus and pons-medulla; and they decreased binding by 60% in the cerebellum. Higher concentrations of Na^+ (100 to 200 mM) were inhibitory in all brain regions. In the striatum, the Na^+-stimulated binding was less than maximal at these concentrations. These effects of Na^+ are consonant with the proposal that these are at least two different types of [^{3}H]cocaine binding in the brain: one is stimulated by the presence of 25-50 mM Na^+, occurs in the striatum, and is related to dopamine uptake; a second one is relatively insensitive to concentrations of Na^+ up to 50 mM, occurs in all brain regions examined so far, and is related to serotonin uptake.

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108.19 CENTRAL METABOLISM OF ANGIOTENSINS: REMOVAL OF AMINO ACID END PRODUCTS BY A HIGH-AFFINITY UPTAKE SYSTEM. C.G. Cámara, R.H. Abhold, and J.W. Harding. Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164-6520.

In the central nervous system angiotensins are degraded to their constituent amino acids by membrane-bound peptidases, some of which appear to be associated with angiotensin receptors (Camara, C.G., et al., submitted for publication). Subsequent to the degradation of angiotensins all of their aromatic and branched-chain aliphatic amino acids are removed from the medium by a high-affinity uptake system that is enriched in the synaptosomal fraction. This transport process is independent of Na^+, is enhanced at pH values below neutrality, and is capable of exchanging intracellular with extracellular amino acids. When ^{125}I-tyrosine is used as the substrate the apparent Km of uptake is 3.8 μM and its Vmax is 89 pmoles/min/mg protein. ^{125}I-tyrosine exchange has an apparent Km of 18 μM and a Vmax of 219 pmoles/min/mg protein. This transport system is identical with the leucine-preferring system described in a variety of mammalian cells, and its distribution coincides with that of apparent ^{125}I-AIII binding but differs from that of ^{125}-AII binding and of catecholamine neurons.

At very low substrate concentrations this uptake system is capable of very steep uphill transport which appears to be coupled to a gradient of pH across the plasma membrane.

The available evidence suggests that the proton circulation responsible for the formation of the coupling transmembrane pH gradient is not maintained by a Mg^{2+} ATP-ase but, instead, depends on the activity of an electron transport chain.

108.20 PRIMARY CULTURES OF FETAL RAT BRAIN NEURONS RELEASE AN ANGIOTENSIN II-LIKE PEPTIDE UPON CHEMICAL STIMULATION. J.M. Meyer, K.L. McConchie*, and J.A. Weyhenmeyer. Neural and Behavioral Biology Program and College of Medicine, University of Illinois, Urbana, IL, 61801.

The octapeptide angiotensin II (AII) exerts a potent neuromodulatory effect on neural tissue. There is controversy as to whether the AII acting on the CNS is derived from the periphery or is endogenous to the brain. We have shown that dissociated primary cultures of fetal rat brains have the ability to synthesize a peptide that resembles AII in immunochemical and chromatographic behavior. The aim of this research was to show that AII is released from neurons in primary cultures of fetal rat brains under appropriate stimulatory conditions.

In this experiment primary cultures were set up by removing the brains of 20 day old rat fetuses and dissociating them by mincing and mild trypsinization. Cultures were plated at $5.6x10^6$ cells/60mm Falcon dish, grown for the first 3 days in DME with 10% fetal calf serum, then changed to a serum-free defined medium and taken for experiments on day 8. Cells were incubated in supplemented DME containing 5mM $CaCl_2$ and 0.1% phenylmethylsulfonyl fluoride (PMSF) for 60 minutes and then challenged with high potassium chloride (KCl)DME also containing 5mM $CaCl_2$ and 0.1% PMSF. The different incubation media were collected into an equal volume of glacial actetic acid, heated to greater than 90°C for 5 to 7 minutes, and lyophilized to dryness. The media were reconstituted and brought back to physiological pH. Analysis of the media for the presence of AII was performed by high pressure liquid chromatography (HPLC) using a reverse phase C-18 column and a competitive inhibition radioimmunoassay using a specific AII antibody.

Preliminary evidence indicates an increase in the presence of an AII-like peptide in the high KCl medium over baseline, with highest levels observed after 60 minutes of high KCl incubation. Comparison with AII standards indicate pg levels of AII in the high KCl release medium incubated with the cells for 60 minutes. This suggests that in response to chemical depolarization primary cultures of fetal rat brains have the ability to release an AII-like peptide.

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108.21 ACETYLCHOLINE RELEASE FROM IOTRACHOTIN-PERMEABILIZED RAT CORTICAL SYNAPTOSOMES. M.L. Koenig*, G.P. Miljanich, C.A. Kasal, A.A. Herrera, and W.O. McClure. Section of Neurobiol., University of Southern California, Los Angeles, CA 90089.

Iotrachotin(IOT), a novel cytotoxic factor derived from the exudate of the Caribbean purple bleeder sponge Iotrachota birotulata, has been purified to apparent homogeneity and appears to have digitonin-like effects on synaptosomal membranes. Treatment with IOT of rat cortical synaptosomes previously loaded with ^{3}H-choline chloride resulted in a calcium independent release of ^{3}H-acetylcholine, but did not stimulate release of the cytosolic markers lactate dehydrogenase (LDH) and choline acetyltransferase (CAT). These data suggest that IOT may be used to permeabilize synaptosomal membranes without fear of disrupting them entirely (as appears to be the case with digitonin). The small size and apparent specificity of IOT-induced lesions of the membrane (ATP and 2-deoxyglucose can pass, but LDH and CAT cannot) suggests that IOT might be a useful probe with which to study changes in the vesicular and nonvesicular pools of ACh under varied physiological conditions. Preliminary studies of the effect of IOT on release of ATP from Torpedo synaptosomes indicate that at least two distinct pools of this nucleotide (and presumably ACh) are present and can be separately manipulated. One pool is depleted by depolarization with 50mM K^+ but not by IOT; the other can be depleted by IOT but not K^+. Depletion of the second pool is dependent upon the concentration of IOT. It is possible that the K^+-sensitive pool is "vesicular" and the IOT-sensitive one is "cytoplasmic." The results of a study assessing the relative contributions of these two pools to release of ACh under different physiological conditions will be presented. Work supported by grants from the National Institutes of Health, Nelson Research, the Faculty Research and Innovation Fund of USC, and NIH Training Grant 5T32-AG00093 to CAK.

108.22 EFFECTS OF SARIN UPON HIGH AFFINITY CHOLINE UPTAKE BY RAT BRAIN SYNAPTOSOMES. C.E. Whalley and T.-M Shih. USA Med. Res. Inst. Chem. Def., Aberdeen Proving Ground, MD 21010.

We have previously reported (Trans. Am. Soc. Neurochem., 14(1): 139, 1983) that the in vivo administration of soman decreased high affinity choline uptake (HACU) of rat brain synaptosomes isolated from hippocampus (HIP) and cortex (COR) but increased HACU in striatum (STR). In the present studies, we continued to examine changes in synaptosomal HACU in rat HIP, COR and STR following administration of potent organophosphorus compounds (OPs). In the in vivo study, rats were injected s.c. with 120 ug/kg of sarin and subsequently killed at various time intervals (0.5, 1, 4, 24 and 168 hrs) to measure HACU. Following acute sarin treatment, HACU in HIP was significantly decreased at 1 and 4 hr (19 and 24%, respectively); in COR was significantly decreased at 0.5 hr (21%); while in STR was significantly increased at 1 hr (27%). In addition, the in vitro effect of sarin and soman (concentrations ranging from 10-8 to 10-2M) upon HACU by synaptosomes isolated from STR, HIP and COR was also studied. Only when the respective OP concentration approached 10-3M was there a significant inhibitory effect upon HACU observed. The calculated IC50 concentrations for inhibition of HACU in STR, HIP and COR were 5.0 x 10-3, 2.8 x 10-3 and 4.5 x 10-3M, respectively, for soman and 8.5 x 10-3, 8.0 x 10-4 and > 10-2M, respectively, for sarin. These data suggest that acute sarin and soman treatments produced similar effects upon HACU in different brain areas, although the time-course of these effects was different for the two compounds. However, these effects were probably not due to a direct action of these OPs upon this uptake process since a direct effect was only observed at extremely high OP concentrations in vitro. The differential response of the brain regions may reflect differences in the density of cholinergic innervation and the influence of other neurotransmitter systems.

109 SYMPOSIUM: HOW Ca^{2+} ACTS AS A SECOND MESSENGER IN NEURONS. J.H. Schwartz (Chair), Columbia; M. Pallazollo, Columbia; S. Smith, Yale; M.B. Kennedy, Cal Tech; S. DeReimer, L.K. Kaczmarek, P. Greengard, Yale and Rockefeller Univ.; R. Tsien, UC Berkeley.

Ca^{2+} is recognized as critical for the exocytotic release of neurotransmitters and hormones. Ca^{2+} has also been identified as an important universal second messenger. Regulation depends primarily on the concentration of the free ion, which can be changed either by influx from the environment or by release from sequestered intracellular stores. An important topic to be discussed is how to measure free Ca^{2+} in intact cells and in parts of cells and how those measurements relate to biophysical mechanisms underlying the exocytotic process.

Although changes in free Ca^{2+} govern the processes controlled by Ca^{2+}, control is actually effected by bound forms of the ion. Ca^{2+} regulates the activities of many diverse proteins through calmodulin (CaM). This third messenger is conserved throughout eukaryotic phylogeny. Multiple CaMs with distinctive properties operate in a variety of tissues in the same animal. Do these forms originate from a multi-gene family? Recombinant DNA technology reveals that CaM is encoded by only a single gene in chicken, frog, and Aplysia, but that in all species several mRNAs are produced. While their functional significance is uncertain, the multiple mRNAs may play a role in producing the post-translational modifications that result in the variety of CaM molecules.

Phosphorylation is the most prominent mechanism regulating the action of cell proteins. Two different enzymes that carry out Ca^{2+} control are the Ca^{2+}/CaM-dependent kinase; and the diacylglycerol/C kinase. One Ca^{2+}/CaM kinase, which is a major component of the vertebrate postsynaptic density and the principal kinase of brain, has been characterized in both vertebrates and Aplysia. It phosphorylates some of the same protein substrates (including Synapsin I and MAP2)as the cAMP-dependent kinase does, and so provides a mechanism interfacing control of neuronal function by Ca^{2+} and by cAMP, the other major second messenger. The properties of the lipid-dependent kinase will also be discussed. This enzyme, which has been studied in vertebrates and Aplysia, has been implicated in mediating secretion in platelets, neutrophils, PC12 cells, and synaptosomes.

110 WORKSHOP: RECENT DEVELOPMENTS ON THE MEDULLARY, HYPOTHALAMIC AND SPINAL CONTROL OF AUTONOMIC FUNCTION: CHARACTERIZATION OF "TRANSMITTER-SPECIFIC" PATHWAYS. J.B.Cabot(Chairperson), SUNY at Stony Brook; L.W.Swanson, Salk Institute; D.J. Reis, Cornell Univ. Med. Sch.; A.D. Loewy, Washington Univ. Sch. Med.; R.P. Elde, Univ. Minnesota.

It is well established that the ventrolateral and ventromedial medulla provide critical links in the central regulation of autonomic function. Yet, it has only been quite recently that there has been major forward progress in defining the anatomical and neurochemical substrates underlying previously defined physiological functions. Along the way, many of the old constructs are slowly being modified and new hypotheses are rapidly appearing.

Dr. Swanson will discuss recent data on the reciprocity of chemically defined ("transmitter-specific") connections between medulla, hypothalamus and the nucleus of the tractus solitarius (NTS). Specifically, evidence establishing catecholamine projections from the A1 and A2 noradrenergic cell groups to the paraventricular nucleus of the hypothalamus (PVN) will be presented and integrated with observations on peptidergic inputs (oxytocin and vasopressin) to NTS arising from PVN.

Dr. Reis will present data on the role of ventrolateral medullary C1 (adrenaline-containing) and A1 (noradrenaline-containing) neurons in the regulation of cardiovascular function. C1 cells project directly to the spinal sympathetic preganglionic neuropil (intermediolateral cell column, IML) and appear to be responsible for maintaining tonic levels of arterial blood pressure as well as mediating vasodepressor responses from baroreceptors nerves. In contrast, the region of the A1 cells appears to exert tonic sympathoinhibition, perhaps through interactions with C1 neurons.

Dr. Loewy will discuss recent evidence for a descending substance P containing pathway arising from ventral medulla and terminating in the IML. It is hypothesized that this projection is important for tonic vasmotor control. Anatomical, physiological, pharmacological and ^{3}H-SP receptor binding data will be presented.

Dr. Elde will discuss the hypothesis that the viscerotopic organization of the IML is not only manifest at the cellular level, but also at the level of neurotransmitter receptors. The IML is known to receive dense catecholaminergic and sertoninergic projections from the brain stem. In line with these data, evidence will be presented which shows that alpha$_2$ adrenergic and LSD binding sites are especially enriched over IML neurons innervating the adrenal medulla.

DEVELOPMENT AND PLASTICITY: TROPHIC AGENTS I

111.1 NERVE GROWTH FACTOR INCREASES CHOLINE ACETYLTRANSFERASE ACTIVITY IN THE BASAL FOREBRAIN AND NEOSTRIATUM OF NEONATAL RATS: W.C. Mobley, G. Tennekoon*, K. Buchanan* and M.V. Johnston. Div. N.P., Walter Reed Institute of Research, Washington, DC 20307; Dept. of Neuro., Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205 and Depts. of Ped. and Neuro., Univ. of Mich., Ann Arbor, MI 48104.

Recent studies have demonstrated a response to nerve growth factor (NGF) by the central cholinergic neurons of neonatal (Gnahn et al., Dev. Br. Res. 9, 45 (1983)) and lesioned adult rats (Hefti et al., Br. Res. 293, 305 (1984)). We report our investigation of NGF's effects on these neurons, including results achieved with a highly purified preparation.

Mouse NGF was prepared by a modification of the method of Mobley et al. (Bioch. 15, 5543 (1976)). Purity was 92% by isoelectric focusing (IEF) and 93% by sodium dodecyl sulfate (SDS) gel analyses. Renin activity varied from nondetectable to less than 2 parts in 10,000 of that in submaxillary gland homogenates. Neonatal rats were injected intracerebroventricularly (icv) with 30 μg of either NGF or cytochrome c (as control) in 10μl phosphate buffered saline on postnatal days 2,4,6 and 8 and killed on day 12. Increases in choline acetyltransferase (ChAT) activity were detected in septum (220% increase), hippocampus (53%), substantia innominata (140%), and neostriatum (94%). Neither peripherally injected NGF nor icv injections of either angiotensin I or II delivered in the same dose and schedule affected ChAT activity. A series of individual doses of 1,3, or 10 μg of NGF, delivered in the same manner and schedule, described a dose response-relationship with ChAT activity in septum, substantia innominata, neocortex and neostriatum.

In an attempt to exclude an effect on ChAT activity of impurities in the NGF preparation, the material was further purified via a preparative electrofocusing column (pH gradient 6.5-9.5) and then by gel filtration over a Sephadex G-75 column (120 x 0.8cm) in 2N acetic acid. Purity was 96% by IEF and 95% by SDS gel analyses. This sample was equipotent to the original in its ability to increase ChAT activity. NGF had no effect on tyrosine hydroxylase activity in locus coeruleus or on glutamic acid decarboxylase activity in neocortex, hippocampus or neostriatum. These results indicate that NGF has a prominent and selective effect on ChAT activity in the basal forebrain and neostriatum of neonatal rats.

111.2 ALTERED NERVE GROWTH FACTOR (NGF) RECEPTORS IN PC12 CELL MUTANTS LACKING RESPONSES TO NGF. Steven H. Green, Russell E. Rydel and Lloyd A. Greene*. Dept. of Pharmacology, NYU Med. Ctr., New York, NY 10016.

PC12 cells were mutagenized with EMS, plated at low density, and, after the growth of small colonies, exposed to NGF. Clones lacking a neurite outgrowth response to NGF were selected by visual inspection. Four of 24 clones so obtained apparently lack any responses to NGF as evidenced by the failure of NGF to promote survival in serum-free medium, to induce ornithine decarboxylase (ODC) (a transcription-dependent response), or to induce phosphorylation of specific proteins (a transcription-independent response). ODC induction and phosphorylation responses to epidermal growth factor (EGF) or dibutyryl-cAMP are not impaired. PC12 cells, like sympathetic and DRG neurons have both low and high affinity NGF receptors. These, as well as NGF internalization, were measured by previously described methods (Bernd & Greene, 1983, Soc. Neurosci. Abstr. 9: 842). The mutants have normal or somewhat reduced levels of low affinity receptors (60-110% of parent PC12) and greatly reduced levels of high affinity receptors (~20% of normal PC12). They internalize NGF at greatly reduced levels as compared to parent PC12 cells (1-10%). This is not due to a general defect in endocytosis of bound ligand since binding and internalization of EGF are normal in the mutant cells. The defects in binding and uptake appear not to lie in the receptor itself: treatment of the mutants with Wheat Germ Agglutinin (WGA) results in a loss of 50-75% of the low affinity receptors and a 3 to 5-fold increase in high affinity sites, an effect of WGA also seen in normal PC12 cells. The mutants also have 2.5 to 5-fold greater rates of NGF internalization following WGA treatment. Another mutant line in which total receptor number is ~10% of that in the parent PC12 line, but which retains normal ratios of high to low affinity sites and of internalized to external ligand, possesses NGF responses. These data suggest that it is the high, rather than the low affinity, NGF receptors that mediate physiological responses to, and internalization of, NGF and that the non-responsive mutants appear to be defective in a mechanism that converts low affinity sites to high. It is this defect, and not the absolute number of receptors, that is most closely associated with the mutants' lack of response to NGF. These mutants promise to be of great value in the elucidation of the mechanisms of receptor conversion and response to NGF. Supported by USPHS Grant NS16036.

111.3 IMMUNOCYTOCHEMICAL CO-LOCALIZATION OF NERVE GROWTH FACTOR (NGF) AND EPIDERMAL GROWTH FACTOR (EGF) IN MOUSE SUBMANDIBULAR GLANDS. A.Y. Watson*, J.A. Rhodes*, J. Anderson*, K. Siminoski*, J. Mole*, and R.A. Murphy (SPON: T.E. Phillips). Dept. of Anatomy, Harvard Med. Sch., Boston, MA 02115 and Dept. of Biochem., Univ. Mass. Med. Sch., Worcester, MA 01605

NGF and EGF are produced by granular tubule cells (GTC) in mouse submandibular glands but contradictory results have been reported on their cellular and subcellular localization. In this study, NGF and EGF were co-localized by high resolution immunocytochemical methods using light and electron microscopy. Cell structure and protein antigenicity were best preserved when mice were perfused with 4% paraformaldehyde, 0.1% picric acid in 0.05M cacodylate buffer. Tissues were not post-fixed in O_sO_4 but otherwise were routinely processed for plastic embedding. Methoxide-etched sections were treated with rabbit antiserum raised against EGF or against 2.5S NGF isolated by routine methods or by reverse phase high pressure liquid chromatography. Sections were then stained with fluorescein-labeled goat anti-rabbit IgG. Examination of serial sections revealed a similar pattern of staining for both antigens. All GTC, and no other cell type including striated duct cells, were immunoreactive. Within GTC, staining was confined to secretory granules. These results were confirmed by ultrastructural analyses. Antibodies to NGF and EGF were directly coupled to 8.6 nm and 17.9 nm colloidal gold particles respectively and thin sections incubated with both antibodies. All moderate to large-sized secretory granules were immunoreactive for both growth factors. GTC did contain, however, a population of small-sized vesicles scattered throughout the cytoplasm that were not immunoreactive for either molecule.

In summary, we detect no differences in the cellular and subcellular distribution of NGF and EGF in mouse submandibular glands. Both molecules are exclusively located in secretory granules of all GTC. In addition, the use of plastic-embedded material for fluorescence microscopy and colloidal gold labels for electron microscopy permit high resolution analysis of growth factor-containing cells and may be useful for localizing NGF or EGF in non-salivary tissues.

111.4 ISOLATION BY REVERSE PHASE HPLC OF AN IMMUNOREACTIVE PROTEIN THAT CO-PURIFIES WITH NERVE GROWTH FACTOR. K. Siminoski*, J. Anderson*, A.Y.Watson*, J.A. Rhodes*, J.Mole*, and R.A.Murphy. Dept. of Anatomy, Harvard Med. Sch., Boston, MA 02115, and Dept. of Biochem., Univ. Mass. Med. Sch., Worcester, MA 01605

In the course of immunoblot characterization of rabbit antisera to NGF, a protein (MW 14,200) which reacts strongly with NGF antibodies was detected in purified preparations of both 2.5s - and ß-NGF. This protein accounts for approximately 2% of the total protein in both preparations of NGF and can be distinguished from NGF on SDS-containing polyacrylamide gradient gels (13-22%) or on acetic acid-urea gels. This molecule has been partially purified by reverse phase HPLC. 2.5S NGF in 0.1% trifluoroacetic acid (pH 2.5) was applied to sequential preparative and analytical C_{18} uBondapak columns and eluted with an acetonitrile gradient (0-60%). The protein chromatographed slightly ahead of NGF. By RIA, it showed an 11% cross-reactivity to 2.5S NGF and stimulated neurite outgrowth in the sensory ganglion bioassay at concentrations between 500-800 ng/ml. It is unclear whether these activities are intrinsic to the protein or are due to contamination by NGF. Under identical conditions, HPLC-purified NGF was 91% reactive in the RIA and was biologically active at 12-50 ng/ml. Amino acid analyses suggest that the contaminating protein and NGF are chemically dissimilar, although rabbit antibodies which had either been raised against contaminant-free HPLC-purified NGF or which had been affinity-purified using HPLC-purified NGF reacted with this molecule on immunoblots of SDS gels. Amino-acid sequence analysis has been unsuccessful, suggesting a blocked amino terminus. Attempts to sequence proteolytic fragments of the protein are underway and monoclonal antibodies are being used to determine whether the molecule is a precursor or anomalous form of NGF or a protein unrelated to the growth factor.

111.5 INTERACTIONS BETWEEN SYMPATHETIC AND SUBSTANCE P-CONTAINING SENSORY NEURONS IN THE RAT IRIS. U.Otten, H.P.Lorez*, G.Weskamp*, F.Businger*, L.Hedler* and D.K.Meyer*. Dept. of Pharmacology, Biocenter of the University, Basel, Switzerland; Hoffmann-LaRoche & Co.Ltd, Basel, Switzerland; Dept. of Pharmacology, University of Freiburg, Freiburg, West-Germany.

Target-derived nerve growth factor (NGF) is essential for the regulation of survival, development and maintenance of sympathetic neurons as well as of a large population of primary sensory neurons. Levels of NGF in target organs can be markedly increased after sensory and sympathetic denervation of the target.

We have investigated the interactions between sensory and sympathetic nerve fibers in the rat iris by biochemical and immunohistochemical methods. The rat iris is known to be innervated by nerves containing substance P (SP)-, somatostatin-, vasoactive intestinal polypeptide- and enkephalin-like immunoreactivity in addition to the noradrenergic and cholinergic innervation. SP, mainly localized in trigeminal sensory neurons, and noradrenaline, present in sympathetic nerves originating from the superior cervical ganglion, were used to monitor innervation of the iris after sensory or sympathetic nerve lesioning. Administration of capsaicin to neonatal rats resulted in a dose-dependent decrease of iris SP in the adult animals which was due to the permanent loss of SP-containing sensory fibers. On the contrary, noradrenaline in the iris almost doubled due to an increase both in the number of innervating nerves and in the noradrenaline content per nerve fiber.

Unilateral surgical removal of the superior cervical ganglion of adult rats led to the complete loss of noradrenaline and to a gradual increase in SP (+120%) in the iris within six weeks which is at least in part due to an increase in the number of nerve fibers.

These results indicate that important regulatory interactions occur between SP-containing trigeminal sensory and sympathetic neurons in the iris. Measurement of endogenous NGF in the iris by an enzyme-linked immunosorbent assay provided evidence that sensory and sympathetic nerve fibers compete for an NGF-like factor within this target organ.

Supported by the Swiss National Foundation for Scientific Research (Grant 3344-083).

111.6 DORSAL ROOT GANGLION NEURONS REQUIRE TROPHIC SUPPORT FROM THEIR CENTRAL PROCESSES: EVIDENCE FOR A ROLE OF RETROGRADELY TRANSPORTED NERVE GROWTH FACTOR (NGF) FROM THE CENTRAL TO THE PERIPHERAL NERVOUS SYSTEM. Henry K. Yip and Eugene M. Johnson. Dept. of Pharmacology, Washington Univ. School of Med., St. Louis, MO 63110.

Axonal injury of the peripheral process of dorsal root ganglion (DRG) in adult animals leads to a profound reaction in the DRG neurons. In contrast, section of the dorsal root proximal to the DRG does not initiate significant morphological change in the ganglion cells. In the previous study, we have shown that injury of the peripheral process produced a profound cell loss (40-50%) in the DRG of newborn rats. However, no information has been avaliable on the effects of dorsal root section in developing DRG. Here we report that six days after dorsal rhizotomy on newborn rats, there was a 50% decrease in neuronal number in L_5 DRG. A combined central and peripheral lesion of the sensory process resulted in a greater decrease in neuronal number (70%). Daily injection of NGF (20 µg) from day 0 to day 5 completely prevented the cell loss produced by these lesions at the end of the treatment period.

In this study, we also demonstrated that ^{125}I-NGF was retrogradely transported to the DRG via the central process after ^{125}I-NGF was injected into the dorsal spinal cord of adult rats. Significant accumulation of radioactivity was observed in the ipsilateral L_5 DRG, but not contralateral DRG, 4 hr after the injection, reached a maximum at 18 hr. and returned to the baseline by 36 hr. Autoradiographic examination of the L_5 DRG from the injected side showed a clear localization of radioactivity in a small number of neurons. Injections of ^{125}I-cytochrome c or ^{125}I-NGF in the presence of excess unlabelled NGF produced no transport of radioactivity to the ipsilateral DRG. These results indicate that the retrograde transport of ^{125}I-NGF in the central process is receptor-mediated and is as selective as that in the peripheral sensory system.

In conclusion, our study provides evidence indicating that some trophic support for developing sensory neurons is provided through the central process. This is presumably due to the uptake and retrograde transport of a trophic factor by the nerve terminals of the central process. The data suggest that NGF may be the trophic factor. Supported by NIH grants NS 19071 and HL 20604.

111.7 CHOLINERGIC SYMPATHETIC NEURONS REQUIRE NGF DURING DEVELOPMENT. S.C. Landis and J.R. Fredieu*. Department of Neurobiology, Harvard Medical School, Boston, MA 02115

Most sympathetic neurons are noradrenergic, and it is well established that they require NGF during development for survival. A small minority of principal neurons in sympathetic ganglia are cholinergic, including those that innervate eccrine sweat glands. We undertook to determine whether these cholinergic sympathetic neurons also require NGF during development.

Two day old rat pups received a single injection of goat antiserum (200mg lyophilized antiserum/kg) raised against 2.5S NGF isolated from male mouse salivary glands (antiserum generously provided by M. Schwab). At three weeks, the sympathetic catecholaminergic innervation of the iris and salivary gland was essentially absent, as judged by catecholamine fluorescence and tyrosine hydroxylase immunocytochemistry. The anti-NGF treatment thus was successful in eliminating the sympathetic innervation of these target tissues.

The mature sympathetic cholinergic innervation of sweat glands in rat foot pads is characterized by prominent acetylcholinesterase (AChE) staining and vasoactive intestinal polypeptide-like immunoreactivity (VIP-IR). In rats subjected to anti-NGF administration, however, neither AChE nor VIP-IR could be detected in the morphologically normal sweat glands of foot pads at three weeks. These observations suggest that cholinergic sympathetic neurons, like adrenergic sympathetic neurons, require NGF during postnatal development for normal differentiation.

111.8 BIOASSAY AND DISTRIBUTION OF PARASYMPATHETIC NEUROTROPHIC ACTIVITY IN NORMAL AND NEOPLASTIC TISSUES. Thomas L. Wallace* and Eugene M. Johnson, Jr. Dept. of Pharmacology, Wash. Univ. Med. School, St. Louis, MO 63110.

Non-neoplastic tissues from seven animal species and neoplastic tissues of non-human and human origin were screened for parasympathetic neurotrophic activity (NTA). Extracts were prepared by homogenizing solid tissues or by sonicating cultured cells in 5 mM tris-HCl, pH 7 containing phenylmethylsulfonyl fluoride (2 mM) as a protease inhibitor. The 12,000 x g supernatants were incubated directly with embryonic (E10) chicken ciliary ganglia, Eagles MEM containing serum (10% v/v), and KCl for 3 days. NTA was evaluated by assaying choline acetyltransferase (CAT) activity and by microscopic examination of neuronal survival. The highest concentrations of NTA were found in normal lung tissue and in several neoplastic tissues of neural or neuroendocrine origin. Pig lung was a superior source because of its high NTA and ready availibility, and the NTA was very stable. Thus, pig lung was used to systematically characterize the bioassay conditions. Lung extract alone sustained neurons, but could not maintain CAT activity, whereas both lung extract and high concentrations of KCl were required to maintain CAT activity. There was a good correlation between levels of CAT and survival of neurons when ganglia were incubated with both lung extract and high concentrations of KCl. However, KCl by itself under these conditions did not sustain either neuronal survival or CAT activity. Lung extract exerted trophic effects on ciliary ganglia from chickens only of embryonic ages 7 to 12. It was also essential for survival of dissociated ciliary neurons cultured in the absence of non-neuronal cells, but could not support sympathetic or sensory neurons in vitro. These findings demonstrate a simple bioassay and relatively rich tissue source (pig lung) of parasympathetic NTA that may provide a basis for purification of the trophic substance(s). (Supported by NIH grant 5-T32-NS07129 and a Muscular Dystrophy Association Fellowship to T.L.W.).

111.9 PROLIFERATION AND CHARACTERIZATION OF ISOLATED OLIGODENDROCYTES CULTURED IN A CHEMICALL DEFINED MEDIUM. R.P. Saneto, R. Cole* and J. de Vellis. MRRC/NPI UCLA Los Angeles, CA. 90024.

Factors which regulate the proliferation of oligodendrocytes (oligos) remain obscure. Although the use of primary culture has enhanced our knowledge of oligo function, the inducement of cell proliferation in isolated culture has not been previously reported. We report here, for the first time a serumless chemically defined medium (ODM) that reproducibly induces cell proliferation of isolated primary oligos.

Purified populations of oligos were prepared from postnatal rat cerebral cortex as previously described by us (McCarthy and de Vellis, J. Cell Biol. 85, 890). Cells were seeded in medium containing 10% fetal calf serum. After 18 h the cells were washed 3x in serum-free medium and ODM was subsequently added. The presence of optimal concentrations of hormones, insulin, transferrin, and fibroblast growth factor (FGF), induced isolated oligos to undergo cell proliferation. Insulin and transferrin individually exerted little effect on cell maintenance, while FGF alone elicited a survival of 70% of the cell number when compared to sister cultures containing serum-supplemented medium. In combination the supplements acted synergistically, producing a 3 fold increase in cell number at the end of a 5 d growth period, when compared to sister cultures in serum-supplemented medium.

After the 5 d growth period, cells in ODM were judged by biochemical and immunological criteria to be 95-98% oligos. These cells were inducible for the enzymes glycerol phosphate dehydrogenase (GPDH), lactate dehydrogenase (LDH), and 2',3'-cyclic nucleotide 3-phosphohydrolase (CNPase). Utilizing immunological methods, the oligo specific markers GPDH, galactocerebroside (GC), and myelin basic protein (MBP) were detected. Cultures were found to be approximately 95% positive for GPDH, 90% for MBP, and 60% for GC. The fibroblast marker protein fibronectin was not found, while only 2-5% of the cells expressed the astrocyted marker protein glial fibrillary acidic protein. These criteria strongly suggest that the cells undergoing proliferation are oligodendrocytes. (Supported by NIH grants HD 05615 and HD 06576, and DOE Contract DE-AM03-76-SF00012)

111.10 CONTROL OF EXPRESSION OF THE BETA NERVE GROWTH FACTOR GENE IN SYMPATHETIC EFFECTOR ORGANS. D.L. Shelton and L.F. Reichardt Div. of Neuroscience, UCSF. San Francisco, CA. 94143

Beta Nerve Growth Factor (NGF) is a protein necessary for normal development and maintenance of sympathetic and sensory neurons in vivo and in vitro. Evidence has accumulated which indicates that NGF is required at growing tips of axons and when present there, is bound, internalized, and transported retrogradely to the cell body. This has led to the hypothesis that NGF is produced by target tissues of the responsive neurons, but so far this has been impossible to demonstrate.

Using an assay capable of detecting 10 femtograms (fg) of mRNA encoding NGF (NGFmRNA)(Reichardt and Shelton, these abstracts), we have surveyed tissues with varying densities of sympathetic innervation for their content of NGFmRNA as an indication of their levels of synthesis.

There are extremely high levels of NGFmRNA in male mouse salivary gland and rabbit prostate, two tissues known to have anomolously high levels of NGF. Tissues which have a dense sympathetic innervation, such as iris, heart atria, heart ventricle, and spleen have relatively high levels of NGFmRNA. Those tissues with a paucity of sympathetic innervation, such as muscle and thymus, have much lower levels. When the innervated spleen capsule is isolated and assayed separately from the uninnervated splenocytes, it is found to contain almost all the NGFmRNA of the entire organ.

An iris denervated either in vivo or by growth in vitro rapidly accumulates NGF. It was not previously known whether this reflects an increase in synthesis or a change in removal or degradation of NGF. We have found a large increase in NGFmRNA content when irides are placed into culture. This suggests that the accumulation of NGF seen upon denervation is due, at least in part, to an increase in synthesis.

In addition to target tissues, NGFmRNA was found in elements of the adult peripheral nevous system. Sensory and sympathetic ganglia and sciatic nerve all contained readily detectable amounts. This may help explain why adult sympathetic and sensory neurons are relatively refractory to treatments which cut off the supply of NGF from the periphery.

NGF has recently been shown to have effects on the CNS of mammals. We find levels of NGFmRNA in the CNS higher than can be accounted for by the sparse sympathetic innervation of the cerebral vasculature. There are up to 5-fold differences in NGFmRNA content between different regions of the CNS, further arguing against vasculature as the only source, and suggesting that endogenous NGF may play some role in normal adult CNS function.

111.11 SYMPATHETIC NEURON DENSITY DIFFERENTIALLY REGULATES TRANSMITTER PHENOTYPIC EXPRESSION IN CULTURE. J.E. Adler and I.B. Black. Division of Developmental Neurology, Cornell Univ. Med. Coll., N.Y., NY 10021.

Sympathetic cell aggregation appears to play a critical role in expression of transmitter traits during embryonic development in vivo (e.g. Cochard et al., Devel. Biol. 71, 100, 1979). To study the effects of cell density and aggregation in detail, we examined dissociated, pure sympathetic neuron cultures grown at varying cell concentrations in fully-defined, serum-free medium. After one week at a density of 7-8000 neurons/35 mm dish, moderate levels of tyrosine hydroxylase (TH) activity (728 pmoles/dish/h) and substance P (SP; 20 pg/dish) were measured. In contrast, choline acetyltransferase (CAT) activity was undetectable. Increasing neuron density 4-fold resulted in a 4-fold increase in TH activity and, consequently, no change in TH per neuron. In contrast, SP increased 30-fold, resulting in a 7-fold increase in SP per neuron. Further, CAT activity, initially detectable at a density of 15,000 neurons/dish, increased 6-fold when this cell concentration was doubled. Thus, cell density differentially affects expression of different transmitter traits. Moreover, medium conditioned by high density cultures failed to reproduce these effects on low density cultures, suggesting that diffusible factors were not involved and that cell contact may be critical.

Time-lapse phase-contrast microscopy of high density cultures demonstrated neuronal migration and progressive aggregation, that did not occur in low density cultures. Our observations suggest that cell contact may mediate differential expression of transmitter traits. We are studying potential underlying mechanisms.

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111.12 PURIFIED MOTONEURONS RESPOND TO TWO DISTINCT ACTIVITIES IN MYOTUBE CONDITIONED MEDIUM. A.L. Calof and L.F. Reichardt. Div. Neurosci., UCSF Sch. of Med., San Francisco, CA 94143

We have purified spinal motoneurons from chick embryos, using retrograde transport and fluorescence-activated cell sorting techniques, in order to study the factors that affect their survival and development. Motoneurons rapidly extend neurites when plated onto polylysine-coated dishes that have been exposed to conditioned medium from cultures of chick myotubes (MCM). Enzymatic analysis and density sedimentation of this substratum-binding, neurite outgrowth promoting activity from MCM indicate that it is similar to neurite outgrowth-promoting factors which are present in media conditioned by many cell types, and which appear to be complexes containing heparan sulfate proteoglycan and laminin (Lander et al., Soc. Neurosci. Abstr. 10).

In addition to its outgrowth-promoting activity when applied to the culture substratum, MCM in the culture medium of motoneurons enhances their survival over periods of >2 days in culture. This apparent trophic activity might be due to the motoneurons' need for a continuous supply of the substratum-binding factor (SBF), which they might take up and use up during the course of longer-term culture. A supply of SBF could be provided by the MCM added to the motoneuron culture medium. To test this, the SBF was partially purified from MCM by precipitation in 45% saturated ammonium sulfate and gel filtration on Sepharose CL-6B. The SBF fraction was added back to the medium of motoneuron cultures every 2-3 days for 6 1/2 days. Motoneuron cultures supplemented in this way fared no better than cultures grown without supplement. Thus, a continuous supply of the SBF does not appear to be sufficient for long-term survival of motoneurons.

The apparent trophic activity of MCM could be due to a general medium conditioning effect. Media conditioned by other cell types might then be able to substitute for MCM in promoting motoneuron survival. To test this, motoneurons were grown for 1 week on partially-purified SBF in growth medium supplemented with MCM and/or conditioned media from chick spinal cord cells (SCCM) or fibroblasts (FCM). MCM, SCCM, and FCM all enhance motoneuron survival over 1 week, but MCM is most effective. SCCM and FCM also weakly inhibit neurite outgrowth and/or survival at 24 hr. This inhibition can be overcome by the addition of small amounts of MCM. Thus, MCM appears most effective in promoting motoneuron survival because it contains a substance(s) that SCCM and FCM contain less of, or lack altogether.

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111.13 NEUROTROPHIC EFFECTS OF ADULT AMPHIBIAN EXPLANT-CONDITIONED MEDIUM. H.T. Whelan, P.C. Letourneau*, and K.F. Swaiman*. Div. of Pediatric Neurology, and Dept. of Anatomy, University of Minnesota Medical School, Minneapolis, MN 55455.

Spinal cord regeneration, even through adulthood, occurs in urodele amphibians, such as ambystoma tigrinum(the tiger salamander). In our previous experiments we found evidence of a protein produced by adult salamander heart which stimulates neurite outgrowth. This effect must be further characterized, and its relevance to central nervous system regeneration in salamanders has yet to be clearly demonstrated.

The tissue surrounding spinal cord is likely to participate in molecular interactions following axonal injury with special significance in neuronal regeneration. Thus, the regenerating tail,"blastema" at the site of cord transection may also produce neurotrophic substances. However, crude homogenates of the blastema do not stimulate neurite outgrowth in vitro. Indeed, blastema homogenate appears to be neurotoxic. This may have several explanations: 1)homogenation may destroy neurotrophic factors; 2)crude homogenate contains many substances, some of which may be antagonistic to neurotrophic factors, or toxic to neurons in culture; 3) perhaps inhibitory as well as stimulatory neurotrophic factors exist which serve to guide and direct(counter-regulate) axonal regrowth in vivo.

Experiments with salamander tissue explants were thus performed to evaluate the substances secreted by living tissue without homogenation. Neurite outgrowth and cell survival were evaluated in dissociated neuron cultures(from chick embryo dorsal root ganglia and fetal mouse cortex) treated with spinal cord-,blastema-,or heart-conditioned medium prepared by incubating tissue explants from adult salamanders 2 to 8 weeks post cord transection(by tail amputation).

Neurite outgrowth was enhanced by heart-and spinal cord-conditioned medium, but neurite outgrowth and cell survival were inhibited by blastema-conditioned medium. Ultrafiltration of the blastema-conditioned medium with a 10,000 dalton molecular weight cut-off Amicon filter apparatus produced 2 fractions. The "concentrate"(M.W.$>$10,000 daltons) continued to be neurotoxic in vitro. The "ultrafiltrate"(M.W.$<$10,000 daltons) produced extensive neurite outgrowth in vitro.

These results suggest that adult salamanders produce substances during spinal cord regeneration with stimulatory and inhibitory neurotrophic effects. Experiments utilizing two-dimensional electrophoresis and autoradiography of labelled proteins in conditioned medium are planned to further characterize factors unique to CNS regeneration.

112.1 POLYNEURONAL AND INTERSEGMENTAL INNERVATION OF BODY MUSCLES IN THE ZEBRAFISH. Monte Westerfield, Paul Z. Myers*, and Judith S. Eisen, Institute of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

We have examined the pattern of motor innervation of body muscles in adult zebrafish to learn if muscle fibers 1) receive input from more than one motoneuron (polyneuronal innervation) and 2) receive input from more than one spinal segment (intersegmental innervation).

Muscle fibers in the ventral half of body segments 5-10 in the trunk were studied. The pattern of innervation of an individual fiber was determined by recording end plate potentials (EPPs) while stimulating the spinal nerve of each segment with suction electrodes. The degree of polyneuronal innervation was determined by varying the amplitude of the stimulating current and counting the number of discrete EPP amplitudes. In the normal physiological saline containing 1.8 mM Ca^{2+}, the majority of EPPs were suprathreshold and elicited an action potential in the muscle fibers. Since this obscured the observation of larger amplitude EPPs, most measurements were made in a reduced Ca^{2+} (0.5-0.9 mM) saline.

The majority of muscle fibers were polyneuronally innervated. Approximately 40% (n=72, in the reduced Ca^{2+} saline) of the fibers received input from two motoneurons, 26% received three inputs and 26% were singly innervated. A few fibers (8%) were innervated by four motoneurons and no fibers with more than four inputs were observed. In almost all cases (93%, n=208, in normal and reduced Ca^{2+} saline) muscle fibers were innervated exclusively by motoneurons from their own spinal segment. In the few fibers with intersegmental innervation, a single input came from an axon in an immediately adjacent segment.

These results suggest that spinal cord motoneurons in the zebrafish are restricted in their choice of postsynaptic targets by 1) sharing individual muscle fibers with no more than one or two other motoneurons and by 2) confining their synapses to fibers within their own segment. The origins of these restrictions are being examined during initial growth of the motor axons (see Eisen, Myers and Westerfield in this session).

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112.2 SEGMENTALLY SPECIFIC GROWTH OF MOTOR AXONS IN LIVE ZEBRAFISH EMBRYOS. Judith S. Eisen, Paul Z. Myers*, and Monte Westerfield. Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

The axial musculature of adult zebrafish is arranged segmentally and muscle fibers in each segment receive innervation primarily from motoneurons within the corresponding spinal segment (Westerfield et al., this session). In order to determine how this segmental specificity arises, we watched motoneurons in the living animal as they grew axons and innervated their target muscle fibers. Motoneurons were visualized by filling an early precursor blastomere with a fluorescent dye (rhodamine or fluorescein) coupled to dextran. Motoneurons were observed from the first time at which they were recognizable by their characteristic size and location in the spinal cord. We concentrated our efforts on a single class of neurons, the large primary motoneurons, of which there are only 2-4 per hemisegment (Myers, Soc. Neurosci. Abstr. 1983, 9:848).

Somata of primary motoneurons were first identified in the spinal cord at 18 hrs. post-fertilization. The first axons left the spinal cord between 18-19 hrs., and could be labeled by retrogradely transported horseradish peroxidase shortly thereafter. The length of an individual axon in the periphery at early times (between 20-50 hrs.) was not correlated with the rostro-caudal position of that cell in the animal. As it left the cord, each axon appeared to follow a stereotyped pathway. The axon grew along the medial surface of the developing muscle in the middle of the segment. When the axonal growth cone reached the region of the horizontal septum separating the dorsal and ventral muscle masses, it formed a large varicosity and often paused for up to several hours. When it resumed growing, the growth cone followed one of a small number of stereotyped pathways to give rise to cells with characteristic, and identifiable morphologies. In most cases the axon of each primary motoneuron was confined within a single segment (95%; n=20 motoneurons) during the time of our observations (up to 50 hrs.). All of the axonal branches were maintained during the period of observation.

From our observations we conclude that the pattern of innervation of the axial musculature in the zebrafish initially develops in a segmentally specific fashion.

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112.3 GROWTH CONES OF ZEBRAFISH POSTERIOR LATERAL LINE SENSORY NEURONS ARE CLOSELY ASSOCIATED WITH THEIR MIGRATING TARGET CELLS. Walter K. Metcalfe, Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

The development of the posterior lateral line system of young zebrafish (Brachydanio rerio) was studied in order to understand how the developing axons of sensory neurons are able to locate their peripheral targets. These targets are hair cells of neuromasts. Electron microscopy has revealed chemical synapses from hair cells onto terminals of sensory neurons in five day old larvae. The sensory neurons are bipolar cells whose somata are located in a postauditory ganglion. Peripheral axons of the sensory neurons extend a considerable distance to terminate within neuromasts.

Scanning electron microscopy of embryos at different developmental stages demonstrated that the first six "primary" neuromasts arise in a strict rostrocaudal sequence during the period between 33 and 48 hours post-fertilization. These neuromasts are distributed from the sixth myotome to the base of the tail.

Examination of living embryos, histological sections, and scanning electron micrographs shows that the primary neuromasts arise directly from cells of a migratory primordium. The premigratory primordium is first seen as a postauditory placode immediately adjacent to the developing sensory ganglion in 20 hour embryos. The primordium then migrates caudally between the inner and outer layers of the epidermis along the transverse myoseptum at a rate of about 100 μm/hr. The migration to the base of the tail is complete at about 39 hours. Clusters of cells are deposited from the trailing end of the primordium as it migrates caudally. These cell clusters give rise directly to the primary neuromasts. Thus, the origin of the primary neuromasts of zebrafish is similar to the origin of primary neuromasts in amphibians (Harrison, '04; Stone, '33).

The development of sensory neurons was studied by labeling these cells with horseradish peroxidase. At 20 hours, before the primordium has begun to migrate, some sensory neurons already possess both peripheral and central axons, and the growth cones of the peripheral axons are within the premigratory primordium. Later, sensory neuron growth cones are within the actively migrating primordium.

These results demonstrate that although the growth cones of the sensory neurons grow over a considerable distance to their final destination, they are never very far from their target cells, which migrate with them and may even lead them. Supported by NIH grant NS 17963.

112.4 ADHESION BETWEEN OPTIC AXONS AND NEUROEPITHELIAL ENDFEET IS MEDIATED BY NEURAL CELL ADHESION MOLECULE (NCAM). U. Rutishauser and J. Silver. Dept. of Developmental Genetics and Anatomy, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

NCAM has been shown previously to be a ligand in the formation of bonds between surface membranes of cells which express this molecule on their surface. These cells include virtually all nerve and striated muscle cells and their precursors, and recently the molecule has also been identified on cultured astroglial cells (Noble, M., Bock, E., and Rutishauser, U., in preparation). We report here that NCAM antigenic determinants are localized specifically on the endfeet of the neuroepithelial cells which line the optic pathway and, in the disk and stalk regions, differentiate into glial cells. Fascicles of optic axons are found in close proximity to these cells and it has been proposed that the endfeet constitute a substrate for neurite elongation (Silver, J. and Sapiro, J., J. Comp. Neurol 202: 521, 1981). To test the possibility that NCAM on neuroepithelial endfeet might be involved in guidance of optic axons, we injected anti-NCAM Fab into the eyecup of 84 hour chick embryos and 24 hours later analyzed the route of retinal ganglion cell axons which subsequently grew out of the eye. These experiments revealed that the Fab caused the optic nerve axons to abandon the endfoot region of the neuroepithelial margin and become dispersed throughout the intermediate zone of the optic stalk. As the axons grew out of the region containing the Fab, that is, further proximally into the nerve, they were able to re-establish their association with endfeet and except for a disruption of local fiber order, assumed their normal locations. These results suggest that NCAM on neuroepithelial endfeet is a factor in the positioning of retinal ganglion cell axons as they grow toward the tectum.

112.5 GUIDANCE OF OPTIC AXONS BY A PREFORMED ADHESIVE PATHWAY ON NEUROEPITHELIAL CELL ENDFEET. J. Silver and U. Rutishauser. Dept. of Developmental Genetics and Anatomy, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

What are the factors that direct the growth of optic axons along their tortuous path at the marginal edge of the neural tube? Previous studies have shown that retinal ganglion cell growth cones associate preferentially with marginal neuroepithelial cell endfeet at all points along their pathway. The continuing addition of growth cones at only one surface, the outer limiting membrane, may play a role in positioning optic axons at the periphery as well as generating orderly topographic patterns of axons within the nerve. We have shown that in embryonic chicks, the close association between axons and neuroepithelial endfeet may be due to an adhesive interaction mediated by NCAM. However, the question remains as to whether this molecule also plays a role in governing the overall trajectory of axons toward their central targets. Here, we report that in embryonic chicks, neuroepithelial cells express NCAM on their marginal endfeet before the first wave of optic fibers is generated. Furthermore, NCAM-producing endfeet are distributed in a surface pattern that closely mimics the route that the earliest axons will eventually take in the nerve, chiasm, and along the rostral wall of the tectum.

If NCAM plays a role in promoting adhesion between growth cones and neuroepithelial endfeet, then antibodies to NCAM should alter this interaction. Electron microscopic analysis of the chick optic pathway on embryonic day 4.5, 24 hours after intraocular injection of anti-NCAM Fab, revealed that growth cones in the distal stalk had dislocated from the endfoot region and were found within aberrantly located fascicles deep within the stalk's dorsal rather than ventral tier.

These observations suggest that a preformed pathway of NCAM confined to marginal endfeet of a discrete subpopulation of neuroepithelial cells may play a role in providing radial as well as long distance guidance cues to optic axons. Supported by NSF (BNS-8218700) and NIH (NS-15731)

112.6 THE ROLE OF THE ENVIRONMENT IN GUIDING INTERSECTING AXON TRACTS IN THE MAMMALIAN TELENCEPHALON. M.H. Hankin* and J. Silver (SPON: K. Alley). Dept. Dev. Genetics and Anat., Case Western Reserve University, Cleveland, OH 44106.

What are the forces acting at points of intersection of axon tracts that determine directionality? In order to answer this question the ontogeny of the corpus callosum (CC) and its perforating fibers (PF) was examined morphologically in a series of timed mouse embryos (C57BL/6J).

Beginning on embryonic day (E) 15, a preaxonal stage for both fiber tracts, a sequence of alterations in the spatial pattern of the non-neuronal environment occurs at the point of intersection. Coronal forebrain sections contain a matrix-filled, planar interface between cortical plate and intermediate zone. The matrix is a highly structured extracellular material between radial glial processes and is composed of a trilaminar core surrounded by an amorphous coat. The possible function of this material in axonal growth and guidance is unknown. The interface pathway is only present along the route of migration for the PF from cingulate cortex to the septum. Prior to the arrival of the first callosal axons on E17, the planar interface is closed at the corticoseptal junction. This occurs when subependymal cells migrate medially through the intermediate zone to join the ventral edge of the cortical plate, thereby forming a dense, cellular barricade. The barricade is the unfused and thickened rostral extension of the glial sling (Silver et al., 1982) and we propose that it is responsible for turning the callosal axons across the PF and toward the opposite hemisphere. These results show that the intersection of the CC and PF occurs as a result of (i) the different times of fiber tract formation, and (ii) the change in configuration of the subependymal environment.

Since the subependymal cells that line the lateral ventricles are continuous with those of the sling-barricade, they collectively form an extended pathway for callosal fibers as they migrate within and between the hemispheres. Is there an affinity between callosal fibers and subependymal cells? Discrete HRP labeling of callosal fibers in E17-18 embryos suggests that growth cones add at the subependymal surface, ie. later adding callosal growth cones "undercut" their predecessors. This pattern of fiber addition (in direct contrast to that found in the spinal cord and optic nerve where fibers add peripherally) supports the hypothesis that subependymal cells provide a pathway for growing callosal fibers. (Supported by NSF grant BNS 8218700 and NIH grant NS 15731)

112.7 OPTIC FIBERS PROJECT TO PUTATIVE CHOLINERGIC SITES AFTER ABLATION OF THEIR NORMAL TARGETS. T. A. Reh. Department of Biology, Princeton University, Princeton, NJ 08544.

Surgical removal of the major targets of retinal ganglion cells in Rana pipiens tadpoles resulted in anamalous optic fiber projections. Bilateral optic tectal and diencephalic nuclei ablations were carried out in midlarval tadpoles, and animals survived for one to two months. Animals received intraocular injections of ^{3}H-proline or HRP two days prior to sacrifice, and the brains were processed with standard histochemical and autoradiographic techniques.

The optic fibers took several new trajectories through the CNS following the surgery. Since the optic chiasm was usually not disrupted, the ganglion cell axons typically entered the CNS in the normal manner. The majority of fibers followed their normal path in the optic tracts, to the dorsolateral position normally occupied by their tectal and diencephalic targets. In many animals, large neuromas formed at this location; however, a substantial number of axons continued to grow caudally into the n. isthmus or the dorsolateral fasciculus of the medulla and spinal cord, adjacent to the trigeminal tract. A second group of fibers grew rostrally from the optic chiasm forming a projection to the ventrolateral forebrain.

These nuclei and CNS tracts in which the aberrantly projecting optic fibers are found, all share, with the retinal ganglion fibers themselves, acetylcholinesterase activity and α-bungarotoxin binding sites. Thus, there appears to be a selective association of optic fibers for other putative cholinergic CNS structures, when their normal targets have been removed. Previous studies of anomalous trajectories of retinal fibers in adult frogs and goldfish have proposed that the optic axons are following the degenerating debris left by tectal efferents after the tectal removal. This explanation seems unlikely to account for the present results for two reasons. First, injections of HRP into the tectum failed to reveal any significant numbers of tectal efferents in the dorsolateral spinal cord or the ventro-lateral forebrain. Second, embryonic removal of tectum, prior to the outgrowth of axons from the tectal cells, results in a similar pattern of anomalous fiber pathways. These results suggest that an association between axons of similar pharmacological character may be a factor in axon guidance and target selection. Supported by NIH EY055179.

112.8

WITHDRAWN

112.9 FORM, ULTRASTRUCTURE, AND SELECTIVITY OF GROWTH CONES IN THE DEVELOPING PRIMATE OPTIC NERVE: 3-DIMENSIONAL RECONSTRUCTIONS FROM SERIAL ELECTRON MICROGRAPHS
R. W. Williams and P. Rakic, Section of Neuroanatomy, Yale University School of Medicine, New Haven CT 06510

Five hundred serial ultrathin sections were cut in the transverse plane from the optic nerve of a 39-day rhesus monkey embryo. The number of axons in the nerve at this age was 7,500, merely 0.26% of the peak of 2.85 million reached at mid-gestation (Rakic & Riley 1983, Science 219:1441) and 0.63% of the adult value of 1.2 million. A group of 40 retinal ganglion cell growth cones (GCs) and 160 axons were traced sequentially through micrographs taken near the ventral margin of the nerve. Twenty-four GCs confined to the series were measured and reconstructed with an image analysis system.

The surface areas of GCs ranged from 100 to 300 μm^2, volumes from 15 to 30 μm^3, and lengths from 10 μm to more than 50 μm. The leading segment of all GCs spread into large sheet-like structures termed lamellipodias. These were 0.1 to 0.3 μm thick and up to 15 μm wide and often formed sheaths around axon fascicles. Roughly one-third of the surface area of the lamellipodias was apposed to glia and two-thirds to neurites. Spike-like projections (filopodia) were rare and none were longer than 2 to 3 μm. More than one-fourth of the GCs had branches that entered adjacent fascicles. In contrast, no optic axons branched, indicating that some lamellipodias withdraw (Williams & Rakic 1983, Invest. Ophthalmol. Vis. Sci. 25:125). The edges of the lamellipodias were connected by short linear junctional specializations (resembling punctae adherens) with other GCs, axons, and glial cells. The terminal 10 μm of GCs was free of microtubules and did not contain aggregates of vesicles, mitochondria, or ribosomes. However, this portion contained many microfilaments and a small amount of smooth endoplasmic reticulum. Although GCs made contact with up to 60 neurites, their stems did so with only 8 to 10 neurites. Surprisingly, 60% of the GCs failed to retain contact with any single neighbor from their lamellipodias back to their stems. GCs lost contact with 50% of their neighbors over a distance of less than 10 μm. Most growth cones took unique paths and acquired new sets of neighbors; they often crossed from one fascicle to another. Thus, contrary to prevailing views, in vivo GCs in the primary visual pathway of primates are sheet-like structures that do not grow along the same set of neighbors for significant distances. (Research supported by the NEI.)

112.10 CENTRAL NERVOUS SYSTEM (CNS) NEURONS GROW AND DIFFERENTIATE RAPIDLY IN THREE-DIMENSIONAL (3-D) CULTURE.
P.W.Coates. Dept.Anat.Texas Tech Univ.HSC Sch.Med. Lubbock,TX 79430.

The feasibility of using 3-D collagen substrate gels for culture of CNS and PNS explants and dissociated neurons has been established (Coates, Anat.Rec.208:14A,1984). The present report provides quantitative and qualitative data on growth and differentiation of dissociated CNS neurons on that substrate. Cerebral hemispheres from d.10-11 chick embryos were dissociated by trituration and enzymatic digestion. 3-D gels prepared from native collagen in tissue culture dishes were hydrated with M-199+1% PSF+10% FCS. 1-5X10^5 cells were plated onto 3-D gels or onto modified plastic culture dishes (Primaria) which simulate conventional substrates,i.e., polylysine, collagen or ECM. Thin collagen, plastic and glass have been used as well. Cultures were incubated at 37°C in a humid atmosphere (95% air+5% CO_2). A Bioquant II Image Analysis System was used for quantitation and statistical analysis of neuron growth parameters on d.1, 2 and 3 after plating. To avoid potential cell-cell contact effects, only single neurons not in contact with other neurons or glia, with at least one process longer than the neuron soma diameter, were used. Cultures were examined with phase contrast microscopy, LM of Nissl stained whole gels, and scanning and transmission EM. Neurons on Primaria did not in any case meet criteria for quantitation within 3 days. In contrast, neurons in 3-D attached rapidly (minutes-hours), strongly adhered to and promptly initiated process outgrowth into the gels. Two fundamentally distinct classes of processes were quickly established which became more complex over time: extremely long processes interpreted as axons, and one or more shorter processes interpreted as dendrites. Within hours many neurons became 'polarized', exhibiting bi- and multipolar morphologies characteristic of the in situ CNS region they were derived from. Indices of neuron growth and differentation in 3-D culture increased by 1-5 fold over the test period. These included number of: primary processes; branch points; segments; growth cones; as well as total length of dendrites and axons alone, or combined as total per neuron. Some axons measured >1400 um by d.3. The data demonstrate that single neurons in 3-D culture have the capacity for rapid, prolific growth and quick expression and maintenance of morphologically differentiated features. These base-line data will be used for comparison in future experimental manipulation of the system. (Partial support from a TTUHSC BSRG.)

112.11 NEURITE GROWTH AND FILOPODIAL INTERACTION STUDIED WITH VEC-DIC MICROSCOPY AND THE HVEM.
H.T. Tsui, K.L. Lankford* and W.L. Klein. Inter. Grad. Prog. Neurosci. and Dept. Neurobiology and Physiology, Northwestern University, Evanston, IL 60201

Neurite outgrowth requires the transport of cellular components to the growth cone regions as well as active interaction of the growth cones with the surroundings. We have made use of two powerful techniques, the video-enhanced contrast-differential interference contrast (VEC-DIC) microscope and the high voltage electron microscope (HVEM) to examine filopodial interaction and intracellular transport related to neurite growth.

Observation of living cultures of dispersed chick retina neurons with the VEC-DIC microscope revealed movements of small particles in the growth cone regions. These particles seemed to be transported between neurites and growth cones along particular pathways in a synchronous manner, so that frequently several particles were transported sequentially in the same direction at similar rates. Retrograde transport of particles was usually followed by neurite retraction or a reduction of growth cone area. These particles probably corresponded to vesicles of 40 to 200 mm in growth cones of whole mount neurons examined under the HVEM.

Specific interaction among filopodia was suggested by VEC-DIC microscopic examination of filopodial movements. Large circular movements of motile filopodia were usually arrested and replaced with small vibratory movements when filopodia were in close proximity with other filopodia. Frequently these filopodia were not seen to be in direct contact as observed under the VEC-DIC microscope. However, when examined with the HVEM, filopodia were often found to be connected to each other through strands of fine extracellular filaments. These extracellular structures had a distinct filamentous form but were of slightly variable dimensions. They were between 10 to 17 mm in width and 30 to 100 mm in length. Extracellular filaments also seemed to attach isolated filopodia and flat regions of neurites to the substrate. Similar extracellular filaments were also detected in freeze-dried preparations and thick sections of retina cultures, suggesting they were not artifacts of the drying procedure. These filaments may be involved in cellular recognition or adhesion mechanisms associated with filopodial interaction.
(Supported by NIH Grant NS18490 to WLK and NIH 00570-13 to Biotechnology Resources at HVEM Lab at University of Wisconsin, Madison)

112.12 EFFECTS OF GRAFT ORIGIN AND SITE OF TRANSPLANTATION ON REVERSAL OF CONGENITAL HYPOGONADISM BY GNRH TRANSPLANTS.
G.J. Kokoris, M.J. Perlow, M.J. Gibson, E.A. Zimmerman, A.J. Silverman, H.M. Charlton*, and D.T. Krieger. Mt. Sinai School of Med., New York, N.Y. 10029 (G.J.K., M.J.G., D.T.K.); Univ. of Illinois, Chicago, IL 60657 (M.J.P.); Columbia Univ., New York, N.Y. 10032 (E.A.Z., A.J.S.); Univ. of Oxford, Oxford, U.K. (H.M.C.).

We have reported that grafts of normal fetal preoptic area (POA), a site of GNRH-containing neurons, when transplanted into the third ventricle of adult mice of the hypogonadal (HPG) strain reversed their hypogonadism and corrected many of the endocrine and reproductive deficits present in these animals. Immunocytochemistry revealed GNRH-containing cells within the transplant, as well as GNRH-positive fibers projecting from the graft into the median eminence (ME) of the host.

Since GNRH cells in the normal adult are also found in the accessory olfactory bulb (AOB), we compared adult male HPG mice which received either AOB or POA transplants from 17d normal fetal donors. 2 months after surgery, 6 of 7 males with POA grafts showed a significant increase in gonadal weight. In these animals, GNRH fibers innervating the ME and GNRH neurons within the transplant were observed. Only 1 of 6 animals receiving AOB transplants exhibited a significant increase in gonadal weight. This animal showed a large number of GNRH-reactive cell bodies and fibers innervating the ME. The pattern of innervation was comparable to that of successful POA grafts. To date, 2 other animals with AOB grafts have been studied immunocytochemically. Only a few GNRH- reactive cells in the transplant and GNRH fibers in the ME were seen.

In 9 animals, we examined the effects of a site of transplantation distant from the ME by unilaterally placing fetal POA grafts into the lateral ventricle of adult HPG males. Although after 2 months none of these animals showed gonadal growth, upon immunocytochemical examinationgrafts were seen to contain numerous GNRH cells. Immunoreactive GNRH fibers could be traced leaving the graft ventrally to join the adjacent stria terminalis and thence coursing towards the ME, as well as the septum and organum vasculosum of the lamina terminalis. However, no fibers were seen entering the ME.

In conclusion, those animals with either POA or AOB transplants that exhibited gonadal growth also showed innervation of the host ME by GNRH fibers exiting the transplant. Perhaps with a longer survival period, GNRH fibers from the lateral ventricle transplants could also innervate the ME in sufficient numbers to facilitate gonadal growth.

113.1 **A cDNA CLONE ENCODING NEUROPEPTIDES EXPRESSED IN APLYSIA ABDOMINAL GANGLION NEURON L11.** R.Taussig* and R.H. Scheller (SPON:H.Muller).Dept. of Biol. Sci.,Stanford Univ., Stanford,CA 94305.

Single nerve cells can use more than one substance as extracellular chemical messengers. Classical transmitters have been shown to coexist in the same neuron as neuroactive peptides. In addition, multiple neuroactive peptides which are thought to be coreleased are often encoded in the same precursor, assuring their stoichiometric synthesis. The abdominal ganglion of the gastropod mollusk Aplysia californica contains a number of identified neurons which are cotransmitter candidates. One of these cells, L11, is cholinergic and probably uses neuroactive peptide transmitters. Differential screening techniques using radioactively labeled cDNA probes were employed to isolate cDNA clones from a λgt-10 cDNA library constructed from poly-A+ RNA purified from the abdominal ganglion. Clones derived from RNAs differentially expressed in neurons L11, R15, and bag cells were isolated. Sequence analysis of the L11 specific clone suggests that it encodes a 14.7 KD protein which is the precursor for neuroactive peptides. Southern and Northern blot analysis reveal that the polyA+ RNA transcript is about 1.2 KB and there are 1-3 copies of this gene in the Aplysia haploid genome.

Antibodies directed against various portions of the precursor were produced from synthetic peptides conjugated to carrier proteins. Immunohistochemical studies using these antibodies reveal that L11 is the major site of immunoreactivity in the abdominal ganglion. An immunoreactive process emminates from L11 and exits the ganglion via the siphon nerve. In addition, several other neurons located in the lower right quadrant of the abdominal ganglion stain with these antibodies, although with less intensity, and each sends a process into the branchial nerve. Several small unidentified cells in the cerebral, buccal, and pleural ganglia also show reactivity with these antibodies.

113.2 QUANTITATION OF mRNA IN INDIVIDUAL NEURONS IN HUMAN BRAIN. W.S.T. GRIFFIN, R. COX*, and M.R. MORRISON. Depts. of Cell Biology and Neurology. Univ. Tx. Hlth. Sci. Ctr., Dallas, Tx., 75235

We have quantitated specific mRNA levels in both rat and human brain at various times postmortem by hybridization and translation analysis. We found that although there is an overall loss of RNA with increasing postmortem interval, the relative proportions of abundant mRNAs which remain are identical to those present immediately postmortem. However, analysis of RNA isolated from brain homogenates cannot show whether the rate of postmortem RNA degradation is similar in all cell types or whether some are affected more than others. Only by in situ hybridization can this be ascertained. To determine the distribution of total polyadenylated mRNAs among the various cell types at different intervals postmortem, we have stored adult postmortem rat cerebellum at room temperature for either 4 or 16 h before tissue processing for in situ hybridization. The results were compared with in situ hybridization to fresh rat cerebellum and to human hippocampus obtained 4 h postmortem. Polyadenylated mRNAs in the Bouin's fixed paraffin sections were hybridized to [^{3}H] polyuridylate (39,500 cpm per section; 3 Ci/mmole) at 50°C for 1 h, washed extensively at stringencies necessary to obviate nonspecific binding, autoradiographed, and stained. We found that even after 16 h postmortem the integrity of the tissue was such that neuropathological assessment could be made, and that the autoradiographic grains were still localized primarily over cell bodies. For example, areas containing axon terminals were relatively free of grains. As we expected from our previous studies (Morrison and Griffin, 1981, Anal. Biochem. 113, 318-324), loss of mRNA was proportional to postmortem interval and similar in extent to loss of translatable mRNA (i.e., grain density decreased by 30% between 0 and 4 h and by 50% in cerebellum stored for 16 h). Visual inspection indicated that loss of mRNA was similar in the different cell types. In the human hippocampus 4 h postmortem, morphology was maintained and the background grain density was very low. Each cell type was covered with grains, with no evidence of mRNAs leaking into the interstitial space. These studies show that meaningful quantitation of mRNA (by autoradiographic grain counting) in individual cell types is possible up to 16 h postmortem. Thus, in situ hybridization can be used to identify cell types with aberrent mRNA levels in diseases such as Alzheimer's disease. Funded by HD14886; AI14663.

113.3 CONSTRUCTION AND CHARACTERIZATION OF THREE AGE- AND REGION-SPECIFIC cDNA EXPRESSION LIBRARIES OF THE MOUSE BRAIN. W.Wille, A.Unterbeck*, UAO Heinlein*, K.Olek*+, B.Müller-Hill* & H.Schaal* (SPON: ENA). Inst. f. Genetics, Univ. Köln, D-5000 Köln 41 and +)Inst. f. Human Genetics, Univ. Bonn, D-53 Bonn 1, Fed. Rep. Germany

The complexity of the poly(A)+mRNAs of the mammalian brain is significantly higher than in any non-neural tissue . On the other hand, many of the brain specific mRNAs are present only in low copy numbers (Milner RJ & Sutcliffe JG,Nucl Acid Res 11:5497, 1983). Although age-, genotype- and location-specific proteins have been characterized in the mouse brain (Heinlein UAO & Wille W, submitted), almost all genes involved in the histogenesis of the brain have to be defined by their corresponding mRNA sequences.

In order to identify genes with a development-dependent regulation in the mouse cerebellum, we constructed 3 cDNA libraries, two cerebellar (postnatal day 2 and adult) and one cerebral cortex library.

RNA has been isolated from all three tissues by a modified technique according to SL Bernstein et al. (J Neurogen 1:71, 1983) and the poly(A)+mRNA has been purified by oligo(dT)-cellulose chromatography. The bulk of the poly(A)+mRNA ranged from 300 to 2300 nt in length. All three mRNA-populations have been translated in vitro using the reticulocyte lysate system. The translation products have been analyzed by 1-& 2-D-SDS-PAGE.

The poly(A)+mRNAs were transcribed into cDNA and cloned in the pcD vector system, which allows cloning in E.coli and also promotes expression of the cDNA segments in mammalian cells (Okayama H & Berg P, Mol Cell Biol 3:280, 1983). The minimal estimation of the yield is 70,000 colonies per library, of which at least 70% contain inserts. More than 25% of the inserts are larger than 500 nt in lenght.

The characterization of the libraries in respect of 'housekeeping', brain- & cerebellum-specific sequences, and the use of the libraries in screening experiments will be presented.

113.4 **EXPRESSION OF THE ELH GENE FAMILY IN APLYSIA.** J. R. Nambu*, A. C. Mahon*, R. Taussig*, R. H. Scheller. (SPON:,C. Shatz) Dept. of Biological Sciences, Stanford University, Stanford CA., 94305.

This study was initiated in order to investigate the tissue specific expression exhibited by members of the ELH gene family in Aplysia.

Transcription of the ELH genes was examined through the isolation and characterization of homologous cDNA clones from both abdominal ganglion and atrial gland cDNA libraries. Several clones were identified and analyzed via restriction enzyme mapping and DNA sequencing. The existence of two distinct types of bag cell transcripts was demonstrated; both encode the ELH precursor and both are homologous to the previously characterized ELH genomic clones. In addition, these clones also contain 5' untranslated exons which had not been described within the genomic clones. One type of cDNA is roughly 1.30 kb and contains 190 bp of novel 5' untranslated sequence. The second type of cDNA is 1.15 kb and posesses only the first 40 bp of this 5' sequence. Characterization of atrial gland cDNAs suggests that they are organized in a similar fashion as the bag cell transcripts.

These findings demonstrate the existence of at least three discrete exons within the corresponding genes. In order to elucidate the genomic arrangement of these segments and to determine how they give rise to the cDNA types, an Aplysia genomic DNA library was screened with probes made from the novel 5' sections of the cDNA clones. Several positive recombinants were identified, including one, clone ELH 18, that is known to contain a single A peptide gene. Regions of this clone and two others which hybridized to the 5'-end cDNA probes were sequenced. The entire 190 bp 5' untranslated region was found to occur in all three genomic clones, implying the existence of a single intron within these genes. In ELH 18, some 5 kb separates the two exons and thus transcription would be initiated well upstream of the precursor coding region.

Further characterization of the overall structure and organization of the various ELH gene family members is currently being pursued in order to determine whether alternative arrangements of these exons exist in the genome. Such information is necessary for a complete understanding of the patterns of expression exhibited by these genes whose products influence egg-laying behavior in Aplysia.

113.5 CHARACTERIZATION OF GENES CODING FOR THE CATECHOLAMINE BIOSYNTHETIC ENZYMES. E.E. Baetge, D.J. Reis and T.H. Joh. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

We have recently proposed that the catecholamine (CA) biosynthetic enzymes tyrosine hydroxylase (TH), dopamine B-hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT) share similar protein domains in their primary structure, and that these enzymes might therefore share common gene coding sequences (Joh et al., Cold Spring Harbor Symp. Quant. Biol. Vol. 48, 1983). To further test this hypothesis, cDNA clones were generated for DBH and PNMT and used to analyze the structural relationships among the mRNAs and genes coding for these enzymes.

DBH and PNMT cDNA clones cross hybrid-selected both DBH and PNMT mRNA in positive hybrid selection assays. Northern blot analysis also shows cross-hybridization. Southern blots suggest that both DBH and PNMT are encoded by single genes which may be linked. Furthermore, a genomic clone for DBH containing a 12 Kb insert has been isolated from a human DNA library. When the clone is subjected to Southern blot analysis and hybridized with either DBH or PNMT cDNA probes, PNMT is observed to specifically cross-hybridize to the DBH genomic DNA.

Partial DNA sequence analysis of PNMT and DBH cDNAs has revealed the existence of exact DNA matches of 95, 120 and 121 bases. More interesting is the finding that two of these matches contain an internal repeat of 63 bases. Translation of open reading frames into amino acid sequence revealed a composition high in arg, lys and pro which compares favorably to the amino acid composition analysis previously performed on the purified enzyme proteins.

These data strongly support our hypothesis that the catecholamine enzymes share DNA sequence homologies and suggest the possibility that the CA enzyme gene(s) may have evolved from a common ancestral precursor. (Supported by NIH Grants NS19002, MH24289 and HL18974.)

113.6 ISOLATION AND CHARACTERIZATION OF A cDNA CLONE TO HUMAN TYROSINE HYDROXYLASE: EVIDENCE FOR DNA SEQUENCE HOMOLOGY AMONG THE CATECHOLAMINE SYNTHESIZING ENZYMES. J.M. Carroll, E.E. Baetge, D.J. Reis and T.H. Joh. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

We have postulated that the catecholamine enzymes share similar gene coding sequences (Joh et al., CSH Symp. Quant. Biol. 48, 1983). This hypothesis is supported by the finding of sequence homology between cDNAs for dopamine-β-hydroxylase (DBH) and phenylethanolamine-N-methyltransferase (PNMT). We sought to establish if tyrosine hydroxylase (TH) also shares sequence homology with DBH and PNMT. We report the isolation of a cDNA clone for human neuroblastoma TH and characterization of its cross hybridization with DBH and PNMT clones.

Polysomal poly A^+ mRNA was prepared from human neuroblastoma cells, SK-N-BE(2), and enriched for TH mRNA by size fractionation on agarose gels containing methylmercuric hydroxide. The mRNA extracted from gel slices exhibiting the highest levels of immunoprecipitable TH was used to direct cDNA synthesis. The cDNA was inserted into the PstI site of pBR322 by G-C tailing and the recombinant plasmids used to transform the DH1 strain of E. coli. We screened the tetracycline-resistant library with a 1400 base pair DBH cDNA probe. This allowed for the selection of 200 colonies which were then analyzed by positive hybridization selection. Of the first thirty-six plasmids tested, one contained an insert which strongly selected an mRNA from BE(2) coding for a 60 kd protein, which was subsequently immunoprecipitated with TH antibody. This clone, TH189, contained an insert of approximately 400 bp which could be isolated from the plasmid by digestion with the restriction enzyme PstI. Northern blot analysis of mRNA from human BE(2), rat PC12 and bovine adrenal medulla revealed hybridization of TH189 to mRNA of 1900 nucleotides. This is identical in size to mRNA recognized by other cDNA probes to TH (Lewis et al., J. Bio. Chem., 1983; Lamouroux et al., PNAS, 1982) and is sufficient to code for a 60 kd protein. Southern blot analysis demonstrated cross hybridization of TH189 to two previously identified DBH clones and one PNMT clone.

These data support the hypothesis that regions of homology exist among three catecholamine synthesizing enzymes, TH, DBH and PNMT. Finally, this TH clone can now be used along with our DBH and PNMT cDNA clones to complete the investigation of the structure and regulation of the catecholamine biosynthetic pathway at the gene level. (Supported by Grants NS19002, MH24285 and HL18974.)

113.7 NERVE ENDING PROTEINS MAY SHARE DOMAINS WITH PHENOTYPIC SPECIFIC ENZYME: A SIMILAR GENE HYPOTHESIS. T.H Joh, M. Docherty*, H. Bradford*, E.E. Baetge and D.J. Reis. Cornell Univ. Med. College, New York, NY 10021 and Imperial Coll., London, England*.

The catecholamine biosynthetic enzymes tyrosine hydroxylase (TH), dopamine B-hydroxylase and phenylethanolamine N-methyltransferase share similar protein domains in their primary structures and share common gene coding sequences (Joh et al., CSH Symp. Adv. Biol. Vol. 48). To assess whether these common peptide sequences are shared by a distinct yet functionally related protein, we examined the crossreactivity of TH antibodies to a membrane protein in dopaminergic neurons as revealed by complement-mediated lysis of dopaminergic synaptosomes (SS).

Synaptosomes were prepared from the rat corpus striatum (400-1000 ug protein/ml) and incubated with: (a) TH-antibodies plus complement; (b) TH-antibodies alone; or (c) complement alone, in a total volume of 0.5-1.0 ml for 30 min at 37°C. Lactate dehydrohgenase (LDH) activity was revealed in the supernatant as a cytoplasmic marker enzyme. Sodium dependent high affinity uptake of dopamine (DA), choline, GABA and norepinephrine were measured in SS. Incubation of the SS only with TH-antibodies and complement released LDH into the medium (12% of total LDH in synaptosomes). This treatment blocked (to 40%) the high affinity uptake of DA but not choline, GABA or norepinephrine. This selective lysis of DA terminals of corpus striatum by TH antisera indicates that the antisera specific for TH, the neurotransmitter biosynthetic enzyme, is recognizing a protein in DA nerve terminals. Similar experiments with antibodies to glutamate decarboxylase and choline acetyltransferase caused selective lysis of the GABAergic and cholinergic subpopulations of the SS, respectively (Doucherty et al., submitted for publication). Together the results indicate that antibodies to a neurotransmitter synthesizing enzyme selectively recognizes membrane proteins(s) of the specific subpopulation of synaptosomes which contain that neurotransmitter synthesizing enzyme, and suggest that the neurotransmitter biosynthetic enzyme and the specific nerve endings protein(s) share similar protein domain(s). This implies that the enzyme and the protein of the nerve endings share common gene coding sequence(s).

More importantly, these results suggest that the coordinate expression of both the neurotransmitter enzyme and the nerve ending protein may define the phenotype of the neuron. (Supported y NIH Grants NS19002, MH24285 and HL18974.)

113.8 SUPRAOPTIC VASOPRESSIN AND DYNORPHIN mRNAs VISUALIZED BY IN SITU HYBRIDIZATION: ADVANTAGES OF SYNTHETIC OLIGONUCLEOTIDE PROBES. G.R. Uhl, G.O. Hackney*, H. Zing*, G. Heinrich* and J.F. Habener*. Depts. of Neuroscience and Neurology, Johns Hopkins University, Sch. of Med., Baltimore, MD 21205, Dept. of Neurology, Massachusetts General Hospital, Boston, MA 02114 and Howard Hughes Medical Institute, Harvard Med. Sch. Boston, MA 02114.

In situ hybridization can quantitate regional neuropeptide gene expression, and may provide a marker for the activity of specific peptidergic neuronal systems. The method can even allow study of differential expression of co-localized neuropeptides, such as vasopressin and dynorphin in supraoptic neurons. To study such differential regulation we need to develop evidence for hybridization specificity greater than that demonstrated in studies using single nick-translated cloned cDNA probes. We report use of multiple chemically-synthesized cDNA probes to localize vasopressin and dynorphin mRNAs in situ in rat supraoptic nucleus, and to allow increased confidence in hybridization specificity.

$AT^{32}P$-labelled, gel-purified vasopressin probes provide excellent hybridization in rat supraoptic nucleus. Several features of this hybridization argue for its specificity. Hybridization is localized to this known vasopressin-synthesizing area, while other brain areas in the same tissue sections show negligible labelling. This feature alone has been used to argue for specificity in single-cloned-probe studies. We can also adduce additional signs of selectivity. Several vasopressin probes directed against different portions of the gene yield positive staining of the same areas. Indeed, signal/noise ratios from 40- and 45-base cDNA probes are as favorable as those from a nick-translated cloned cDNA probe. Conversely, probes directed against somatostatin or glucagon genes are ineffective in this region. Finally, competition experiments with excess unlabelled homologous probe substantially reduce hybridization.

Hybridization with a 42-base dynorphin probe also yields staining in the supraoptic nucleus, though with lower signal levels that correspond to the lower levels of dynorphin peptide immunoreactivity noted here. Labelling these two co-localized peptide mRNAs with in situ techniques of proven specificity can provide a means for monitoring their differential expression in varying physiological states.

113.9 MULTIPLE mRNA SPECIES CONTAINING VIP SEQUENCES. I. Gozes, M. Bodner*, Y. Shani* and M. Fridkin*. Departments of Hormone Research and Organic Chemistry, The Weizmann Institute of Science, 76100 Rehovot, Israel.

VIP (vasoactive intestinal peptide) is a peptide which exhibits both neurohormone and neurotransmitter actions in the nervous system and in endocrine cells. In view of the many bioactivities of the peptide, it is plausible that several controlling mechanisms exist for its generation. To identify mRNAs containing VIP sequences, we chemically synthesized a battery of oligodeoxynucleotides as hybridization probes specific for mRNA species containing VIP sequences. The probes were produced in a stepwise manner, using the deoxynucleoside phosphoramidite approach. These specific probes were then radioactively labeled, using the enzyme polynucleotide kinase, and subsequently hybridized to mRNA, which had been resolved on denaturing agarose gels. Employing this approach, we identified, in rat brain, two major RNA species containing VIP sequences, one of ∿1600 bases and a second of ∿7000 bases. These two RNAs have limited structural similarity, namely, only one oligodeoxynucleotide probe (among several tested) exhibited cross-hybridization to both of them. The expression of the ∿1600-base poly(A)-rich mRNA is developmentally regulated and reaches a peak in the mature cerebral cortex in accordance with the VIP distribution. The ∿7000 RNA is mainly found in the poly(A)$^-$ RNA fraction and is enriched in nuclear preparations. The two mRNA fractions show specific tissue distribution. Indeed, some human tumors producing VIP contain only the ∿7000-base RNA, in contrast to the normal rat brain. In addition, a ∿1300-base VIP coding mRNA was described in human neuroblastoma, suggesting that different cells contain different VIP-mRNA species. In situ hybridization experiments coupled with VIP-gene cloning will probably lead to clarification of this issue. Indeed, using the specific oligodeoxynucleotide probes, we have identified a recombinant phage inserted with human genomic DNA that is positive for the prohormone sequences. Characterization of the VIP's genomic organization is now in progress.

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113.10 MYELIN BASIC PROTEIN (MBP) GENE EXPRESSION IN CULTURED DEVELOPING RAT BRAIN OLIGODENDROCYTES. N.K. Zeller*, T.N. Behar*, M. Dubois-Dalcq, R.A. Lazzarini*. Laboratory of Molecular Genetics, National Institutes of Health, Bethesda, MD 20205.

We have used the techniques of in situ hybridization and immunolabeling to correlate MBP expression with oligodendrocyte growth and differentiation in culture. For in situ hybridization, a cDNA clone of the rodent MBP gene was labeled with dATP ^{35}S to a final specific activity of 1×10^9 dpm/µg. Cultures grown on specially treated coverslips were fixed with paraformaldehyde, treated with 0.2 N HCl, post-fixed, dehydrated, and hybridized at 30°C for 20 hrs with 10^6 dpm of denatured probe. After stringent washes the cells were overlaid with photographic emulsion and developed after 3 days. For immunolabeling, both a monoclonal antibody and goat polyclonal serum to the small MBP were used. Brains from fetal and newborn rats were dissociated and grown in medium enriched with 5% fetal calf serum, various hormones, and growth factors. MBP was first detected 7 days after culturing 2 day-old rat brain, equivalent to 9 days post-natal rat age. MBP specific mRNA was detected 2 days before MBP was found by immunolabeling. At 11 days post-natal age, when 5% of the cells were MBP positive, process-bearing cells were shaken off the confluent layer and reseeded, resulting in a 10 to 15-fold enrichment in oligodendrocytes. The number of autoradiographic grains on the oligodendrocytes minus background, indicative of the amount of MBP mRNA present in the cells, increased about 10-fold between 9 and 14-17 days post-natal age. At that time, in the enriched cultures, the number of MBP positive cells by immunolabeling closely correlated with the number of cells expressing MBP mRNA. After 17 days, however, the number of MBP positive cells and the amount of MBP mRNA per cell declined. In contrast to the 2 day-old rat brain cultures, 16 day-old fetal brain monolayers contained rare cells expressing MBP RNA 7-9 days before MBP could be identified immunologically. Neurons will mature and develop for some time in culture from fetal brain but not from newborn brain, and may therefore account for these differences in MBP gene expression. Thus cultured oligodendrocytes appear to be able to differentiate in a time frame very similar to what is observed in vivo in terms of expression of MBP.

113.11 EXPRESSION OF THE THY-1 GLYCOPROTEIN GENE IN NEURONAL CELLS BY DNA-MEDIATED GENE TRANSFER. H.A. Ingraham*, K.A. Lewis*, G.M. Lawless* and G.A. Evans* (SPON:F.H.C. Crick). The Salk Institute, La Jolla, California 92138.

We have developed a system for studying the expression of a cloned brain-specific gene by DNA-mediated gene transfer. Thy-1 is an allelic cell-surface glycoprotein which was classically described as a marker for mature neurons, glial cells and T lymphocytes. In mouse nervous tissue, Thy-1 levels are developmentally regulated, increasing by up to one hundred fold during the first few weeks of life. The gene encoding the Thy-1 glycoprotein was isolated from a recombinant library constructed with BALB/c mouse genomic DNA. To study tissue-specific regulation of this cloned gene, we then constructed a plasmid containing the mouse Thy-1 gene and the bacterial xanthine-guanine phosphoribosyl transferase gene (Eco-gpt) as a selectable marker which confers resistance to mycophenolic acid. This construction was introduced into the rat neuronal cells B50 and B103, mouse neuronal cells alphaC and Et, mouse glial cells betaCFA, and mouse fibroblasts by either DEAE/dextran-facilitated DNA uptake or electric field-mediated gene transfer. Stable transformants were selected for resistance to mycophenolic acid and analysed for the presence of the cloned Thy-1 gene integrated into the host genome. Southern blotting experiments demonstrated that 2 to 4 copies of the cloned gene were present in each transformed cell. Cell-surface Thy-1 expression was determined by flow microfluorimetry using allele-specific anti-Thy-1 monoclonal antibodies. Cell-surface Thy-1 expression was 10 to 50 fold greater in transformed neuronal and glial cells than in fibroblasts with equivalent gene copy number. Northern blot analysis shows a similar increase in an mRNA species of 1800 nucleotides, consistent with the known size of the Thy-1 mRNA. This study demonstrates regulated expression of a cloned gene which is specific for neuronal and glial cell lines. Further experiments have localized the DNA sequences within the Thy-1 gene responsible for the control of gene expression in brain cells.

113.12 SYNTHESIS AND CLONING OF COMPLEMENTARY DNA TO POLYADENYLATED RNA FROM CHICK EMBRYO SPINAL CORD: IDENTIFICATION OF TISSUE SPECIFIC SEQUENCES. J.G. Dickson*, S.E. Coulson*, J. Kenimer* and F.S. Walsh*, Institute of Neurology, Queen Square, London WC1N 3BG, UK.

In order to study expression and regulation of genes involved in the development of the vertebrate spinal cord we have constructed a cDNA plasmid library representing expressed sequences of 7-day embryonic chick spinal cord. Double-stranded cDNA synthesized from spinal cord poly(A)$^+$ RNA was annealed into the Pst-1 site of PBR322 using dG- dC homopolymeric tails and annealed vector-cDNA then used to transform competent E. Coli HB101 cells (4×10^4 transformants per µg DNA). To identify sequences exhibiting tissue-specific expression patterns the library was screened by colony hybridization of 1) ^{32}P-labelled cDNA probes synthesized from poly(A)$^+$ RNA of spinal cord and liver tissues, and 2) spinal cord cDNA depleted of sequences complementary to liver poly(A)$^+$ RNA (cDNA difference probes). For screening with spinal cord versus liver cDNA probes (5×10^9 dpm per µg), randomly selected colonies were replicated in ordered arrays and duplicate nitrocellulose filters processed for colony hybridization. In the case of difference probe analyses, ^{32}P-labelled spinal cord cDNA was hybridized with excess liver poly(A)$^+$ RNA to a C_0t value of 2000, and cDNA-RNA hybrids removed by hydroxyapatite chromatography. The single-stranded fraction representing sequences unique to spinal cord tissue was then used to probe the spinal cord cDNA library. Some 3-5% of clones in the library were found to represent sequences expressed in spinal cord, but not detectable in liver tissue. Plasmid DNA isolated from 10 putative spinal-cord-specific clones was restricted with Pst-1 enconuclease and subjected to agarose gel electrophoresis. All plasmids were linearized by Pst-1 digestion and 5 had excisable cDNA inserts of >300 bp. Thus we have identified expressed sequences of 7-day chick embryo spinal cord which are not present in poly(A)$^+$ RNA of liver tissue. Further studies are required to determine the molecular nature and sequence of corresponding RNA species. In addition to studies on tissue-specific gene expression, the approach described here may be applied to analysis of cellular subpopulations from spinal cord e.g. motorneurons, for which suitable bulk separation methods exist.

114.1 MULTIPLE MECHANISMS INVOLVED IN SOMATOSTATIN'S INHIBITION OF ADRENOCORTICOTROPIN RELEASE FROM MOUSE PITUITARY TUMOR CELLS. Terry Reisine and Ronald D. Sekura*, Section on Pharmacol., Lab. Clinical Sci., NIMH and Lab. Develop. and Mol. Immunity, NICHD, Bethesda, MD 20205

Somatostatin (SRIF) can inhibit the release of pituitary hormones by a direct action on the adenohypophysis. In a tumor cell line of the mouse anterior pituitary (AtT-20/D16-16), SRIF blocks the stimulation of adrenocorticotropin (ACTH) release. This cell line is useful in studying the specific mechanisms of action of SRIF in controlling the secretion of ACTH since it consists of a homogenous population of corticotrophs and has a high density of SRIF receptors. SRIF prevents the release of ACTH from these cells elicited by forskolin, K^+ and 8-bromo-cAMP (8-BCA). Each of these secretagogues increases ACTH release by way of different mechanisms. The potent activator of adenylate cyclase, forskolin, stimulates ACTH release and cAMP production and SRIF blocks these effects with equal potency. SRIF prevents forskolin from increasing ACTH release by activating a guanine nucleotide inhibitory protein (N_i). This was shown by using pertussis toxin which is an agent that catalyzes the ADP-ribosylation of a subunit of N_i and abolishes the inhibition of forskolin-stimulated cAMP synthesis and ACTH release by SRIF. K^+ also stimulates ACTH release but does not affect cAMP formation. SRIF blocks K^+-evoked ACTH release; however, pertussis toxin pretreatment does not remove SRIF's inhibition of K^+-stimulated ACTH release. K^+ may heighten the secretion of ACTH by elevating Ca^{++} influx or mobilization. SRIF's inhibition of K^+ but not forskolin-evoked ACTH release is overcome by increasing extracellular Ca^{++} concentrations, suggesting that SRIF alters some Ca^{++} mediated event in blocking the secretion of ACTH induced by K^+. 8-BCA stimulates ACTH release probably by activating protein kinase. SRIF's inhibition of 8-BCA-stimulated ACTH release is not prevented by pertussis toxin treatment or by increasing extracellular Ca^{++} concentrations. Interestingly, prior exposure of AtT-20 cells to SRIF desensitizes SRIF's inhibitory effect on forskolin-stimulated cAMP accumulation and ACTH release but does not alter SRIF's inhibition of K^+ or 8-BCA-stimulated ACTH secretion, indicating that SRIF's various mechanisms of affecting ACTH release are regulated differently. These studies suggest that SRIF acts through at least three mechanisms to inhibit ACTH secretion.

114.2 **EFFECTS OF 6-HYDROXYDOPAMINE ON THE AUTORADIOGRAPHIC DISTRIBUTION OF THYROTROPIN-RELEASING HORMONE (TRH) RECEPTORS IN RAT CNS.** S. Manaker, A. Winokur and T.C. Rainbow, Depts. of Biology, Psychiatry, and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

Thyrotropin-releasing hormone (TRH) and its receptors have been demonstrated to be widely distributed throughout the CNS. However, little is known about the regulation of brain levels of TRH and TRH receptors. Treatment with 6-hydroxydopamine (6-OHDA), which destroys catecholaminergic nerve terminals, has been reported to increase the TRH content of specific brain regions. We now report that treatment with 6-hydroxydopamine results in a marked decrease in TRH receptor levels in specific nuclei and subregions of rat brain.

Four male Sprague-Dawley rats (180-200 g) were injected in the right lateral ventricle with 400 µg of 6-OHDA dissolved in a vehicle of 0.15 M NaCl and 1% ascorbate. Four paired control rats were injected with vehicle alone. Three days later, all rats were decapitated, their brains rapidly dissected and frozen. Coronal sections 32 µ thick were cut at -18°C, thaw-mounted onto subbed slides, and stored at -70°C until use. Slides were warmed to 25°C, preincubated for 10 min in Tris/$MgCl_2$/BSA buffer (pH 7.4), immediately chilled to 4°C and allowed to air dry. Sections were then incubated with 300 µl of ice-cold buffer containing 20 µM bacitracin and 10 nM of (^{3}H)-MeTRH (3-methyl-histidine-TRH). Nonspecific binding was defined as the binding of (^{3}H)-MeTRH in the presence of 10 µM TRH. After a 2-hr incubation, slides were washed with ice-cold buffer 4 times for 30 sec each, dipped in ice-cold distilled water to remove buffer salts, rapidly dried on a 60-70°C slide drier, and apposed to LKB tritium-sensitive Ultrofilm for 2 mos. Density values were converted into molar amounts using tritium brain mash standards.

Treatment with 6-OHDA decreased TRH receptor levels in specific nuclei and subregions of rat brain. The septohypothalamic nucleus, rhinal cortex, facial nucleus, subfields of the hippocampus, and specific nuclei of the amygdala, thalamus and hypothalamus all contained decreased (25%-75%) concentrations of TRH receptors compared to paired control animals. Other nuclei and subregions of brain, including the olfactory bulbs, limbic forebrain, spinal cord, and nuclei of the hypothalamus and brainstem were unchanged in TRH receptor concentrations. In no structure was an increase in TRH receptor concentration noted.

These data suggest two hypotheses: 1) Some TRH receptors are located presynaptically on catecholaminergic nerve terminals; and/or 2) Some TRH receptors down-regulate their concentrations in response to elevated levels of TRH. Studies are presently underway to evaluate these hypotheses.

114.3 CORTICOTROPIN-PEPTIDE REGULATION OF INTRACELLULAR CYCLIC AMP LEVELS IN CORTICAL NEURONS IN CULTURE. S. Weiss*, M. Sebben* and J. Bockaert* (SPON: B. Rouot). Centre CNRS-INSERM de Pharmacologie-Endocrinologie, 34033 MONTPELLIER Cedex, FRANCE

Previous studies have provided evidence for adrenocorticotropic hormone (ACTH) effects on a wide variety of behaviors. However, the precise sites of action and the mechanisms by which these effects may be mediated have yet to be clearly elucidated. While ACTH was shown to augment cyclic AMP (cAMP) levels in glial cells isolated from whole brain, other studies found that ACTH peptides were without effect on cyclic nucleotide metabolism in slices of cerebral cortex or in homogenates of whole brain. In the present study, our objective was to determine whether ACTH peptides regulate cAMP levels in neurons of the cerebral cortex. Cultures of neuronal cells from 14-15 day old mouse embryos were generated from the cerebral cortex and grown for 6 days in serum-free media. Using antibodies against neurofilament and glial fibrillary acidic proteins, these cultures were demonstrated as almost exclusively neuronal in nature. cAMP levels were determined by pre-incubation of cortical neurons with ^{3}H-Adenine and subsequent examination of the conversion of ^{3}H-ATP to ^{3}H-cAMP. ACTH peptides stimulated cAMP synthesis 3-fold in a dose-dependent manner; stimulation was complete within 5 min of exposure to agonist. Neurohormone efficacy was augmented by 0.1 µM forskolin (which was ineffective alone); potency was unaffected. The order of potency (EC_{50}) for increasing intracellular cAMP levels was as follows: ACTH(1-24) (10 nM) > alpha-MSH (100 nM) > ACTH(1-10) (1 µM) > ACTH(4-10) (5 µM). The hexapeptide ACTH(4-9) as well as ACTH(11-24) were inactive at concentrations as high as 10 µM. In addition the neuropeptides met- and leu-enkaphalin were without effect on basal or hormonally-stimulated cAMP synthesis. In order to determine whether distinct receptors for ACTH are present on cortical neurons, saturating concentrations of the peptide were co-incubated with either vasoactive intestinal polypeptide (VIP) or the beta-adrenergic agonist isoproterenol (INE). The response to combinations of VIP and ACTH, INE and ACTH or INE and VIP were significantly greater although not completely additive, than the response to any one agonist alone. These findings suggest the existence of distinct neuronal target sites in the cerebral cortex for corticotropin peptides involved in the regulation of intracellular cAMP.

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114.4 AUTORADIOGRAPHIC DISTRIBUTION OF CHOLECYSTOKININ (CCK) AND SUBSTANCE P RECEPTOR BINDING SITES IN HUMAN BRAIN. R. Quirion[1], C. Csonka[1]*, T.V. Dam[1]*, P. Etienne[1], Y. Robitaille[1]*, N.P.V. Nair[1] and P. Gaudreau[2]. [1]Douglas Hospital Research Centre, Verdun, Canada H4H 1R3 and [2]Hopital Notre-Dame.

Using membrane preparations, Hays et al (Brain Res. 22: 452, 1981) have reported the presence of CCK receptors in basal ganglia and cortex of human brain. The existence of SP receptor binding sites in human brain as yet to be reported. We now described the visualization of human brain CCK and SP receptors using an in vitro autoradiographic technique. Normal human brains were obtained and processed as described before (Etienne et al, submitted). Whole frozen brain hemispheres were cut into 30 µm thick sections and mounted on subbed slides, dessicated and then processed for autoradiography. For CCK, frozen slide-mounted sections were preincubated for 15 min at 23°C in 50 mM Tris.HCl, pH 7.7 containing 5 mM $Mg Cl_2$ and 0.2% BSA followed by a 120 min incubation at 23°C in 50 mM Tris.HCl, pH 7.7 plus 5 mM $Mg Cl_2$, 0.35 mM bacitracin, 0.2% BSA, 1.0 mM dithiothreitol and 25 pM N$^{\alpha}$[^{125}I-desaminotyrosyl] CCK 26-33 (CCK-8). For SP, sections were preincubated for 15 min at 23°C in 50 mM Tris.HCl, pH 7.4 plus 0.2% BSA followed by a 30 min at 23°C in 50 mM Tris.HCl, pH 7.4 plus 0.2% BSA, 40 µg/ml bacitracin, 4 µg/ml leupeptin, 2 µg/ml chymostatin, 3 mM $MnCl_2$ and 0.1 nM [^{125}I]BHSP. At the end of the incubation, slides were washed in cold incubation buffer. Incubated slides were then exposed to LKB Ultrofilm for 2-15 days. High densities of CCK binding sites are located in the cingulate cortex with moderate concentrations in temporal and insular cortices. Moderate densities of sites are also seen in the claustrum, caudate, putamen, nucleus accumbens and amygdaloid body. Low densities of sites are observed in the internal capsule, globus pallidus, thalamus and hypothalamus. SP binding sites are highly concentrated in caudate, putamen, amygdaloid body, colliculi and dentate gyrus. Low densities are seen in most thalamic and hypothalamic areas, and in cerebellum. The autoradiographic distribution of CCK and SP receptors in human brain is similar to those reported in rat and guinea pig brain (Zarbin et al, J. Neurosci. 3, 877, 1983; Gaudreau et al, Peptide, 4, 755, 1983; Quirion et al, Nature, 303, 714, 1983).

114.5 DEHYDRATION INCREASES THE NUMBER OF ANGIOTENSIN II RECEPTORS MEASURED BY QUANTITATIVE RADIOAUTOGRAPHY IN RAT ANTERIOR PITUITARY AND SUBFORNICAL ORGAN. A. Israel*, M.Niwa* and J.M. Saavedra. Section on Clinical Psychopharmacology, Laboratory of Clinical Science, NIMH Bethesda, MD. 20205.

We quantitated angiotensin II (AII) receptors in single, individual rat pituitary glands and brain nuclei after incubation of tissue sections with ^{125}I-Sar^{1}-AII, followed by radioautography at appropriate exposure times, computerized densitometry and comparison with ^{125}I standards carried through the procedure.

The possibility of a dynamic regulation of AII receptors was analyzed in Sprague Dawley (SD) male adult rats,Brattleboro heterozygous (HZ) and homozygous (DI) rats, control and submitted to water deprivation for 18 hr (DI) and 18 hrs and 5 days (HZ and SD). DI rats, unable to synthesize vasopressin, are chronically dehydrated, and similarly to control dehyrated rats, have high plasma AII levels.

Scatchard plots of binding data in the anterior pituitary of control SD rats revealed a binding capacity of 325 fmol/mg protein with a Kd of 2.3 x 10^{-9} M. Dehydration of SD rats increased AII receptors (+ 56 %). Brattleboro rats showed increased AII receptors (HZ: + 44 %, DI: + 55 %) when compared to SD controls. Dehydration induced a further increase in AII receptors in Brattleboro rats (HZ: + 43 %; DI: + 35 %). In contrast, 18 hr dehydration did not produce changes in AII receptors in HZ or SD rats.

There were changes in the number of AII receptors in the rat brain during water deprivation. In SD control rats, the number of AII receptors was 14.88 fmol/mg protein in the subfornical organ, and 9.88 fmol/mg protein in the paraventricular nucleus. No AII receptors were found in the nucleus supraopticus. Increases in AII receptors were restricted to the subfornical organ. In SD rats, the increase was 36%. There were no alterations in the paraventricular nucleus.

Our results demonstrate that AII receptors in the anterior pituitary and subfornical organ are up-regulated in conditions of acute and chronic dehydration. The subfornical organ mediates central actions of AII, including thirst. Increased AII receptors in this structure could represent an amplification of the homeostatic response to dehydration. A possible physiological role for anterior pituitary AII receptors during dehydration in the rat can also be postulated.

114.6 SUBSTANCE P (SP) RECEPTORS ARE PRESENT IN AUTONOMIC AND RESPIRATORY NUCLEI IN THE SPINAL CORD (SC). C.G. Charlton* and C.J. Helke (SPON. B. I. Gold), Dept. of Pharmacology, Uniformed Services Univ. of the Health Sci., Bethesda, MD 20814.

A central neuronal system under tonic GABA inhibition (Keeler and Helke, this meeting) mediates cardiovascular controls and may be comprised of SP cell bodies in the ventral medulla that projects to the intermediolateral (IML) cell columns of the SC (Helke et. al., Brain Res., 243: 147, 1982) where SP is probably released as a transmitter. To determine the possible roles for SP in the SC, ^{125}I Bolton-Hunter-SP (^{125}I-BH-SP) was used to study SC binding sites for the peptide.

Cell membrane preparation and autoradiography of SC slices were used to study SP binding and to determine the specific localization of the binding sites. The binding of SP was time dependent, membrane-concentration dependent, saturable, reversible, and of high affinity. The IC_{50} for SP was 0.46 nM, as compared with 0.95, 60, and 150nM for physalaemin, eledoisin, and kassinin. Four putative antagonists of SP were < 0.0001 as potent as SP in inhibiting ^{125}I-BH-SP binding. IC_{50}s were 5, 7.5, 7.0, and 45 μM for D-Pro^{2},D-Trp^{7,9}-SP; D-Pro^{2},D-Phe^{7},D-Trp^{9}-SP; D-Arg^{1},D-Pro^{2}, D-Trp^{7,9}, Leu^{11}-SP, and D-Pro^{4},D-Trp^{7,9,10}-SP(4-11), respectively. The lumbosacral segment bound 3 times more SP than the cervical and thoracic segments, although the IC_{50} for the cervical segment was 0.06 of that for the lumbosacral and thoracic segments.

Autoradiography revealed discrete regional and segmental binding. High density binding sites occurred in the IML, the medioventral ventral horns (MVVH), the dorsal horns (DH), and lamina X. The IML binding, which may be associated with cells of origin for autonomic preganglionic fibers, was localized in the thoracic and the sacral segments. The MVVH binding site was distinct in the midcervical segment and corresponded with the phrenic motor nucleus. In the DH SP receptor density increased from the cervical to the sacral where it is 22% higher. Density in lamina X region however was: thoracic > sacral > lumbar > cervical.

The data suggest more than one binding site for SP in the SC. These occur in distinct loci and may represent a site-specific physiological role for SP. Sites in the IML and phrenic nucleus may transduce autonomic and respiratory effects. (Supported by NIH grant #NS19317).

114.7 SUICIDE TRANSPORT DEFINES THE PRESENCE OF SUBSTANCE P RECEPTORS ON AUTONOMIC AND SOMATIC MOTOR NEURONS. C.J. Helke, C.G. Charlton* and R.G. Wiley. Dept. of Pharmacol., Uniformed Services Univ. of the Health Sci., Bethesda, MD 20814 and Dept. of Neurology, Vanderbilt Univ., Nashville, TN 37202.

Substance P (SP) receptors were visualized in brain (Helke et al., Neuroscience, in press) and spinal cord nuclei (Charlton et al., this meeting) that contain cholinergic efferent neurons projecting to somatic muscles or to autonomic ganglia. To learn whether the SP receptors were localized on the cell bodies of these efferent neurons in the rat, we used the technique of suicide transport (Wiley et al., Science 216:889, 1982) combined with light microscopic autoradiographic localization of ^{125}I-Bolton-Hunter labeled SP (^{125}I-BH-SP) binding sites. Ricin, a toxic lectin from castor beans, was injected unilaterally into a somatic motor nerve (hypoglossal), parasympathetic preganglionic nerve (vagus), and sympathetic ganglion (superior cervical). Selective motor neuron destruction was confirmed by assessing cell loss in the appropriate CNS nucleus (the hypoglossal nucleus, the dorsal motor nucleus of the vagus and nucleus ambiguus, the intermediolateral cell column, respectively) in the thionine-stained coronal sections. SP receptor autoradiograms were quantitated by computerized densitometry.

Two weeks after ricin injections into the hypoglossal nerve, a selective unilateral loss of neuronal somata and an 80% reduction in the density of specific ^{125}I-BH-SP binding were seen in the hypoglossal nucleus. Likewise, cervical vagus administration of ricin reduced the number of neurons in the dorsal motor nucleus of the vagus and in the nucleus ambiguus, and reduced (35% and 70%, respectively) the SP binding in both nuclei. Injections of ricin into the superior cervical ganglion also reduced both the number of cell bodies in the intermediolateral cell column of the upper thoracic spinal cord and diminished the binding of ^{125}I-BH-SP in this nucleus. No other brain and spinal cord areas were affected.

These data demonstrate that SP binding sites are located on cell bodies of efferent motor neurons of both the autonomic and somatic nervous system suggesting that SP can influence the activity in each of these systems. In addition, the data show that suicide transport is a useful technique in elucidating the cellular location of neurotransmitter receptors. (Supported by NIH Grant #NS 19317 to C.H. and VA Merit Review Grant to R.W.)

114.8 NEUROTENSIN STIMULATES CYCLIC GMP FORMATION IN NEUROBLASTOMA CLONE N1E-115 AND RAT CEREBELLUM. J.A. Gilbert*, M. McKinney, and E. Richelson. Mayo Foundation, Rochester, MN 55905

Neurotensin, an endogenous tridecapeptide, stimulates the production of intracellular cyclic GMP in neuroblastoma clone N1E-115 in a dose related fashion with an ED_{50} of 13 ± 5 nM (mean ± S.E.; n=5) (Gilbert, J.A., and Richelson, E., Eur. J. Pharmacol., 99:245, 1984). With an assay in which cyclic [^{3}H]GMP was isolated chromatographically from cells labeled with radioactive precursor prior to stimulation, we showed that this induction of cyclic [^{3}H]GMP reached a maximum within 30 sec and required the presence of Ca^{2+} in the incubation medium. In accordance with studies relating the structure of neurotensin and its analogs and fragments to biological activity, neurotensin(8-13) was more potent than native neurotensin in stimulating the formation of cyclic [^{3}H]GMP, with an ED_{50} of 0.3 ± 0.1 nM (n=3), while neurotensin(1-8) had no effect on cyclic [^{3}H]GMP production (n=3).

A binding assay was developed employing incubation of intact N1E-115 cells with [^{3}H]neurotensin in physiological buffer with 1% BSA for 20 min at 0° followed by separation on glass fiber filters pretreated with 0.1% polyethylenimine. Under these conditions [^{3}H]neurotensin demonstrated saturable, specific binding in the concentration range of 1-16 nM. This binding displayed linearity with cell number/tube. Scatchard analyses indicated the presence of one binding site with a K_D of 8 ± 1 nM and a B_{max} of 140 ± 40 fmole/10^{6} cells (n=4) for the radioligand. Preliminary experiments have indicated that the ability of neurotensin fragments to inhibit the binding of radioligand to the clone was similar to their ability to stimulate intracellular cyclic GMP production, with neurotensin(8-13) being ~ten-fold more potent than neurotensin, and neurotensin(1-8) having essentially no ability to displace the labeled peptide.

Finally, cyclic GMP formation was measured in 260 x 260 μ slices from Sprague-Dawley rat cerebellum which were incubated for 1 hour at 37° with oxygenated Krebs buffer prior to receptor stimulation under the same conditions. Cyclic GMP was isolated chromatographically and measured with a commercial radioimmunoassay kit. Upon correcting for recovery of added cyclic [^{3}H]GMP, we demonstrated that neurotensin stimulated the production of as much as 13 pmole/mg protein of cyclic GMP, which was 86% above basal level ($P<.005$; n=3). (Supported by Mayo Foundation and USPHS Grant MH 27692).

114.9 SOMATOSTATIN RECEPTOR BINDING IN CEREBRAL CORTEX OF POST-MORTEM CONTROLS AND ALZHEIMER'S DISEASE PATIENTS.
Barbara Petrack and Andrew J. Czernik.*
Neuroscience Research, Research Department, Pharmaceuticals Division, CIBA-GEIGY Corp., Summit NJ 07901.

Somatostatin (SS) receptor binding has been characterized in rat cerebral cortex using the nonreducible SS analog ^{125}I-CGP 23996 as radioligand (1). We have now used the same radioligand to determine SS receptor binding in samples from post-mortem cerebral cortex (obtained from Dr. Peter Davies). Synaptosomal membranes were prepared from individual 100-200 mg samples of tissue and assayed using procedures that were esablished for rat brain, with incubations for 120 min at 25°C (1). CGP 23996 inhibited binding with nanomolar potencies, whereas leu-enkephalin, TRH, Substance P, neurotensin and bombesin did not affect binding at 1 μM.

Because cortical SS levels are markedly reduced in Alzheimer's disease (AD)(2), we tried to determine if SS receptor binding is also affected. Post-mortem samples of cerebral cortex including some from patients with AD were obtained from the Douglas Hospital Research Centre Brain Bank, Verdun, Quebec. ^{125}I-CGP 23996 binding to synaptosomal membranes from these samples was determined at 10-12 ligand concentrations between 0.28 nM - 31.4 nM. Scatchard plots were nonlinear, but analyses of the data did not indicate two binding sites. K_D and Bmax values for each sample were determined by Gauss-Newton weighted nonlinear regression analysis. The values were then subjected to cluster analysis but the results did not distinguish two populations. The mean K_D and Bmax ± s.e. for the 15 samples (with confidence limits) are: K_D = 5.05±0.47 nM (4.12-5.98); Bmax = 437.06±31.62 fmoles/mg protein (374.63-499.50). These values are similar to the K_D and Bmax that have been reported for rat brain (K_D = 2.4 ± 0.1 nM; Bmax = 450±30 fmoles/mg protein)(1). Of the 15 samples, 8 were then identified as controls, 5 as AD brains and 2 from patients who died of hepatic coma (HC). The Bmax values were reduced approximately 60% in 2 of the 5 AD samples, whereas, the values were similar to those of controls in the other 3AD and the 2 HC samples.

Our studies demonstrate the presence of SS recognition sites in post-mortem cerebral cortex and suggests the possibility that a sub-population of AD patients might exhibit a reduced number of SS binding sites, as reported for muscarinic binding sites (3).

1. Czernik and Petrack (1983) J. Biol. Chem 258:5525-5530.
2. Davies et al. (1980) Nature 288:279-280.
3. Wood, et al. (1983) J. Neurol. Sci. 62:211-217.

114.10 CALCITONIN GENE RELATED PEPTIDE: HIGH AFFINITY BINDING SITES IN RAT BRAIN.
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Calcitonin Gene Related Peptide (CGRP) is a 37 residue novel neuropeptide predicted on the basis of alternative processing of the primary transcripts of the calcitonin gene. This peptide has been detected immunohistochemically in the central and peripheral nervous system of the rat. Immunoreactive CGRP is released from cultured rat trigeminal ganglion cells, and potent in vivo actions have been reported for centrally (icv) and peripherally (iv) administered r(rat) CGRP.

Here we characterize specific high affinity binding sites in rat brain cortex using [^{125}I-His^{10}]-rCGRP as radioligand. Specific binding (defined as total bound minus bound in the presence of 100 nM unlabeled peptide) to 30,000 g membrane pellets was rapid and temperature dependent. Routine experiments were carried out at 23°C in 50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 2 mM EGTA in a final volume of 0.5 ml containing 0.1-0.5 mg protein/tube (linear increase of specific binding up to 1 mg protein/tube) bound and free ligand were separated by filtering on Whatman GF/C filters. Under these conditions equilibirum specific binding was reached after 60 min and remained stable for more than 2 h. After addition of excess unlabeled ligand specific binding was fully reversible. Boiling of the membrane preparation or pH 5 totally abolished specific binding. From competition studies a dissociation constant K_D of 0.4 nM and an estimated receptor concentration of 0.1 fmol/ g membrane protein were obtained using the program LIGAND for analysing the data. The linear analogs devoid of the disulfide bridge, [acetamidomethyl-Cys^{2,7}]-rCGRP and -human CGRP bound with much lower affinity than the native peptides (K_D ~10 nM). Neither the fragments rCGRP(1-14) and [Tyr^{23}]-rCGRP(23-37) nor the homologous peptides human and salmon calcitonin in concentrations up to 100 nM competed with the radioligand.

Significant high affinity binding was also detected in rat hypothalamus, hippocampus, striatum, thalamus, cerebellum, was scant in brainstem, and not detectable in anterior pituitary, kidney, and blood cells.

In summary the described CGRP binding sites being saturable, of high affinity and specifity for the intact CGRP molecule, and tissue specific, exhibit properties expected of physiological receptors. They seem to be different from calcitonin binding sites in rat brain.

114.11 A BEHAVIORALLY POTENT FRAGMENT OF VASOPRESSIN (AVP 4-9) DOES NOT BIND TO BRAIN VASOPRESSIN RECEPTORS.
K.A.Smith*, L.E.Cornett*,D.M.Dorsa (SPON: E. Powell). GRECC, VA Medical Center and Depts. of Med. and Pharmacol., Univ. of Washington, Seattle, WA 98108 and Dept. of Physiol. and Biophys., Univ. of Arkansas for Med Sci., Little Rock, AR 72205.

Recent reports suggest that the major metabolite of arginine vasopressin (AVP) when exposed to brain membranes is AVP 4-9, and that this fragment is much more potent than AVP in preventing extinction of a conditioned avoidance response in the rat. Our group and others have provided evidence for the presence of binding sites for ^{3}H-AVP in various brain regions including hindbrain, amygdala, and septum. The present studies were designed to determine if the enhanced behavioral effect of AVP 4-9 is due to an interaction with ^{3}H-AVP binding sites or with a separate receptor for this peptide in the CNS.

Adult Sprague-Dawley rats (n=36) were sacrificed and brain tissue rapidly removed and the septum and amygdala were dissected. Tissues from all animals were pooled, and crude membrane extracts from each brain region were prepared, frozen and stored at -70°C, and thawed for use in radioligand assays. Non-specific binding for ^{3}H-AVP (S.A.=62Ci/mmole) and ^{35}S-AVP 4-9 (S.A.=23Ci/mmole) was defined by the presence of 1 μM unlabelled AVP and 1μM unlabelled AVP 4-9, respectively.

Saturation experiments using ^{3}H-AVP yielded a dissociation constant of 0.7±0.3nM and a receptor density of 12±4fmole/mg protein in septal tissue. In contrast, ^{35}S-AVP [4-9] binding to septal membranes appeared to be specific, but saturation was not observed in concentrations up to 150nM. ^{3}H-AVP binding (5nM) was not displaced by unlabelled AVP 4-9 metabolite (10^{-7} to 10^{-9}M) in membranes prepared from the amygdala. ^{35}S-AVP 4-9 binding (10nM) was not reduced by unlabelled AVP (10^{-6} to 10^{-9}M) in septal membranes.

In conclusion, it appears that AVP 4-9 metabolite does not bind with appreciable affinity to ^{3}H-AVP binding sites in brain. Binding specific for AVP 4-9 can be demonstrated in brain membranes but does not yet fulfill the criteria necessary for indentification of a separate receptor for this peptide.

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114.12 INHIBITION OF ^{125}I-ANGIOTENSIN II BINDING TO ANGIOTENSIN II RECEPTORS BY ANGIOTENSIN II ANALOGS WITH AMINO TERMINAL EXTENSIONS.
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The natural occurrence of an amino terminal extended angiotensin II (Ang II) in frog skin (crinia Ang II), the recent determinationof a pre- sequence for angiotensinogen (Ohkuba et al., Proc. Nat. Acad. Sci. 80: 2176, 1983) and the observation of a family of angiotensin I-like peptides in dog cerebrospinal fluid which may represent amino terminal extensions of the peptide (Husain et al., Circ. Res. 52: 460, 1983) prompted an investigation of the ability of amino terminal extended angiotensin II (Ang II) analogs to bind to Ang II receptors. Three Ang II analogs were synthesized and studied: Crinia Ang II, Ala-Pro-Gly-[Ile^3,Val^5] Ang II; Thr-Ala-Gly-Ang II (3+); Val-Ser-Leu-Thr-Ala-Gly-Ang II (6+). The 3+ and 6+ analogs correspond to the pre-sequence for liver angiotensinogen described by Ohkubo et al. Inhibition of ^{125}I-Ang II binding by these Ang II analogs was measured in the brain as well as the adrenal of the rat. Estimates of inhibition constants, (IC_{50}s) for each analog were determined by Hill plots of inhibition of specific (one μM Ang II displaceable) ^{125}I-Ang II binding over a concentration range of 0.05 to 1000 nM. Results are expressed as IC_{50} ratios of the amino terminal extended Ang II analogs to the IC_{50} for Ang II. The concentration ratios for 3+ Ang II, crinia Ang II and 6+ Ang II in the rat brain were 2.3, 4.7 and 7.0, respectively. In the rat adrenal, the amino terminal extended Ang II analogs were again less potent than Ang II with dose ratios of 7.5, 8.4 and 35 for 3+ Ang II, crinia Ang II and 6+ Ang II, respectively.

These results indicate that the affinity for Ang II receptors of Ang II analogs in which the chain length has been extended at the amino terminus remains relatively high. Since some amino terminal extended angiotensins occur naturally, and evidence from this laboratory suggests that higher molecular weight angiotensins may occur in mammalian central nervous systems, amino terminal extended angiotensins may be an important component of the brain renin angiotensin system. (Supported by USPHS Grants HL-6835 and HL-27568).

115.1 QUANTITATIVE AUTORADIOGRAPHIC LOCALIZATION OF SEROTONIN RECEPTORS IN THE HUMAN BRAIN POST MORTEM. Anat Biegon, Dept. of Isotope Research, The Weizmann Institute of Science Rehovot Israel.

The human brain possesses binding sites with the characteristics of S_1 and S_2 receptors. We have studied the character and regional distribution of these receptors on 32μ thick sections from frozen right hemispheres obtained at autopsy. The sections were cut on a Bright cryostat at -20°C and cold mounted onto gelatin coated glass plates (12cm x 12cm x 2mm). They were dried at 30°C and stored at -20°C before use. For saturation and displacement experiments, the sections were covered by the appropriate ligand and drugs; washed and then wiped off the glass plate for direct counting in the scintillation counter. ^{3}H-5HT was employed to study the S_1 receptor, following the experimental conditions we have published for the rat brain (Biegon et al., Brain Res. 242: 197, 1982). Saturation experiments revealed a kd of 4nM;in competition experiments, serotonin itself was the best competitor, with IC_{50} in the nM range. Mianserine competed in the μM range and Atropin not at all. ^{3}H-ketanserine in 0.17 M Tris. HCl, pH 7.7, was applied to the sections for 1 hour at room temperature followed by 2 x 20 min. washes in ice cold buffer. A kd of 0.2nM was obtained in saturation experiments. Competition studies showed mianserine to have an affinity in the nM range, serotonin in the μM range and no competition with atropin. These results are in excellent agreement with the rat brain. For localization experiments, we used 2nM ^{3}H-5HT and 10μM cold 5HT to define non specific binding to the S_1 receptor. S_2 receptors were labeled with 1.5nM ^{3}H ketanserine, with 100μM mianserine for nonspecific binding. The sections were dried and apposed against LKB ^{3}H-ultrofilm for 60 days. Autoradiograms were analysed using a computerised image analysis system. The two receptors have completely different distribution patterns in the human brain. The highest concentrations of S_1 receptors appear in the hippocampus, followed by the outer layers of the cortex. The globus pallidus had substantial amounts of label, with relatively lower levels over the caudate and putamen. S_2 receptors were concentrated in the inner layers of the cortex and in the hypothalamus, and the globus pallidus was remarkably poor in binding sites. A detailed analysis of the distribution shows gross similarities but also significant differences when compared to the rat brain.

115.2 AUTORADIOGRAPHIC LOCALIZATION OF CNS SOMATOSTATIN RECEPTORS IN NORMAL RATS AND HUMANS AND ALZHEIMER DISEASE PATIENTS. V.T. Tran, G.R. Uhl, D.C. Perry, D.C. Manning, P.J. Whitehouse, W.W. Vale, M.H. Perrin, J.E. Rivier, D.L. Price, J.B. Martin, and S.H. Snyder+. Dept. of Neuroscience, Johns Hopkins University, Sch. of Med., Baltimore, MD 21205, Dept. of Neurology and HHMI, MGH, Boston, MA 02114, Lab. of Peptide Biology, Salk Institute, La Jolla, CA 92037.

Somatostatin (SS) receptors, sites at which the peptide may influence neuroendocrine, motor and higher cortical functions, may be identified in homogenate-binding and receptor autoradiographic studies. We report detailed receptor mapping in rat brain to document possible sites of SS action. Since SS levels are selectively altered in Alzheimer's (AD), Parkinson's and Huntington's diseases, we have also examined human SS receptor binding in regions implicated in these neurodegenerations.

In rat and human brain ^{125}I-[Leu^8-D-Tryp^{22}, Try^{25}]SS-28 (^{125}I-LTT) labels pharmacologically-specific SS receptors, whose distributions parallel SS immunoreactivity. In rats, high grain densities are present in deeper laminae of cerebral cortex, specific hippocampal zones, stria terminalis, subfornical organ, lateral amygdala, interpeduncular nucleus, locus coeruleus, lateral septum and medial habenula. Moderate densities are found in hypothalamic, other amygdaloid, substantia nigra, zona incerta, nucleus accumbens, and ventral tegmental areas, as well as in the bed nucleus of the stria terminatis, floor of the fourth ventricle, periaqueductal grey, vagal and solitary tract nuclei. White matter and cerebellar cortex display very low binding.

Human SS receptor binding is dense in cerebral cortex with greatest binding in deeper layers. High densities are also found in substantia nigra and caudate. Moderate binding is seen in the locus coeruleus and parabrachial nuclei. White matter shows very low binding levels.

Preliminary examination of cerebral cortex from AD patients reveals apparent augmentation of binding, with most marked changes occuring in the deepest cortical laminae. Extension of these studies to other AD cases and other neurodegenerations may allow determination of the roles of SS receptor alterations in these diseases.

115.3 Quantitative Autoradiography of [^{125}I] LSD Binding Sites in Rat Brain. Marian T. Nakada* and Thomas C. Rainbow(SPON:A.Stein).Department of Pharmacology, University of Pennsylvania, Phila. PA 19014

[^{125}I] ligands are very useful for quantitative receptor autoradiography because their high specific radioactivities and energies of decay allow the production of autoradiograms in hours, as opposed to weeks or months for [^{3}H] ligands. There is also little or no differential grey-white matter absorption for ^{125}I. We report here the use of [^{125}I]LSD to localize S-2 serotonin receptors and D-2 dopamine receptors in rat brain. Frozen 32u thick brain sections were labeled in vitro with [^{125}I]LSD and exposed for 24 hr against LKB Ultrofilm to generate autoradiograms. Non-specific binding was defined as the labeling in the presence of 1 uM D-LSD. Scatchard analysis of the binding of [^{125}I] LSD to Layer IV of cortical area 2 indicated that the binding was to a single population of sites, with a Kd of 0.17±.01 nM and a Bmax of 84.8±3.8 fm/mg protein (N=3). The binding of [^{125}I] LSD to Layer IV was potently inhibited by the specific S-2 antagonist ketanserin (Ki=7 nM) and slightly inhibited by the selective D-2 antagonist sulpiride. Based on these results, the binding of [^{125}I]LSD to slide-mounted sections appears to be preferentially to S-2 receptors with some binding to D-2 dopamine receptors,in general agreement with the findings of Hartig et al. Autoradiograms of [^{125}I]LSD binding revealed that high concentrations of [^{125}I] LSD sites (40-60 fm/mg P) were observed in Layer IV of the cerebral cortex, caudate-putamen, claustrum, olfactory tubercle, nucleus accumbens, ependyma, mammillary nucleus and inferior olive. All other brain regions had lower levels of binding sites. Non-specific binding was an anatomically uniform 20% of total binding. Co-incubation of sections with sulpiride to block binding to D-2 receptors resulted in a uniform 20-30% reduction in the amount of specific [^{125}I]LSD binding, with no qualitative difference in the pattern of labeling. However, co-incubation with 300 nM ketanserin to block S-2 receptors resulted in a pattern of binding that was similar to previous descriptions of the location of D-2 receptors, with high levels of residual binding in caudate-putamen, olfactory tubercle, nucleus accumbens and inferior olive. Our results indicate that [^{125}I] LSD is a suitable ligand for quantitative autoradiography of both S-2 and D-2 receptors, and that there is a strong anatomical correspondence between these receptors,perhaps implying a functional interaction.Supported by NS19597.

115.4 A HIGH SALT EXTRACT OF RAT DIAPHRAGM EXTRACELLULAR MARTIX (ECM) ENRICHED IN SYNAPTIC BASAL LAMINA INCREASES THE NUMBER OF α-BUNGAROTOXIN BINDING SITES ON CULTURED EMBRYONIC CHICK MYOTUBES. K.F. Barald,G.Phillips*,J.Jay*,I.F.Mizukami*,D.C.M. Chu*,I.Hill* & L.A. Polacek*, Dept. Anatomy & Cell Biology, University of Michigan Med School, Ann Arbor, Michigan,48109.

A number of laboratories have reported that fractions of nerve, nerve-associated material or extracts of synaptic basal lamina (sbl) from electric fishes affected the organization of acetylcholine receptors (AChR) as measured by α-bungarotoxin (BTx) binding. We report here evidence that a purified preparation of mammalian synaptic ECM had a similar effect on cultured embryonic chick myotube BTx binding sites.

Preparations of ECM from end-plate enriched (EP-matrix) and non-end plate regions (NEP-matrix) were prepared by the method of Wicha et al*. Both EP- and NEP-matrix supported the growth of chick embryonic myotubes in culture. Myotubes plated on EP- or NEP-matrix had twice as much protein,nuclei per unit area and lactate dehydrogenase activity as myotubes grown on rat tail collagen substrates.

15 μg/ml of a high salt extract of EP- but not NEP-matrix added to cultures for 8 hrs after addition of 10^{-8}M ^{125}I-BTx also increased the numbers of clusters of BTx binding sites. Treated myotubes had 3.8 ± 0.2 (s.e.m.; n=25) times the number of clusters seen on controls or on cultures to which boiled or trypsinized extracts were added. An extract of Torpedo ECM kindly provided by L.L. Rubin of Rockefeller U. increased the number of clusters by 4.3± 0.2 (sem n=15)times.

A monoclonal antibody was made to the EP-matrix by a modification of Matthew's(personal communication) in vitro hybridization method. NEP-matrix was first injected into mice; after 3 days, an immunosuppressive drug was administered. Spleens were explanted to culture 21 days later over an EP-matrix lawn in the presence of mitogens. The monoclonal antibody selectively labeled a component enriched the the s.b.l. of rat muscle and was co-localized with rhodamine BTx binding The antibody did not affect the clustering of BTx binding sites.

In summary, we have produced an ECM preparation from a mammalian muscle that improves the culture of embryonic myotubes. An extract of this ECM affects the organization of putative AChR's. We are presently investigating the relationship of the s.b.l. associated antigen to the orgainzation of AChR's in vivo and in vitro. (Supported by USPHS grants NS17017 &NS17262, and grants from the MDA and Dysautonomia Fnd. to K.F.B.

*Wicha et al (1982) Proc. Natl. Acad. Sci.(USA) 79: 3213.

115.5 MONOCLONAL ANTIBODIES TO TORPEDO NICOTINIC ACETYLCHOLINE RECEPTOR LABEL DROSOPHILA CENTRAL NERVOUS SYSTEM TISSUE WITH REGIONAL SPECIFICITY. Bruce Chase*, Janet Holliday*, James Reese*, Edward Hawrot, and Linda L.Y. Chun* (SPON: Robin J.J. Benson). Depts. of Biology and Pharmacology, Yale University, New Haven, CT and Dept. of Neurology, Mass. General Hospital, Boston, MA.

Using several different techniques we have been examining the cross-species reactivity of mouse monoclonal antibodies (mAbs) raised against purified Torpedo electric organ acetylcholine receptor (AChR). Frozen sections of Drosophila melanogaster heads were examined by indirect immunofluoresence for binding of several of these monoclonal antibodies whose subunit specificity has been determined by western blotting. One mAb, designated 21.1A.16.42, is directed to the β-subunit of Torpedo AChR but does not cross-react with mammalian muscle AChR. This antibody strongly stains synaptic regions within the lamina and medulla of the optic lobe as well as the neuropil of the entire central nervous system (CNS). Another mAb, 23.55.13, which is directed against the α-subunit of Torpedo AChR and which does cross-react with mammalian muscle endplates, shows similar but less intense staining of the neuropil region.

mAb 27.43.37, which also cross-reacts with mammalian endplates but is directed to a second epitope on the α-subunit of Torpedo AChR, primarily stains axonal tracts in the entire optic lobe (lamina, medulla, and lobula plate) as well as the chiasma. The staining of another mAb to a third epitope on the Torpedo α-subunit is apparently restricted to the outer tips of the photoreceptor cells in the anterior retina. This mAb, 27.34.52, also cross-reacts with mammalian muscle AChR. Yet other mAbs to the α-subunit failed to stain any structures within the Drosophila CNS.

The demonstration of anti-Torpedo AChR mAb binding to specific regions in Drosophila brain suggests a conservation of epitopes between fish electric organ AChR and undefined antigens within the Drosophila CNS. Efforts to characterize the Drosophila antigens responsible for the observed immunofluorescent staining patterns are in progress. Supported by NIH GM 32629, the American Parkinson Foundation, and the PMA Foundation.

115.6 AUTORADIOGRAPHIC LOCALIZATION AND DEMONSTRATION OF TRANSPORT OF SEROTONIN AND IMIPRAMINE RECEPTORS IN THE CNS. T.M. Dawson,* E.W. Snowhill,* J.K. Wamsley (SPON: J.W. Conlee). Depts of Psych and Pharm, Univ Utah Sch of Med, SLC,UT 84132

Autoradiographic techniques for the microscopic localization of receptors have been applied to differentially localize 5HT-1, 5HT-2, and imipramine receptors. We have utilized these techniques to compare the distribution of 5HT-2 and 5HT-1 receptors and to demonstrate the transport of 5HT-2 and imipramine receptors in the brain.

High concentrations of 5HT-1 receptors (labeled for autoradiography with [^{3}H]-serotonin) were identified in the following areas: globus pallidus, septal nuclei, supraoptic nucleus, suprachiasmatic nucleus, substantia nigra zona reticulata, dentate gyrus, entorhinal cortex, cingulate cortex and the choroid plexus. In contrast, 5HT-2 receptors (labeled with [^{3}H]-ketanserin) were found in high concentrations in the following areas: laminae 4 of the cerebral cortex, caudate-putamen, nucleus accumbens; anterior, paraventricular, anterior ventromedial, and the posterior hypothalamic nuclei, mammillary nuclei, geniculate nuclei and the substantia nigra zona compacta. The areas that contained relatively high specific binding for 5HT-1 receptors in general contained low specific binding for 5HT-2 receptors and vice versa.

In an attempt to demonstrate the flow of serotonin and imipramine receptors, the medial forebrain bundle (MFB) was lesioned. Brain sections from control (non-lesioned) animals showed no 5HT-1, 5HT-2 or imipramine receptors in the area of the MFB. Lesioned animals, however, showed a dense accumulation of 5HT-2 and imipramine receptors surrounding the MFB lesion with the caudal aspect of the lesion showing the largest accumulation of receptors. We then performed a radiofrequency lesion of the dorsal raphe (using a stereotaxically placed electrode) prior to the MFB lesion in a separate group of animals. Examination of tissues from this group showed an accumulation of 5HT-2 and imipramine receptors only rostral to the MFB lesion.

This is the first evidence of transport of a "drug receptor" in the brain. Thus imipramine receptors (thought to be presynaptic receptors responsible for the inhibition of serotonin reuptake) and a subpopulation of 5HT-2 receptors (thought to be presynaptic autoreceptors) are both undergoing the process of orthograde axonal transport to terminals in the forebrain from the serotonin containing neuronal perikarya found in the dorsal raphe.

115.7 RECIPROCAL RELATIONSHIP BETWEEN MUSCARINIC RECEPTORS HAVING HIGH AFFINITY FOR CARBACHOL OR PIRENZEPINE IN THE RAT BRAIN. W.S. Messer, Jr.* and W. Hoss (SPON: J.R. Farrar) Center for Brain Research, University of Rochester Medical Center, Rochester, NY 14642.

Whereas classical muscarinic antagonists such as atropine and quinuclidinyl benzilate (QNB) bind uniformly to muscarinic receptors in brain, both agonists such as carbachol (CCH) and oxotremorine and selective antagonists such as pirenzepine (PZ) and gallamine distinguish different subtypes of the receptor. Further, binding studies with homogenates show that there are regional differences in the affinities of selective ligands for muscarinic receptors. To examine these regional differences in more detail, we have measured the ability of PZ and CCH to compete for {^{3}H}-l-QNB binding using autoradiographic techniques. Coronal sections (24μ) were mounted on microscope slides, incubated with various concentrations of{ ^{3}H}-l-QNB in the presence and absence of competing ligands for 1.5 hr and rinsed twice with buffer. One section from each slide was removed for scintillation counting. The slides were subsequently exposed to X-ray film for 7 days at 0°C. The resulting autoradiograms were examined by inspection and by microdensitometry. Scatchard analysis of the binding data indicated a Kd value of 0.6 nM and a Bmax of 250 fmoles/section with 95% specific binding. Inhibition curves for both CCH and PZ were flattened, suggesting the existence of multiple sites for these ligands. Examination of the autoradiograms showed that CCH completely inhibited binding in brainstem areas including superior colliculus and periaqueductal gray and diencephalic structures including septal and thalamic nuclei at concentrations that inhibited overall QNB binding by less than 10%. Structures such as the molecular layer of the dentate gyrus can be visualized even at concentrations of CCH that inhibited QNB binding by greater than 75%. In contrast, PZ had a high selectivity for the molecular layer of the dentate gyrus at concentrations which inhibit overall QNB binding by 10%. Thalamic nuclei and superior colliculi are still visible at the concentrations of PZ which inhibited binding by 59%. The observed regional selectivity in muscarinic receptors having high affinity for CCH or PZ suggests that these pharmacological differences may result from underlying regional differences in the distribution of muscarinic receptor subtypes. Supported in part by GM 07136.

115.8 ADENOSINE RECEPTORS OF CEREBRAL MICROVESSELS. R.N. Kalaria* and S.I. Harik (SPON: S.H. Kori). Dept. of Neurology, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

Recent evidence derived from in vivo and in vitro work on cerebral microvessels indirectly suggests the presence of adenosine receptors on membranes of cerebral blood vessels. We have assessed in a direct manner, the presence of adenosine (A) receptors on cerebral microvessels obtained by bulk separation using ligand binding techniques, and present evidence that cerebral microvessels are richly endowed with A_2 receptors but are essentially devoid of A_1 receptors.

Cerebral microvessels were obtained by bulk separation from pig cerebral cortical mantles. The ligands, [^{3}H] N^6-cyclohexyladenosine (CHA) and [^{3}H]5'-N-ethylcarboxamide adenosine (NECA) were used to label A_1 and A_2 receptors, respectively. Specific [^{3}H]CHA binding was defined as total binding in the presence of [^{3}H]CHA minus non-specific binding in the presence of 20μM cold CHA or 100μM N^6-L-phenylisopropyl)adenosine (L-PIA). Since NECA binds to both A_1 and A_2 receptors, the specific [^{3}H] NECA binding to A_2 receptors was defined as that occurring in the presence of 100 nM CHA or in preparations previously treated with N-ethylmalemide (NEM), minus non-specific binding in the presence of 100μM L-PIA. Preliminary investigations show that the specific binding to both A_1 and A_2 receptors appears saturable with an apparent K_D of about 2 and 20 nM, respectively.

The results shown in the table below demonstrate that cerebral microvessels from pig brain have few, if any, A_1 receptors but have a density of A_2 receptors that is similar to that of cerebral cortical membranes. Our findings are compatible with the existence of A_2 receptors in cerebral microvessels as suggested by investigations using cyclic AMP generation.

	A_1 binding	A_2 binding	
	[^{3}H]CHA (fmol/mg prot.)	[^{3}H]NECA (fmol/mg prot.) +100nM CHA	NEM-treated
cerebral cortex	303 ± 10	257 ± 18	191 ± 22
microvessels	35 ± 1	256 ± 31	175 ± 22

Values represent means ±SEM of 3-5 separate determinations of specific binding at ligand concentrations of 12 nM for CHA and 90 nM for NECA.

115.9 POSSIBLE RELATIONSHIP BETWEEN ADENOSINE UPTAKE SITES AND A TRANSMITTER ROLE OF ADENOSINE IN RAT CNS. J.D. Geiger*, F.S. LaBella and J.I. Nagy. Dept's of Pharmacology and Physiology, Univ. of Manitoba, Winnipeg, Man. R3E 0W3

An important neuroregulatory role of adenosine in the CNS is suggested by behavioral, electrophysiological and pharmacological observations. However, the neural systems specifically involved have not been identified. One approach to their identification hinges on the hypothesis that neurons which utilize adenosine as a transmitter may exhibit a propensity to release, take up and metabolize this nucleoside. We compared levels of adenosine deaminase, adenosine receptors labeled by [^{3}H]cyclohexyladenosine ([^{3}H]CHA) and adenosine uptake sites labeled with [^{3}H]nitrobenzylthioinosine ([^{3}H]NBI).

[^{3}H]NBI, in a conventional radioligand binding assay using rat CNS membrane preparations, bound to a single class of high affinity sites with a K_D of 0.12nM and B_{max} of 107 fmoles/mg protein. Adenosine inhibited [^{3}H]NBI with a higher affinity than other nucleosides or nucleoside bases. IC_{50} values for adenosine, thymidine, uridine, guanosine, cytidine or adenine were 0.03, 2.7, 4.3, 5.1, 11.0 and 40 mM, respectively. IC_{50} values for the metabolically stable adenosine analogs CHA, (+)phenylisopropyladenosine (PIA), (-)PIA, adenosine, 2-chloroadenosine and adenosine-5'-ethylcarboxamide were 8.8, 10.2, 29, 33, 135, 590 uM; while the values for 2-deoxyadenosine, inosine and hypoxanthine were 0.24, 1.8 and 2.4 mM, respectively. [^{3}H]NBI binding sites were not affected by methylxanthines, guanine and adenine nucleotides.

The regional distribution of [^{3}H]NBI binding sites was examined for 29 CNS regions as well as autoradiographically. The binding levels (fmoles\mg protein) ranged from 233 in thalamus to 44 in anterior pituitary and correlated well with levels of adenosine deaminase. Lesion studies were performed in attempts to localize the [^{3}H] NBI binding sites. Kainic acid injections into striatum resulted in a reduction of 48% in adenosine receptors and 28% in uptake sites.

These results indicate that [^{3}H]NBI binds to adenosine uptake sites in the rat CNS and may be a valuable marker for neural systems which use adenosine as a transmitter-like substance. (Supported by the Medical Research Council of Canada, Manitoba Health Research Council and the Manitoba Mental Health Research Fdn.)

115.10 INSULIN BINDING IN THE PERIPHERAL NERVOUS SYSTEM. Merrit L. Quarum,* Robert J. Waldbillig* and Celeste B. Hart*(SPON: G Hope) NIH, Bethesda, MD 20205; Dept. Physiology, AFRRI, Bethesda, MD 20814, NIH, Bethesda, MD 20205

Although there has been a marked increase in the amount of work conducted on insulin and insulin receptors in the CNS there has been little or no attention given to insulin binding in the peripheral nervous system (PNS). The work presented here addressed this issue by comparing insulin binding in the sympathetic superior cervical ganglion (SCG) with that of the liver and whole brain homogenate. It was found that although the SCG is neural tissue it binds insulin in amounts that greatly exceed that of whole brain (6.0% vs 2.0% /50 ug protein). The levels of insulin binding in the SCG and liver were approximately equal.

Structural characterization of the insulin receptor alpha subunit in the above tissue was accomplished using 125 I-insulin crosslinking, SDS polyacrylamide gel electrophoresis and autoradiography. These studies demonstrate that the alpha subunit in SCG insulin receptors have an approximate molecular weight of 136,000, which is significantly higher than the alpha subunit found in whole brain homogenate (Mr= 126,000). The molecular weight of the SCG receptor subunit is identical to that of the hepatic receptor. Work is currently being conducted to determine if there are differences in the insulin receptor kinase activity in these tissues.

These findings may indicate that exposure to blood-borne insulin is a better predictor of receptor structure and the level of binding than is tissue type. The metabolic implications of insulin receptors in the PNS remain to be examined.

115.11 STRUCTURAL HETEROGENEITY IN INSULIN RECEPTORS OF RAT BRAIN. Douglas J. Steel,* Robert J. Waldbillig,* Merrit L. Quarum*(SPON: V. Odom) Univ. of Florida, Gainesville, Fl 32611; Dept. Physiology, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814; National Institutes of Health, Bethesda, MD 20205

This laboratory recently demonstrated that streptozotocin-induced insulinopenia increased insulin binding in circumventricular brain areas where the absence of the blood-brain barrier (BBB) exposes neurons to plasma insulin. In contrast to this effect it was also found that insulinopenia decreased insulin binding in at least one of the non-circum-ventricular brain areas where the BBB prevents the entry of insulin in to the neuropile.

To determine whether circumventricular, non-circumventricular differences in the brain's response to diabetes could be related to regional differences in the structure of the insulin receptor, 125 I-insulin crosslinking, SDS poly-acrylamide gel electrophoresis, and autoradio-graphy were used to determine the apparent molecular weight of the alpha subunit in insulin receptors from olfactory bulb (non-circumventri-cular), area postrema (circumventricular) and liver.

It was found that the liver and the olfactory bulb each contained a single type of alpha subunit, the apparent molecular weight of the olfactory bulb subunit was lower than the weight of subunit found in the liver (126,000 vs 136,000). In contrast to the olfactory bulb and liver, it was found that the circum-ventricular area postrema contained two alpha subunits of different apparent molecular weight. One of these subunits had an apparent molecular weight identi-cal to the alpha subunit in the hepatic insulin receptor; the weight of the second area postrema subunit was identical to weight of the alpha subunit found in the olfactory bulb. The demon-stration of two insulin receptor alpha subunits in the area postrema is consistent with the fact that this area is both brain tissue and exposed to pancreatic insulin.

115.12 SENSITIVITY OF HINDBRAIN CIRCUMVENTRICULAR NEURONS TO PANCREATIC HORMONES. Robert J. Waldbillig*(SPON: D. Livengood) Dept. of Physiology, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814

Although the region of the area postrema is primarily known as the "chemorecepter zone" for the emetic response, it has recently been shown that blood-borne insulin binds to neurons in this and the immediately subadjacent area. This laboratory's finding that low-dose insulin infusions in this area produce a short latency reduction in hepatic glucose output indicates that the region contains insulin-sensitive neurons. The work presented here further examined this issue by characterizing the region's pattern of spontaneous neural activity and by determining the sensitivity of indivi-dual neurons to iontophoretically applied glutamate, insulin, glucose and glucagon.

It was found that in ketamine-anesthetized male Long-Evans rats, many area postrema neurons were spontaneously active, produced small extracellular potentials (typically < 100 uV), and were largely insensitive to stimula-tion by glutamate. In contrast, neurons in the area immediately ventral to the AP were frequently inactive, sensitive to the excitatory effects of glutamate, and produced somewhat larger extracellular potentials (typically between 100 and 200 uV). In large part, glucose and insulin had similar effects and inhibited glutamate evoked activity. Surprisingly, in all regions, insulin was usually ineffective in reducing spontaneous activity. A relatively small population of area postrema neurons producing large extracellular potentials were found to be sensitive to both insulin and glucagon. In these units the effects of these two compounds were uniformly antagonistic.

115.13 NATURE OF FMRF-NH_2 IMMUNOREACTIVITY IN VARIOUS RAT BRAIN REGIONS. H.-Y.T. Yang. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

Cardioexcitatory peptide, phe-met-arg-phe-NH_2 (FMRF-NH_2), was initially isolated from ganglia of macrocallista nimbosa clams. Subsequently, FMRF-NH_2 immunoreactivity (FMRF-NH_2 IR), which is distinct from FMRF-NH_2, was detected in mammalian CNS. The biological role of the mammalian FMRF-NH_2 IR is as yet unclear. However, its occurence in neurons and its excitatory action on brain stem of rat seem to suggest a neuromodulator role for the FMRF-NH_2 IR. Furthermore, we have previously observed that FMRF-NH_2, injected intrathecally, can reduce morphine analgesia. Whether the mammalian FMRF-NH_2 IR is identical to α_1-MSH, NPY, PYY or APP has been debated by various investigators. Because of this, in further searching for the biological role of FMRF-NH_2 IR, we have decided to characterize the FMRF-NH_2 IR in various rat brain regions with an antiserum developed against synthetic FMRF-NH_2. The antiserum cross-reacted almost equally with FMRF-NH_2 and α_1-MSH (bovine) but only slightly with other structurally similar peptides, such as NPY, met^5-enkephalin-arg^6-phe^7 (about 0.1%) and γ-MSH (0.05%). The distributions of FMRF-NH_2 IR (fmol/mg prot) in various rat brain regions are cerebellum (5.4±0.7); medulla oblongata (16±1.3); striatum (5.4±0.6); mid brain (13±1.4); hippocampus (3.5±0.5); hypothalamus (35±3.6); cortex (4.7±0.7); pituitary (102±11); spinal cord (40±2.7). The brain parts and pituitaries were dissected from rats killed by focused microwave irradiation and spinal cords from decapitated animals. The values were calculated from the standard curve prepared with FMRF-NH_2 and do not represent true contents of the peptides. Characterization of FMRF-NH_2 IR by HPLC revealed that there were 3 main immunoreactive peaks with retention time of 28 min (A), 32 min (B) and 40 min (C), which differ from the retention times of FMRF-NH_2, α_1-MSH (bovine) or NPY. Interestingly, the proportion of the three FMRF-NH_2 IR varies greatly in different brain regions. Content of C form is high in spinal cord and medulla oblongata and low in pituitary and hypothalamus. The mid brain contains the 3 forms in almost equal proportion. Because of the high immunoreactivity of the antiserum to the synthetic α_1-MSH (bovine), it is possible that FMRF-NH_2 IR may include α_1-MSH (rat). In fact, bovine brain contains a FMRF-NH_2 IR which seems to coelute with synthetic α_1-MSH in HPLC. The results in this study suggest that biological role of mammalian FMRF-NH_2 IR may not be easily explored by using synthetic FMRF-NH_2 and possible different biological properties of various forms should be considered.

ANATOMY OF MEMORY IN HUMAN AND NONHUMAN PRIMATES

116.1 RATE OF FORGETTING IN H.M.: A REANALYSIS. David M. Freed*, Suzanne Corkin, and Neal J. Cohen* (SPON: W. Jordan). Dept. Psychol., MIT, Cambridge, MA 02139.

Huppert and Piercy (1979) examined the rate of forgetting in amnesia with a picture recognition paradigm that provided patients with additional study time in order to make their initial yes/no recognition performance comparable to that of control subjects. Huppert and Piercy concluded that patients with amnesia due to diencephalic pathology and patients with amnesia resulting from medial temporal-lobe pathology showed dramatically different rates of forgetting. In particular, H.M., with bilateral medial temporal-lobe pathology, demonstrated abnormally rapid forgetting: His yes/no recognition performance fell to chance levels within one week. In order to test the generality of Huppert and Piercy's conclusion, patients with Alzheimer's disease, who also have medial temporal-lobe pathology, were tested with a similar paradigm, but using a two-alternative forced-choice format; they did not show rapid forgetting after their initial performance was matched to that of control subjects (Freed, 1984). Because of the marked discrepancy between the two studies, an attempt was made to replicate and extend Huppert and Piercy's results in H.M. Forgetting was assessed using both two-alternative and yes/no recognition formats, giving H.M. 20 sec of study time and the control subjects only 1 sec. H.M. showed retention significantly above chance levels in both formats. Upon retesting with the same target slides 16 days after the original presentation, H.M. scored 70% correct with the two-alternative format but performed at chance levels with the yes/no format. When yes/no and two-alternative recognition performance were assessed in the same experiment but at different retention intervals than those used by Huppert and Piercy, a decrement in yes/no performance was noted at the 72-hour retention interval, with a rebound in performance at the 1-week retention interval. We had noted a similar decrement and rebound in performance, but at different retention intervals, in certain patients with Alzheimer's disease (Freed, 1984). Our results indicate that H.M. did not display rapid forgetting in either yes/no or two-alternative recognition formats and that there are important differences in memory performance as assessed by the two techniques. The precise time course of forgetting, however, may yield useful information concerning amnesia due to different etiologies.

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116.2 CONSEQUENCES OF RECENT EXPERIENCE WITH FORGOTTEN WORDS IN AMNESIA. John.D.E. Gabrieli,* Neal J. Cohen,* F. Jacob Huff, James Hodgeson,* and Suzanne Corkin. Dept. Psychology, MIT, Cambridge, MA 02139.

The performance of patients with global amnesia can show the normal influence of recent experiences with words despite having profoundly impaired recall or recognition memory of the words (Warrington & Weiskrantz, 1968; Jacoby & Witherspoon, 1982; Graf, Squire, & Mandler, 1984). The etiology and severity of amnesia, however, may play an important role in determining the influence on performance of previous experience with words already forgotten. The present studies, therefore, investigated the occurrence of such memory phenomena in the patient H.M., who is distinctive because of the severity of his amnesia and its unusual etiology, a bilateral resection of medial temporal-lobe structures. In one study, H.M. was presented with words under a variety of encoding conditions that contrasted intentional versus incidental learning, various depths of cognitive processing, and number of repetitions of words. H.M. was severely impaired in the recall and recognition of words, but his completion of 3-letter stems was biased toward the forgotten words. Despite this evidence for dissociation, manipulations of various encoding conditions had parallel effects on H.M.'s accuracy of recognition memory and the extent of his stem-completion bias. Normal subjects (n=24) also demonstrated a correlation across testing conditions between levels of recall, recognition, and bias in stem-completion.

In another experiment, H.M. and normal subjects (n=20) completed orally presented sentences that included semantically disambiguated homophones (e.g. "tail" versus "tale"). When normal subjects were subsequently asked to write each homophone to dictation, their spelling reflected the meaning used in the sentences, whereas H.M.'s spelling did not. In a third experiment in which speed was measured for deciding whether a letter string constitutes a word, H.M. failed to show the normal reduction in latency with repeated items, even upon immediate repetition.

These results support the suggestion that the consequences of recent experience with a word upon subsequent performance can reveal learning effects for forgotten words. H.M.'s performance, however, indicates that these preserved learning phenomena are not entirely independent of the fact-learning memory processes governed by medial temporal-lobe structures.

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116.3 TEMPORAL CONTEXTUAL MEMORY IN AMNESIA. Harvey J. Sagar,* John D.E. Gabrieli*, Edith V. Sullivan, and Suzanne Corkin. Dept. Psychology, MIT, Cambridge, MA 02139.

In normal subjects, the recency and frequency of past events may be processed in memory independently from the content of these events (Hasher & Zacks, 1979). In amnesic syndromes, however, a selective deficit in temporal encoding has been proposed as the basis for impaired memory for content (Kinsbourne & Wood, 1975; Tulving, 1972; Huppert & Piercy, 1978; Hurst & Volpe, 1982). Milner (1971), however, found that patients with unilateral temporal-lobe excisions showed normal recency performance together with material-specific memory deficits. Because these deficits were mild, the possibility remains that severely amnesic patients would still show the predicted link between memory for temporal context and content. A critical test of the temporal encoding hypothesis of amnesia would be to examine recency and frequency judgements under conditions where recognition is at chance level. Accordingly, we compared item recognition and judgement of temporal context in the severely amnesic patient H.M., who had bilateral medial temporal-lobe resection as treatment for epilepsy. In two temporal ordering tasks, H.M. and control subjects viewed 493 stimuli displayed one at a time every 2 sec; in a verbal test, the stimuli were single words, and in a nonverbal test, wallpaper designs. Two-choice tests both of recognition memory and of recency judgement were presented at 0, 3, 6, 10, 15, 25, 50, 100, and 150 items after stimulus exposure. In a frequency test, 810 words were presented in 30 blocks, each of 27 words. Within blocks, different words were viewed 1, 3, or 5 times. Two-choice recognition tests for item and frequency occurred at intervals up to 30 blocks after stimulus presentation. In control subjects, performance on recency and frequency tasks was inferior to item recognition at all stimulus test delays. H.M., by contrast, achieved scores in recency judgements that were within the range for control subjects, even when his item recognition was at chance performance. Similar results were obtained in the frequency test, except that at longer intervals, he became impaired relative to normal subjects on frequency judgements as well as item recognition. The results suggest that the amnesia of bilateral medial temporal-lobe pathology is not based upon a specific loss of temporal contextual encoding. Further, the findings support the concept that memory for content and temporal context may operate independently.

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116.4 NORMAL PRIMING EFFECTS IN AMNESIC PATIENTS. A. P. Shimamura*, L. R. Squire, and P. Graf* (SPON: I. Grant). Dept. of Psychiatry, Univ. of CA, La Jolla, CA 92093, and Vet. Admin. Med. Ctr., San Diego, CA 92161, and Dept. of Psychology, Univ. of Toronto, Canada.

In a series of experiments, amnesic patients exhibited normal priming despite being severely impaired on tests of recall and recognition. Priming effects are tested without reference to memory testing or to previously presented material. We tested priming ability and recognition memory in patients prescribed bilateral electroconvulsive therapy (ECT). To assess priming, subjects were presented words (e.g., STOVE) and then given word stems (e.g., STO__) that must be completed to form the first words that come to mind. Patients were tested at 45, 65, 85 minutes, and 9 hours after treatment. Recognition performance was at chance when testing occurred 45 minutes after ECT, and it improved during the period 45 minutes to 9 hours after treatment. Word completion, however, was not affected by ECT. These results show that priming is independent of the processes underlying recognition memory.

A second study suggested that priming is responsible for the frequently demonstrated ability of amnesic patients to learn highly related word pairs. At immediate testing patients with Korsakoff's syndrome exhibited good paired-associate learning for highly related word pairs (e.g., TABLE-CHAIR) (71%; 94% correct for controls), but after a 2-hour delay these patients performed no better than baseline guessing (35% correct vs 26% baseline; 80% correct for controls). When word completion was assessed for the first word in each pair (e.g., TAB__), performance also fell to baseline in 2 hours. In another test, subjects were shown related word pairs, without any instructions to learn them, and then were asked simply to "free associate" to the first word of each pair. Amnesic patients and control subjects performed identically (69% correct; baseline= 25%). Thus, amnesic patients showed normal priming of semantically related material.

In all studies, amnesic patients exhibited normal priming. The results indicate that the processes supporting priming are transient and are independent of the processes that support recall and recognition memory. These processes are preserved in amnesia and thus do not depend on the integrity of the brain structures damaged in amnesia.

116.5 PERFORANT PATHWAY PATHOLOGY AND THE MEMORY IMPAIRMENT OF ALZHEIMER'S DISEASE. B.T. Hyman, G.W. Van Hoesen and A.R. Damasio. Depts. of Neurology and Anatomy, College of Medicine, University of Iowa, Iowa City, IA 52242.

The perforant pathway is a large system of axons that arises from the superficially located neurons of the entorhinal cortex (Brodmann's area 28) and ends on the distal dendritic branches of the dentate gyrus granule cells and the hippocampal pyramidal cells. It is the major source of cortical input to the hippocampal formation conveying both sensory specific and multi-modal association cortical information. We have studied the cells of origin, the course, and the terminal zones of the perforant pathway in the brains of 5 patients with Alzheimer's disease (mean age, 78.3 yrs) and 5 elderly non-demented controls (mean age, 77.0 yrs). The brains were stained with thionin for Nissl substance, iron hematoxylin for myelin, and Congo-red for neurofibrillary tangles. Some were stained with silver nitrate to reveal axis cylinders and neuritic plaques and with the Geneser-Jensen and Blackstad method to reveal acetylcholinesterase activity. As compared to the age-matched controls, Nissl stained sections of the Alzheimer brains showed a marked alteration in the cells of origin of the perforant pathway in layers II and III of the entorhinal cortex and Congo-red staining revealed neurofibrillary tangles in these cells. As expected, the perforant pathway was demyelinated and contained evidence of a marked gliosis. The terminal zones of this system were in part occupied by neuritic plaques, of which several stained intensely for acetylcholinesterase. In view of the fact that some of the cells of origin of the perforant pathway are also AChE-positive, these plaques may represent degenerating neuritic terminals of this well-defined anatomic system. Perforant pathway pathology of the magnitude we have observed in the Alzheimer brains, essentially isolates the hippocampal formation from the sensory specific and multimodal association cortices and undoubtedly interferes with normal hippocampal function. There is little argument that hippocampal pathology leads to a striking memory impairment in humans. We therefore suggest that perforant pathway pathology plays a major role in the memory disorder which is the hallmark of Alzheimer's disease. (Supported by NIH grants NS 14944, 1F32EY05720, and PONS 19632).

116.6 TOWARDS AN ANIMAL MODEL OF HUMAN AMNESIA: A FILMED DEMONSTRATION OF NEUROPSYCHOLOGICAL TESTS USED TO EVALUATE MEMORY IN THE MONKEY. L. R. Squire and S. M. Zola-Morgan. Vet. Admin. Med. Ctr., San Diego, CA 92161 and Dept. Psychiatry, Univ. of CA, La Jolla, CA 92093.

A major goal of neuropsychological work on memory has been to establish a model of human amnesia in the monkey. Several memory tasks are now available for the monkey that are sensitive to human amnesia, and which are failed by monkeys with medial temporal lesions and by monkeys with midline diencephalic lesions. Many investigators who work either with clinical patients or with non-primates are unfamiliar with the kinds of tasks that monkeys can be taught to perform. A short film will be presented to illustrate several of these tasks, and data will be presented for four tasks on which monkeys with conjoint hippocampal-amygdala lesions are severely impaired.

116.7 PERFORMANCE OF MONKEYS WITH SEPARATE AND COMBINED LESIONS OF HIPPOCAMPUS AND AMYGDALA ON DELAYED NONMATCHING TO SAMPLE. S. M. Zola-Morgan, L. R. Squire, and D. G. Amaral. Vet. Admin. Med. Ctr., San Diego, CA 92161, Dept. Psychiatry, Univ. of CA, La Jolla, CA 92093, and The Salk Institute San Diego, CA 92138.

The development of an animal model of medial temporal amnesia has been greatly influenced by studies of the noted amnesic case H.M. Studies at NIMH (Mishkin) and in our laboratory, involving monkeys with conjoint hippocampal-amygdala (H-A) lesions that approximated H.M.'s removal, have largely succeeded in establishing an animal model of amnesia. Monkeys were severely impaired on the delayed nonmatching to sample task and on other tasks known to be sensitive to amnesia in humans.

One question still to be answered concerns the relative contribution of the hippocampus and the amygdala. One view has been that separate damage to hippocampus (H) or amygdala (A) produces only a mild impairment on the nonmatching task, whereas the combined H-A lesion results in severe impairment. Another view has been that H lesions alone can exert a substantial impairment of memory. However, studies supporting the latter view have not included a separate group of monkeys with A lesions to determine if H lesions exert any greater effect on memory than A lesions.

Here we report findings for monkeys with conjoint H-A lesions (N=4), monkeys with separate H lesions (N=4),and monkeys with separate A lesions (N=3). The lesions in the H-A and H groups were done under direct surgical approach, as in previous studies, and necessarily involved damage to overlying medial and ventral cortex. Lesions in the A group were produced using a radio frequency (RF) lesion maker and a stereotaxic approach. Two additional A animals sacrificed immediately after surgery showed near total removal of amygdala, but little or no cortical damage. Monkeys with H-A lesions were severely impaired on the nonmatching task. Monkeys with H lesions were also substantially, though less severely, impaired. Monkeys with A lesions performed normally, even at the longest delay interval of 10 minutes. These findings suggest that the hippocampal formation may make a more substantial contribution to performance on this memory task than the amygdala and that damage to hippocampal formation alone is sufficient to produce a substantial memory deficit. To evaluate the more severe deficit associated with the H-A group, it will be necessary to test monkeys who have received the H lesion together with the circumscribed A lesion described here.

116.8 HYPOTHALAMIC AMNESIA IN MAN J.T. Becker, A. Khan*, and A. Reddy* Alcohol Research Center, Dept. of Psychiatry, Univ. Connecticut School of Medicine, Farmington, CT

Current theories of memory stress the functional significance of the hippocampus and amygdala in the temporal lobe, and the thalamus and hypothalamus in the diencephalon. While damage to the hippocampus, or its major projection areas, alone produces a relatively mild memory defect, combination with damage to amygdala or its projection areas produces a severe amnesic syndrome.

Although there have been some recent reports on the effects of localized lesions in the hypothalamus of monkeys on memory and learning, there have been few reports of their effects in man. In the present study, we report the results of an extensive neuropsychological investigation of a patient three years following the draining of a dermoid cyst in the midline hypothalamus. The patient, T.G., is grossly obese, hyperphagic, polydipsic, polyuric, and has significant impairments in memory function.

T.G. Has relatively normal short-term memory, although his performance deteriorates when memory load is increased. Thus, estimates of primary memory based on free recall tasks are well within normal limits. In addition, his performance on Peterson distractor tasks is normal at short intervals (0-9 secs), but impaired at longer intervals (18-36 secs).

T.G. is impaired in the performance of tests of associative and long-term memory. He has difficulty learning even easy paired-associates, and cannot recall object-location associations. In addition, he shows no primacy effect during free recall tasks.

In contrast to his poor associative learning, T.G.'s recognition memory is only mildly impaired. He has modest deficits in both verbal and tactual recognition memory: poorer than normal but not as affected as alcoholic Korsakoff patients.

These data confirm earlier, less extensive reports of the amnesia-producing effects of hypothalamic damage in man. In addition, the relatively mild form of the memory loss suggests that only part of the anatomical circuitry is damaged. These data support, therefore, the hypothesis that there are two anatomically distinct circuits which interact during normal memory functioning.

116.9 THE EFFECTS OF HIPPOCAMPAL - MAMMILLARY BODY SYSTEM LESIONS ON ASSOCIATION MEMORY IN MONKEYS. R.C.Saunders. Dept. Exp. Psych., Univ. Oxford, Oxford, England.

Recently, in monkeys, it has been demonstrated that lesions restricted to either the hippocampus (H), the fornix (Fx) or the mammillary bodies (MB) results in a recognition memory deficit in tasks analogous to those used with amnesic patients. The present investigation examined the effects of lesions of the H, Fx and the MB on two tests of association memory.

Six monkeys (3 controls and 3 with Fx transection) had been previously trained in a one trial object-reinforcement association memory task (Gaffan, et al., Quart. J. of Exp. Psych., in press). In this task, two objects, one rewarded and the other not, were presented in successive trials. This was followed by a retention test in which the two objects were re-presented simultaneously with the previously baited object rewarded. Thirty new problems were given daily until criterion performance was achieved. The list of problems was then increased in 3 stages, from 1 to 2, then to 3 and finally to 5 problems. As Fx transections were without effect on this task the present study examined in the same monkeys the effects of adding, sequentially, lesions of the MB and the H. Monkeys were re-tested on all stages of the task after each surgery. Results showed that performance on the task was not significantly affected by the addition of MB and H lesions.

In the second experiment eight monkeys (4 controls, 2 with Fx transection alone and 2 with combined Fx, MB and H lesions) were trained on an object discrimination task. However, instead of using single objects as the discriminanda, pairs of objects were used. Four different pairs were formed from different combinations of 4 objects with each object appearing within a rewarded and non-rewarded pair. Monkeys were trained to a high level of accuracy before their memory for the object-pair associations was tested further in a second stage. For this stage, 3 individual objects from the original set of 4 were presented in each trial. The monkeys had to displace the two objects which, as a pair, had been rewarded in the first stage. Animals with combined Fx, H and MB lesions or with Fx transection alone were unimpaired in learning the initial discrimination task; in contrast, they were significantly impaired in the second stage.

These findings suggest that in monkeys at least, a certain type of association memory is impaired after H - MB system damage, while another type remains intact.

116.10 INFERIOR PREFRONTAL CORTEX AND RECOGNITION MEMORY. D. M. Kowalska*, J. Bachevalier and M. Mishkin. (SPON: E. A. Murray). Lab. Neuropsychology, NIMH, Bethesda, MD 20205.

Recognition memory has been shown to be severely impaired following ablations of the ventromedial prefrontal cortex, an area interconnected with thalamic targets of the amygdala and hippocampus, namely, the magnocellular division of n. medialis dorsalis (MD) and the anterior nuclei. In contrast, after lesions of the dorsolateral prefrontal cortex, an area to which the parvocellular division of MD projects, no recognition memory deficits were found (Mishkin and Bachevalier, Neurosci. Abstr., 9:29, 1983). Since the cortex of the inferior prefrontal convexity (IC) is also interconnected with the parvocellular division of MD, we tested whether ablations of this area would likewise fail to affect recognition memory.

Eight macaques were trained in a one-trial-recognition task in which they were required to distinguish a completely novel object from a sample object presented 10 s previously (delayed nonmatching-to-sample). After reaching a criterion of 90 correct responses in 100 consecutive trials, three monkeys received bilateral ablations of IC cortex whereas the remaining animals were kept as unoperated controls. Two weeks later, all animals were retrained on the basic recognition task to a 90% criterion and were then given a performance test with longer delays (30 to 120 s) and list lengths (3 to 10 objects). Unlike the unoperated animals, which reattained criterion in 0 trials, the monkeys with IC lesions needed an average of 740 trials (range: 160 to 1060). Despite their relearning difficulty, however, the monkeys with IC lesions were not significantly impaired in the performance test, on which they averaged 89% correct (range: 84 to 95), as compared to an average of 94% (range: 92 to 95) for the normal controls. The preserved recognition ability after IC ablations suggests that the impairment in relearning the delayed nonmatching principle was due not to a memory loss but rather to the perseverative interference that this lesion is known to induce (Iversen and Mishkin, Exp. Br. Res, 11:376, 1970). This suggestion is supported by an error analysis, which revealed strong position preferences in the operated animals throughout the period of their relearning difficulty.

These findings provide futher evidence that, in the prefrontal region of the monkey, only ventromedial cortex is critical for recognition memory.

116.11 RECOGNITION DEFICIT IN MONKEYS FOLLOWING NEUROTOXIC LESIONS OF THE BASAL FOREBRAIN. T. Aigner*, S. Mitchell, J. Aggleton, M. DeLong, R. Struble, G. Wenk, D. Price, M. Mishkin. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20205 and Depts. of Neurology and Neuroscience, Jonns Hopkins Medical School, Baltimore, MD 21205.

Dysfunction of the cholinergic system of the basal forebrain has been suggested as one explanation for the memory impairment observed in Alzheimer's disease. In our initial attempt to test this hypothesis experimentally (Aigner et al, Soc. Neurosci. Abstr. 9: 826, 1983), we examined recognition memory in monkeys with damage to the nucleus basalis of Meynert (nbM), the major source of cholinergic projections to the neocortex and amygdala. Although no impairment resulted, the animals were found to be more sensitive to the disruptive effects of scopolamine, suggesting that further study of the cholinergic system was warranted. This system includes not only nbM but also the vertical limb of the diagonal band of Broca (dbB) and medial septum (ms), the major sources of cholinergic input to the hippocampus. To assess the contribution of these cholinergic areas to memory, we compared the cynomolgus monkeys previously given lesions of nbM (Group M) and their controls (Group C) with new groups of cynomolgus monkeys given lesions of dbB + ms (Group B) or all regions (Group MB). As before, the animals were trained in delayed nonmatching-to-sample, i.e. to avoid a familiar object, presented as the sample 10 sec earlier, in favor of a novel object with which the familiar one was paired. Electrophysiological recording techniques were again used to map the location of basal forebrain nuclei, and ibotenic acid was injected bilaterally into the designated loci. Two weeks later, behavioral testing was resumed. Groups C, M, and B relearned the task in an average of 100, 125, and 133 trials, respectively, whereas group MB required 540 trials. The animals were then given a performance test in which the delays were extended to 30, 60, and then 120 sec and the lists of objects to be remembered were increased to 3, 5, and then 10. Each condition was tested for 5 consecutive days. The percentage of correct choices, averaged across all conditions, were: C - 92.7%; M - 91.9%; B - 91.6%; and MB - 81.9%. Only the MB group was significantly different from C. The results suggest that combined damage to areas nbM, dbB and ms is necessary to produce impairments in recognition memory in monkeys, perhaps because only such damage causes dysfunction of both the amygdala and the hippocampus (Mishkin, Nature 273: 297, 1978).

116.12 MULTIMODAL AMNESIC SYNDROME FOLLOWING BILATERAL TEMPORAL AND BASAL FOREBRAIN DAMAGE: THE CASE OF PATIENT DRB. A. R. Damasio, P. J. Eslinger, H. Damasio* and G. W. Van Hoesen. Dept. of Neurology, Univ. Iowa College of Medicine, Iowa City, IA 52242.

Patient DRB developed a major amnesic syndrome following herpes simplex encephalitis. His amnesia is both anterograde and retrograde. The retrograde amnesia spans the five decades of his life, sparing only generic (semantic) material devoid of appropriate temporal and spatial placement. The anterograde amnesia encompasses both generic (semantic) and contextual (episodic) material. With the exception of preserved learning of a visual-motor skill (mirror tracing), he has not demonstrated acquisition of any new information since his illness in 1975. In contrast elementary perceptual, intellectual and linguistic abilities remain intact.

Computerized tomography (CT) and single photon emission tomography (SPET) demonstrate extensive bilateral damage to medial and antero-lateral temporal lobe as well as to basal forebrain. The remaining cortices and deep gray and white matter structures appear normal.

Thus the antomical and behavioral characteristics of this patient are significantly different from those of other amnesic patients especially HM, NA, the basal forebrain patients we recently described (Soc for Neurosc Abstracts, 9, 1983, 29) and the classical alcoholic Korsakoff patients. The case of patient DRB provides powerful evidence that: (a) storage and retrieval of previously acquired generic memories and skills do not depend on mesial temporal lobe or basal forebrain structures; (b) supports the hypothesis that learning of new information is dependent on medial temporal lobe structures, (c) demonstrates that acquisition and retention of a visual-motor skill is not dependent on medial temporal lobe and basal forebrain function, and (d) raises the question that lateral temporal lobe and basal forebrain damage, when combined with medial temporal lobe lesions, causes a virtually complete breakdown of contextual memory. Supported by NINCDS Grant PO1 NS 19632-01.

GABA AND BENZODIAZEPINES: BINDING I

117.1 ENDOGENOUS INHIBITORS OF [^{3}H]RO5-4864 BINDING TO PERIPHERAL BENZODIAZEPINE BINDING SITES ALSO INHIBIT [^{35}S]TBPS BINDING IN THE CNS. C. Mantione, M.E. Goldman, B.A. Weissman*, S.M. Paul* and P. Skolnick*, Lab. of Bioorganic Chem., NIADDK, Lab. of Chem., NHLBI, and Clinical Neurosci. Branch, NIMH, NIH, Bethesda, MD 20205.

Benzodiazepines (BZ) interact with specific binding sites in both the brain (central-type), and in various peripheral tissues (peripheral-type). Peripheral-type BZ binding sites (PBS) are also found in brain, and appear to be under neural control in both the pineal and olfactory bulb. Consequently, we have attempted to demonstate the presence of a natural ligand(s) for this site by screening tissue extracts for their ability to inhibit [^{3}H]RO5-4864 binding to PBS. In preliminary experiments, several tissue extracts (e.g. brain and stomach) were found to contain relatively high endogenous inhibitory activity. Subsequent characterization and purification was carried out using rat antral stomach. Tissues were frozen on dry ice and homogenized in 0.5% trichloroacetic acid. The homogenate was heated at 55°C for 15 min, and centrifuged. The resulting supernatant was lyopholized, reconstituted in water, and extracted with ether. The aqueous crude extract produced a dose-dependent inhibition of [^{3}H]RO5-4864 binding without significantly decreasing [^{3}H]diazepam binding. Ultrafiltration studies demonstrate that the crude extract contains about 70% of the inhibitory activity as high M_r material, and the remainder of activity as a fraction with M_r <1000. The potency of the high molecular weight species isolated on Sepadex G-50 (10-15,000 M_r) was decreased by 25% after treatment with pronase or trypsin, but not with carboxypeptidase, suggesting that the material may be a peptide. The low molecular weight material was not affected by proteolytic enzyme treatment. Since RO5-4864 is also an inhibitor (IC_{50} 0.65 μM) of [^{35}S]TBPS binding, we examined whether the PBS inhibior(s) would also interact with this site. The high M_r PBS inhibitor equipotently displaced [^{35}S]TBPS binding to rat cortical membranes by 10-95%, while these concentrations produced <30% inhibition of [^{3}H]RO15-1788 binding to central-type BZ receptors. Reverse phase HPLC of this material yields three regions of activity that inhibit both [^{3}H]RO5-4864 and [^{35}S]TBPS binding. These data demonstrate that endogenous substances are present in rat tissues which recognize a region common to both the high affinity binding sites for RO5-4864, and the picrotoxin site that is a part of the regulatory domain of the GABA receptor/BZ/chloride ionophore complex.

117.2 MODULATION BY GABA AND BENZODIAZEPINES OF A SPECIFIC t-BUTYL-BICYCLOPHOSPHOROTHIONATE (TBPS) BINDING TO CEREBELLAR GRANULE CELLS IN CULTURE. V. Gallo* and A. Guidotti (SPON: W.J. Wojcik). Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

TBPS, a cage convulsant with picrotoxin like activity, binds specifically to rat brain membranes in a saturable fashion[1]. We have now characterized ^{35}S-TBPS binding to intact rat cerebellar granule cells cultured in vitro for 8 days.

The ^{35}S-TBPS specific binding, determined by displacing the radioligand with an excess (10^{-4}M) of picrotoxin, was approximately 70% of the total radioactivity bound to the cells. At 25°, the binding rapidly increases with time of incubation and levels off after 15 min. The amount of binding displaceable by an excess amount of cold picrotoxin or TBPS is the same when the cold ligand is added 15 min before, together or 45 minutes after the addition of ^{35}S-TBPS. ^{35}S-TBPS specific binding was saturable with a Kd of approx. 40 nM and a Bmax of approx. 0.5 pmol/mg prot.

Neither cerebellar astrocytes maintained in culture for two weeks nor a neuroblastoma cell line (N$_{2A}$) exhibited any specific ^{35}S-TBPS binding. ^{35}S-TBPS binding to granule cells was inhibited competitively by TBPS, picrotoxin and n-butylbicyclophosphate (Ki = 2.5×10^{-8}, 5×10^{-8} and 2.5×10^{-6}M respectively).

The binding of ^{35}S-TBPS to intact cerebellar cells was enhanced in a dose-related fashion by muscimol (0.3-5 μM) and was inhibited by bicuculline (0.1-5 μM). The effect of muscimol and bicuculline on ^{35}S-TBPS binding is not competitive.

Muscimol (0.1-5 μM) reversed bicuculline inhibition in a dose-dependent fashion, but failed to reverse picrotoxin-induced inhibition. ^{35}S-TBPS binding was also modulated by benzodiazepines. In fact, muscimol 0.05 μM, failed to reverse bicuculline inhibition in absence of diazepam but it became effective in the presence of 0.01-1 μM diazepam. This benzodiazepine facilitation was concentration-dependent.

These results suggest that ^{35}S-TBPS binds to the picrotoxin site of the GABA-benzodiazepine-ionophore complex and that the mutual interactions between the subunits of the receptor complex can be studied in a system of intact, differentiated nerve cells.

1 Squires, R.F., Casida, J.E., Richardson, M. and Saederup, E. (1983) Mol. Pharmacol. 23, 326-336.

117.3 ISOLATION AND PARTIAL CHARACTERIZATION OF A PUTATIVE ENDOGENOUS LIGAND FOR BENZODIAZEPINE RECEPTORS FROM PORCINE CORTEX. P. J. Syapin, J.F. Van Pelt*, C.A. Meyers*(2), and E.F. Hayes*(3), Dept. of Neurology, University of Southern California School of Medicine, Los Angeles, CA, (2)National Institutes of Health, Bethesda, MD, (3)Scheaffer Institute, Metairie, LA.

The high affinity binding of synthetic anti-anxiety drugs like the benzodiazepines to specific receptors in the brain suggests the existance of a new neurochemical system. Analogous to endogenous opiates binding to brain morphine receptors, this system would be expected to include benzodiazepine receptor endogenous ligands (BZELs) that bind to the same sites as the synthetic benzodiazepines. Many reports in the literature support the existance of endogenous ligands for benzodiazepine receptors, although identification of a universally accepted BZEL is still forthcoming.

We have examined porcine cortex for putative BZEL activity. Fresh pig brains were obtained from a local slaughterhouse and placed in ice within 30 minutes of removal. Within 60 minutes the meninges were cleared and the cortex dissected away from the rest of the brain. Cortex samples were homogenized in cold extraction buffer and ultracentrifuged to obtain a soluble fraction. The soluble material was fractionated by LRP-1 column chromatography followed by HPLC to isolate (3H)diazepam displacing activity. A single peak of displacing activity was present throughout the isolation procedure. The specific activity of the active fraction (units of inhibition/gm weight) increased over one million-fold during isolation. Studies on this putative BZEL revealed an acid extractable, slightly lipophillic, low molecular weight substance (MW= 1000 dalton by Biogel P4 column chromatography) that is resistant to proteolytic digestion. The active substance is a competitive inhibitor of (3H)diazepam binding to mouse forebrain receptors in vitro and resembles an agonist, i.e., is benzodiazepine-like, in the GABA shift assay. The UV absorption spectra in acid showed maxima at both 195 nm and 248 nm; the latter being in the range for heterocyclic compounds. We have been able to rule out the possibility that our active substance is inosine or hypoxanthine, nicotinamide, or beta-carboline-3-carboxylate ethyl ester. Further work is needed to identify the structure of this putative BZEL compound and determine its in vivo actions.

117.4 REGIONAL CIRCADIAN RHYTHMS OF BENZODIAZEPINE AND GABA RECEPTOR BINDING IN RAT BRAIN. M.J.W. Brennan, M.C. Moore-Ede and D. Borsook*. Neurological Unit, Boston City Hospital, Boston, MA 02118

The circadian rhythms in virtually all physiological and behavioral variables are timed by central neural pacemakers which include the suprachiasmatic nuclei of the hypothalamus. Recently we have demonstrated that GABA-mimetics are capable of altering the periods of the activity and temperature rhythms in free-running squirrel monkeys and that GABA inhibits firing of the pacemaker cells in a phase dependent manner. These data suggest that GABA may play an important role in the central control of circadian rhythms. To extend this hypothesis we examined benzodiazepine (BDZ) and GABA receptor binding in different regions of rat brains.

Male Sprague-Dawley rats (250-300g) were entrained to an LD cycle of 14:10, groups were killed at 6-hour intervals over a day and brains dissected. Specific binding of ^{3}H-flunitrazepam (0.5-7.5nM) and ^{3}H-muscimol (1.0-25.0nM) was assayed in washed synaptic plasma membranes from frontal lobes, temporoparietal lobes, hypothalamus, cerebellum and medulla-pons. Total receptor number (Bmax) and affinity (Kd) were determined by Scatchard analysis of the saturation isotherms.

Prominent circadian rhythms in BDZ and GABA receptor numbers were observed in the frontal lobe. Binding was highest at 06h00 and 12h00, corresponding to the period of sleep/low activity (lights on) in the rat; significant (24-31%) decreases in both receptors were noted at 08h00 in anticipation of waking (lights off at 19h00). Binding of ^{3}H-flunitrazepam in the cerebellum was maximal at 12h00, in the middle of the sleep/low activity period, and lowest at 18h00 and 06h00. Again a significant reduction in Bmax (29-42%) occurred from 12h00 to 18h00. No significant circadian fluctuations in receptor affinity were observed.

Our data demonstrate that normal rat brain BDZ/GABA receptors undergo rapid fluctuations in number during the course of a day with changes of 24-42% occurring over a 6-hour period. The specific localization of BDZ receptor rhythms to frontal lobes and cerebellum with parallel rhythms in GABA binding in frontal lobes suggests involvement of BDZ/GABAergic systems in functions which exhibit circadian rhythmicity in these regions. Specific GABAergic projections from hypothalamus to frontal lobe may express the innate rhythmicity of hypothalamic pacemaker neurones which is reflected in cyclical changes in receptor number in the target regions.

117.5 CHRONIC ANTIDEPRESSANTS AND GABA "B" BINDING SITES K.G. Lloyd and A. Pilc* (LERS-Synthélabo, Bagneux, France).

Of the putative classical neurotransmitters, GABA synapses have received the least attention as a candidate either as a biological substrate for the pathophysiology of depression, or a site for the mechanism of action of antidepressant drugs. The limited data available suggests that indeed GABA synapses may play an important role in both of these functions (Cf. Lloyd et al, Pharm. Bioch. Behav. 18, 957, 1983 for references). The present study examined the effects of continuous administration in Alza 2002 minipumps of 5 antidepressants (desipramine, citalopram, viloxazine, amitryptyline and fluoxetine), an MAO inhibitor (pargyline) and a neuroleptic (haloperidol) on various aspects of GABA synaptic function in the rat (Male, Wistar, 250-300 g, 4-6 animals per group) "ex vivo" in the frontal cortex (removed immediately after decapitation and frozen at - 80°C).

Compound, dose mg/kg/day	Days	Activity in % of Saline Treated Rats			
		GAD Activity	GABA"A" Binding	GABA"B" Binding	GABA Levels
Desipramine, 5	6	99	89	140	100
	18	96	105	151**	84
Fluoxetine, 10	6	100	93	184**	109
Amitryptyline, 10	18	92	110	154**	122
Citalopram, 10	18	97	106	172**	71
Viloxazine, 10	18	79	109	188**	134
Pargyline, 20	18	-	-	142*	-
Haloperidol, 0.3	18	-	-	108	-

* $p < 0.05$; ** $p < 0.01$ vs. saline treatment

As can be seen from the Table, only GABA "B" binding was consistently altered by the prolonged antidepressant treatment, an effect which was not observed with haloperidol, or upon a single administration of the compounds (except for viloxazine which induced a 36 % increase, $p < 0.05$). Thus, widely differing classes of antidepressant drugs affect GABA "B" receptor binding in a manner which is consistent with their clinical activity.

117.6 COMPARISON OF [^{3}H]BACLOFEN BINDING TO GABA$_B$ RECEPTORS IN SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE RATS. Rekha Singh* and Maharaj K. Ticku (SPON: A. Modak). Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284.

Several studies have suggested a role for the major inhibitory neurotransmitter γ-aminobutyric acid (GABA), in the regulation of blood pressure. A recent study reported that intracerebroventricular (ICV) administration of GABA produced a greater decrease in mean arterial pressure and heart rate in the spontaneously hypertensive (SH) rat than in age-matched Wistar-Kyoto (WKY) rat (Brennan et al, Life Sci. 33:701, 1983). This differential sensitivity does not appear to be due to differences in GABA$_A$ or central benzodiazepine binding in SH and WKY rats (Thyagarajan et al, Eur. J. Pharmac. 93:127, 1983). Since GABA can also bind to bicuculline-insensitive GABA$_B$ receptors, we investigated the binding properties of GABA$_B$ receptors in SH and WKY rats. The binding to GABA$_B$ receptors was measured by the method of Hill and Bowery (Nature 294:584, 1981), using [^{3}H]baclofen as the radioligand. Specific [^{3}H]baclofen binding to P_2 membranes from various brain regions was compared in age-matched (12 week) SH and WKY rats. The specific binding of [^{3}H]baclofen (20 nM) was significantly higher in the SH rat cerebellum (330 ± 21 vs 228 ± 20 fmol/ mg protein, $p < 0.01$) and hippocampus (133 ± 16 vs 78 ± 17 fmol/mg protein, $p < 0.05$), as compared to WKY rats. In contrast, brain stem of SH rat had lower specific [^{3}H]baclofen binding (138 ± 15 vs 220 ± 21 fmol/mg protein; $p < 0.05$). The specific [^{3}H]baclofen binding was not significantly different in cerebral cortex and hypothalamus of SH and WKY rats. Although nucleus tractus solitarius of SH rat had 25% higher [^{3}H]baclofen binding, it was not statistically significant. Scatchard analysis of the saturation isotherms revealed that [^{3}H]-baclofen binds to two classes of sites in both SH and WKY rats. The higher specific [^{3}H]baclofen binding in cerebellum and hippocampus of SH rats was due to higher density of high affinity GABA$_B$ sites. The affinity of the two sites was not signficantly different between SH and WKY rats.

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117.7 GABA ANTAGONISTS, ANTIDEPRESSANTS, CENTRAL STIMULANTS AND OTHER SUBSTANCES REVERSE THE INHIBITORY EFFECT OF GABA ON THE BINDING OF TBPS TO BRAIN SPECIFIC SITES. R.F. Squires and E. Saederup*. Nathan Kline Inst., Orangeburg, NY 10962.

In the presence of Eccles anions GABA and GABA-A receptor agonists inhibit the binding of ^{35}S-TBPS to picrotoxinin sites located on rat brain membranes (Squires et al., Mol. Pharm. 23:326-336,1983). This inhibitory effect of GABA can be reversed by all known GABA antagonists tested including bicuculline, R5135, Ro5-4864, securinine, strychnine, thebaine and theophylline. Screening of about 700 substances revealed that about 67 could partially or fully reverse the inhibitory effects of 1 µM GABA on TBPS binding. Among these was a predominance of antidepressants or their metabolites: adinazolam (EC_{50} = 42 M, max % reversal = 44%), AHR-0800 (39 µM, 35%), amoxapine (1.6 µM,77%), clodazone (23 µM,54%), dibenzepine (54 µM,50%), deanol (34 mM,62%), doxepin (34 µM, 21%), Imafene (100 µM,43%), mianserine (7.1 µM,26%), methylene blue (30 µM,80%), Noxiptiline (24 µM,30%), quinupramine (23 µM,17%), SKF-10810 (4.3 µM,58%), SQ 10777 (100 µM,24%), thiazesim (16 µM,45%), viloxazine (490 µM,62%) and Zimelidine (24 µM,60%). Although amitriptyline, imipramine, nortriptyline and trazodone are not GABA antagonists in this test they have metabolites which are: cis-10-hydroxy-amitriptyline (48 µM,46%), 2-hydroxy-imipramine (87 µM,46%), cis or trans-10-hydroxy-nortriptyline (140 µM,38%), and 1-(m-chloro phenyl)-piperazine (67 µM,60%). Thirty four (34) other substances categorized as "antidepressant" did not reverse GABA in this test. As yet unidentified active metabolites of these "antidepressants" may be formed in vivo. Other substances placed in the following therapeutic categories were also GABA antagonists in this test: antipsychotics, Clomacran (14 µM,80%), clothiapine (3.9 µM,14%), clozapine (16 µM, 28%), loxapine (4.9 µM,66%), mesoridazine (23 µM,60%), metiapine (3.7 µM,14%), and thioproperazine (8.9 µM,16%). Fifty six (56) other "antipsychotics" were inactive. Antihistamines, clobenzepam (39 µM,32%), methapyrilene (290 µM,58%), pyrilamine (200 µM,80%), thiazinamium (130 µM,48%), tripelennamine (260 µM,48%); antiparasitics, amicarbalide (68 µM,120%), emetine (150 µM,65%), isomethamidium (20 µM,120%), norfloxacin (30 µM,120%), pentamidine (97 µM,62%), pipemidic acid (1.0 mM,120%) and quinacrine (27 µM,110%); diverse, caffeine (2.1 mM,56%), colchicine (260 µM,71%), laudanine (25 µM,67%), laudanosine (50 µM,43%), metoclopramide (1.6 mM,56%), pipazethate (53 µM,61%). Supported in part by grant NS 16442 from NINCDS to RFS.

117.8 SR 95103 A NEW SELECTIVE GABA-A RECEPTOR ANTAGONIST. K. BIZIERE(1), P. FELTZ(2), M. HEAULME(1), S. RESTLE(3), R. SCHLICHTER (2),J.P. CHAMBON (1) and C.G. WERMUTH (3). (1) Centre de Recherches Clin Midy, Groupe SANOFI, rue du Prof. J. Blayac, 34082 Montpellier Cedex, France. (2) Institut de Physiologie et de Chimie Biologique, Lab. de Physiologie, CNRS 309, U.L.P. 21, rue Descartes. 67000 Strasbourg, France. (3) Faculté de Pharmacie, Laboratoire de Chimie Organique, 74 Route du Rhin, 67400 Illkirch Graffenstaden, France.

SR 95103 is a new GABA derivative in which the conformational mobility of GABA is relatively preserved.

In-vitro ligand-receptor interaction studies revealed that SR 95103 specifically displaced radiolabelled GABA from its receptor site with an apparent Ki of 2.2 µM. SR 95103 antagonized the GABA-mediated enhancement of (3H) diazepam binding in a dose-dependant manner for concentrations ranging between 1 µM and 100 µM without affecting (3H) diazepam binding per se. These results suggest that SR 95103 could be a competitive antagonist of GABA at the GABA-A receptor sites.

The characteristics of this competitive inhibition on the GABA-A receptor have been confirmed by measurement of GABA-A activated chloride permeabilities as recorded under voltage clamp conditions in spinal ganglia neurons (groups Aδ and C sensory afferents). Moreover, similar data have been obtained for the GABA receptors located on the neurosecretory terminals in the hypophysis.

To determine the specificity of the interactions of SR 95103 with the GABA-A receptor, the effects of 100 µM SR 95103 on several other central receptor sites were examined, particularly on the GABA-B, the strychnine and the glutamate receptors. SR 95103 did not interact with any of these binding sites. Furthermore SR 95103 did not inhibit sodium dependant synaptosomal GABA uptake and did not affect the activity of GABA-transaminase and glutamate decarboxylase.

Because GABA antagonists cause seizures in various animal species, we examined the convulsant effect of SR 95103. An i.p. administration of SR 95103 elicited tonic-clonic seizures with a threshold dose of 100 mg/kg.

In conclusion, biochemical, electrophysiological and behavioral studies demonstrate that SR 95103 is a new specific and competitive antagonist of GABA at the GABA-A receptor sites.

117.9 CORTICOSTEROIDS DIRECTLY MODULATE AGONIST BINDING TO $GABA_A$ RECEPTORS IN THE CNS. M.D. Majewska*, J.C. Bisserbe* and R.L. Eskay* (SPON: M. Goldman). Neurochemistry Section, Laboratory of Clinical Studies, NIAAA, Bethesda, MD. 20205

Corticosteroids are capable of directly changing neuronal excitability through unknown membranous mechanisms. Possible involvement of membrane GABA receptors in this phenomenon was investigated by examining muscimol, the GABA receptor agonist, binding to synaptosomal membranes in vitro. In bilaterally adrenalectomized (ADX) rats, 14 days post surgery, muscimol binding (MB) at 37° was reduced by 30-50% in several brain areas (cortex, hippocampus, thalamus and cerebellum), as compared to sham operated animals. In contrast, MB was unchanged in the pons medulla region and was enhanced in hypothalamus by 30-40%. Corticosterone (CS) had a biphasic effect on MB in those regions of the brain which demonstrated reduced MB following ADX. Nanomolar concentrations of CS enhanced MB toward that found in control animals (non ADX), whereas, micromolar concentrations of CS lowered MB to that observed in ADX animals. The potency of several steroids in enhancing MB following ADX reduction was as follows: CS>pregnenolone>6-α-methylprednisolone>dexamethasone (no effect). Scatchard analysis of MB binding revealed that the effect of ADX on the cortex, hippocampus, thalamus and cerebellum was due to a reduction in the affinities of GABA binding sites, which were restored to control levels or above by corticosteroids. No significant change in B_{max} was observed. Ethanol at concentrations from 1-15 mM produced an effect on MB in most brain regions similar to that observed following ADX. This effect of ethanol was due to a decrease in the affinities of GABA receptors for their ligand. It is possible that the observed alterations in MB in various brain regions in the presence of corticosteroids or ethanol can be explained via some common mechanism of action.

117.10 "PENTOBARBITAL-SHIFT" OF [^{3}H]-BICUCULLINE METHOCHLORIDE BINDING PROPERTIES PERMITS ASSESSMENT OF AGONIST AND ANTAGONIST STATUS OF $GABA_A$ RECEPTOR LIGANDS. E.H.F. Wong., Neuroscience Research Centre, Merck Sharp & Dohme Research Laboratories, Hoddesdon, Herts., EN11 9BU, U.K.

Sodium pentobarbital was found to enhance the affinity of GABA agonists in displacing [^{3}H]-bicuculline methochloride (BMC) binding, whereas the opposite effect was observed for antagonists. [^{3}H]-BMC binding was determined at 23°C for 30 min. in a previously frozen, extensively washed rat cortical membrane preparation suspended in Krebs buffer (pH 7.4, majority of Cl^- being replaced by SCN^-) with results similar to those reported by Olsen and Snowman (J. Neurochem., 41: 1635, 1983). 200 µM (±) Baclofen failed to show any displacement of [^{3}H]-BMC (2nM) binding. Pentobarbital lowered [^{3}H]-BMC binding, causing a dose-dependent drop in binding affinity with no significant change in receptor density (Wong, et al, Eur. J. Pharm., in press, 1984). At 1mM pentobarbital, with 20-30% lowering of base-line [^{3}H]-BMC binding, the potencies of the $GABA_A$ agonists, muscimol, GABA, isoguvacine, 3-aminopropane sulphonic acid, THIP, imidazole-3-acetic acid and taurine in displacing the binding of 2nM [^{3}H]-BMC were increased by 3 to 6 fold. Conversely, the potencies of the antagonists, R 5135, bicuculline methobromide (BMBr) and strychnine were lowered (up to 5 fold for BMBr). Similar, but smaller, shifts were observed at lower concentrations of pentobarbital which caused no drop in base-line [^{3}H]-BMC binding. The effects of pentobarbital were mimicked by secobarbital. The present result is consistent with the concept of coupling between GABA and barbiturate receptors. This offers a means of assessing the agonist/antagonist status of $GABA_A$ receptor ligands.

117.11 DISSOCIATION AND EQUILIBRIUM STUDIES WITH [^{35}S]t-BUTYLBICYCLOPHOSPHOROTHIONATE (TBPT) BINDING INDICATE THAT CONVULSANT AND DEPRESSANT DRUGS BIND TO DIFFERENT BUT COUPLED SITES AT THE GABA RECEPTOR-IONOPHORE COMPLEX. M.K. Ticku and G. Maksay*. Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284.

The kinetics of [^{35}S]TBPT binding were studied in crude membrane preparations of rat cerebral cortex at 25°C. In an EDTA-treated and dialyzed preparation, the dissociation of [^{35}S]TBPT was biphasic, whereas in a freeze-thawed, washed preparation, it was monophasic ($t_{\frac{1}{2}}$ = 72 min). The dissociation patterns in both the preparations were indistinguishable when studies with completely displacing concentrations of convulsants such as TBPT (10^{-6} M), picrotoxinin (10^{-5} M) or pentamethylenetetrazole (6 x 10^{-3} M). These results suggest that all these convulsants bind competitively to the same (convulsant) sites. This is also supported by Scatchard analysis, since all these convulsants inhibited TBPT binding competitively (Ramanjaneyulu and Ticku, J. Neurochem. 42:221, 1984; Eur. J. Pharmacol. 98:337, 1984). Other dissociation studies were carried out with the freeze-thawed preparation in which the linear on and off rates of [^{35}S]TBPT binding suggest a homogenous population of binding sites. Long incubations, required to reach equilibrium of binding (3 - 4 hr) is not associated with proteolytic degradation of the binding activity. GABA resulted in a greatly facilitated polyphasic dissociation of [^{35}S]TBPT ($t_{\frac{1}{2}}$ = 1.3 and 12 min) by binding allosterically to the GABA recognition site of the receptor-ionophore complex. TBPT dissociation was similarly accelerated by the depressants, such as etazolate, (+)etomidate and the barbiturates (pentobarbital and MPPB), proving that these ligands do not bind to the TBPT sites but to other (depressant) sites. This conclusion is also supported by equilibrium studies since pentobarbital and (+)etomidate inhibit [^{35}S]TBPT binding noncompetitively. The convulsant and depressant S(+)- and R(-)-stereoisomers of N-methyl-5-phenyl-propyl-barbituric acid (MPPB) displayed large stereoselectivity in the acceleration of TBPT dissociation. The accelerating potencies of the barbiturates paralleled their depressant/convulsant activities. These results suggest that depressants bind to allosteric (depressant) sites which are coupled to the convulsant sites at the GABA receptor-ionophore complex.

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117.12 IN VIVO FLUNITRAZEPAM BINDING: ACTIONS OF BUSPIRONE AND CLOZAPINE. P.L. Wood, S.E. McPherson and A. Braunwalder. Neuroscience Res., Res. Dept., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901

The benzodiazepine receptor/gamma-aminobutyric acid (GABA) receptor/chloride ionophore complex possesses a number of intricate regulatory interactions in vivo. Assessment of these interactions in vivo is possible with in vivo flunitrazepam binding.

For example, the neuroleptics clozapine and buspirone both dose-dependently enhance ^{3}H-flunitrazepam binding in vivo in the mouse. To assess the role of the different components of the benzodiazepine receptor complex on these actions, we studied pretreatment with the GABA receptor antagonist bicuculline and the chloride channel antagonist picrotoxin.

Bicuculline at doses of 6 mg/kg which blocked muscimol (5 mg/kg) stimulation of ^{3}H-flunitrazepam binding in vivo also blocked the actions of clozapine. In marked contrast, the actions of buspirone were unaltered. Picrotoxin did not reverse the actions of muscimol or the neuroleptics.

These data indicate that clozapine enhances ^{3}H-flunitrazepam binding in vivo via a GABAergic mechanism. Buspirone, however, acts via some other route which apparently does not involve the chloride channel.

OCULOMOTOR SYSTEM I

118.1 INTERACTIONS OF THE VESTIBULO-OCULAR REFLEX WITH THE SACCADIC SYSTEM DURING COMBINED EYE-HEAD GAZE SHIFTS IN THE MONKEY. R.D. Tomlinson* and P.S. Bahra* (SPON: I. Bruce) Playfair Neuroscience Unit, University of Toronto, Toronto, Canada M5T 2S8.

In an attempt to better understand the mode of interaction between the vestibulo-ocular reflex and the saccadic system, rhesus monkeys were trained to make combined eye-head gaze shifts in order to foveate a target light. The animals were able to move their heads through +/-50 deg in the horizontal plane. Both eye and head position were measured using the search coil technique. Vertical and horizontal eye, head, and target position were digitized on-line at 500 Hz and stored on magnetic tape for further analysis. A brushless servo motor was connected to the head shaft so that the head movement could be perturbed in flight during randomly selected saccadic eye-head gaze shifts. The metrics of both the perturbed and unperturbed gaze shifts were analyzed in order to try to discover the mechanisms by which coordinated eye-head gaze shifts are accomplished.

Analysis of over 5000 unperturbed and 1500 perturbed gaze shifts indicate that the interaction between the head and eye movement systems is much more complicated than previously believed. Unperturbed gaze shifts show that the eye decelerates as the head accelerates so that gaze velocity (eye velocity + head velocity) is maintained constant as if the VOR were functioning normally. However, when the head is suddenly perturbed during the saccade, the trajectory of the ongoing eye movement does not change. When the same perturbation is applied while the eye is stationary, the VOR can be shown to be functioning normally and is able to compensate for the perturbation. Since there is no compensation for perturbations delivered during large saccadic gaze shifts, we conclude that the VOR must be turned off during these eye movements. Thus the apparent interaction between the eye and head movements illustrated by the slowing of the eye as the head speeds up during unperturbed gaze shifts, must represent an interaction at the level of the eye and head motor programs rather than functioning of the VOR as previously believed.

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118.2 INITIAL ORBITAL POSITION AFFECTS THE TRAJECTORIES OF LARGE SACCADES EVOKED BY ELECTRICAL STIMULATION OF THE MONKEY SUPERIOR COLLICULUS M.A. Segraves and M.E. Goldberg National Eye Institute, Bethesda, MD 20205; and Dept. of Neurology, Georgetown Univ., Washington, DC 20007.

Initial orbital position does not affect the trajectories of moderately sized saccades evoked from the superior colliculus (SC) of the monkey (D.A. Robinson,1972), although it has been reported to do so for saccades evoked from caudal SC in the cat (Guitton et al., 1980). We studied the effect of orbital position on saccades evoked by microstimulation of the SC in head restrained trained Rhesus monkeys implanted with scleral search coils. The monkeys were required to fixate various points on a tangent screen while the receptive and movement fields of neurons were measured and then saccades evoked by suprathreshold microstimulation through the recording electrode.

Small saccades (<10°) evoked from rostral SC were generally unaffected by changes in orbital position. At more caudal points with larger and more eccentric receptive fields the direction and amplitude of stimulation-evoked saccades became increasingly dependent upon orbital position. As initial orbital position moved away from the movement field the saccades grew longer. Thus an SC site giving an 18° leftward saccade when the monkey fixated the orbital center gave a 36° saccade when the monkey fixated 27° to the right. In addition, the direction of the saccade rotated; at this same site the saccades evoked from 27° above the center of gaze were roughly horizontal, whereas saccades evoked from 27° below the center of gaze had a 45° upward direction. We frequently have seen reversals of either the horizontal or vertical component, but only rarely both components at a single SC site. The saccades were never directed to an absolute orbital position goal. Instead, the array of termination points was compressed and rotated in a consistent manner relative to the array of initiation points.

The receptive fields and movement fields of the cells did not share this dependence upon orbital position, but instead were consistent with the largest saccades evoked from the site. Thus for some orbital positions, microstimulation evoked saccades that would not by themselves foveate a stimulus in the receptive fields of the neurons at the site. These data imply that the saccade signal from the SC is modified elsewhere in the brain to compensate for the initial orbital position of the eye.

118.3 OCULAR FOLLOWING RESPONSES OF MONKEY: POST-SACCADIC ENHANCEMENT AND DEPENDENCE ON SPATIO-TEMPORAL CHARACTERISTICS OF THE VISUAL STIMULUS. K. Kawano and F.A Miles. Lab. of Sensorimotor Res., National Eye Institute, Bethesda, MD 20205.

Experiments were concerned with ocular tracking responses elicited by brief (100ms), unexpected, ramp movements of the visual scene. Monkeys faced a tangent screen subtending 85^{o}x85^{o} and their eye movements were recorded with the magnetic search coil. A random dot pattern was projected onto the screen via an X-Y mirror galvanometer system. Though ramps were randomized for time of onset, direction and speed (10-100^{o}/s), and tracking was never reinforced, response latencies (L) were invariably short, e.g., mean L to 40^{o}/s ramps was 51.5 $\pm$ 3.1(SD)ms (n=8 animals). However, response amplitudes were strongly influenced by a prior saccade: initial eye acceleration ($\ddot{e}$) was greatest when ramps began immediately after (spontaneous) saccades and declined roughly exponentially as the post-saccadic delay interval was increased (mean time constant, 73ms; mean asymptote, 30%; n=5). Subdividing responses according to the magnitude and/or direction of the antecedent saccade failed to reveal any consistent asymmetries: saccade parameters were irrelevant. We assume that visual tracking helps to stabilize the eyes with respect to the surroundings. We suggest that the transient, post-saccadic enhancement of such tracking serves in particular to reduce the adverse effects of errors in the pulse-step matching of saccades.

Sinusoidal grating patterns were used in place of the random dots in 3 monkeys: contrast, C, was fixed (0.5), while varying spatial frequency, F_S (0.04-$1c/^{o}$), and ramp velocity, V (5-400^{o}/s). Clear short-latency tracking was seen only with F_S<$1c/^{o}$ and L was solely dependent upon temporal frequency, $^{S}F_T$ ($=F_S.V$): tracking was triggered by local contrast changes rather than overall movement of the pattern. For F_T<10Hz, all data could be fitted (r=0.93) by the equation, $L(\text{in ms})=78F_T^{-0.185}$; for F_T>10Hz, L's levelled off at minima of 48-50ms, until responses began to fail with F_T>50Hz. Initial $\ddot{e}$, however, was a complex function of F_S and F_T, and varied from one animal to another.

Reductions in C caused minor decrements in initial $\ddot{e}$ and moderate increments in L, e.g., for $0.27c/^{o}$ gratings moving at 60^{o}/s, reducing C from 0.5 to 0.01 reduced initial $\ddot{e}$ by only 10% while increasing L by nearly 20ms and all data could be fitted (r=0.94) by the equation $L(\text{in ms})=47C^{-0.077}$. The selectivity for low F_S and relative insensitivity to C allow the system to tolerate considerable blur--a most useful property for a visual stabilization mechanism.

118.4 SUPPRESSION OF OKN WITHOUT RETINAL ERROR SIGNALS. J. Pola and H. J. Wyatt. Institute for Vision Research, SUNY State College of Optometry, New York, NY 10010

When a person passively regards a moving textured field, optokinetic nystagmus (OKN) occurs, but if the person looks at a stationary target presented together with the moving field, the OKN is suppressed. To investigate what type of mechanisms contribute to this OKN suppression, we asked subjects to observe a retinally stablized target presented against a large field of spots moving in horizontal sinusoidal motion. The target was stabilized to eliminate "retinal slip" cues which might be used to hold the eye against the field's optokinetic influence.

In one experiment, subjects were asked to actively "look" at the target, which was stabilized at the fovea. Sinusoidal field motion was 0.5 to 1.0 Hz, 30^{o} pk-pk. In spite of the fact that there was no retinal slip, OKN suppression was substantial. In fact, all subjects showed small amplitude slow eye movements in counterphase to the field motion. Preliminary results show that this suppression is found not only at the above midrange frequencies, but also at higher frequencies (e.g. 2 Hz) and at lower frequencies (0.125 Hz and below).

In another experiment, we investigated whether "looking" at the target was necessary for the OKN suppression. Again, the target was stabilized at the retina, but the target was presented at several locations eccentric to the fovea (7.5^{o} to 20^{o}) both horizontally and vertically. The subjects did not try to look at the target, but remained visually passive. We found substantial suppression of OKN with the amount of suppression decreasing with target eccentricity.

These results suggest that at the frequencies employed, OKN suppression does not wholly depend on "retinal slip" cues, or even on specific intent. The fact that the subjects did not have to look at or deliberately attend to the target for the suppression to occur suggests that cues such as relative target-background motion may play a role in suppression.

118.5 CERTAIN COMMON AND DIFFERING FEATURES IN THE SUBCORTICAL EFFERENT PROJECTIONS OF THE FRONTAL AND POSTERIOR PARIETAL EYE FIELDS IN THE MONKEY. G.R. Leichnetz. Department of Anatomy, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

Brodmann's areas 8 and 7, though widely separated in the cerebral cortex, share certain common physiological properties and have been studied to define the framework of connectivity within which each cortical eye field operates in order to shed light on their differing roles in eye movement mechanisms. We have made solid polyacrylamide horseradish peroxidase (HRP) gel implants into both cortical areas, processed the tissue according to the tetramethylbenzidine protocol of Mesulam (1978), and studied the afferent and efferent connections of the two regions. While the areas have a lengthy list of common corticocortical and subcortical inputs, we observed some very notable differences in their subcortical efferent projections.

Anterogradely labelled corticofugal projections from both the frontal eye field (FEF, area 8) and posterior parietal eye field (PPEF, area 7, PG of Von Bonin and Bailey) were followed to the medial pulvinar, stratum intermedium of the superior colliculus and pretectal area. While both regions received inputs from the intralaminar complex, the reciprocal efferent corticothalamic connections from the FEF were clearly heavier and terminated in paralamellar MD, the intralaminar complex (PC, CL, Pf) and VAmc. The PPEF corticothalamic projections ended primarily in LP, but also in LD, in addition to the mutual medial pulvinar projections previously mentioned. The FEF projections to paraoculomotor cell groups like the rostral interstitial nucleus of the MLF, the nucleus of Darkschewitsch, medial accessory nucleus of Bechterew,and dorsomedial parvocellular red nucleus (i.e. both premotor and preolivocerebellar nuclei) were lacking in PPEF cases. The FEF also projected to paramedian basilar and tegmental pons (including the paramedian pontine reticular formation and nucleus reticularis tegmenti pontis), whereas PPEF projected to dorsolateral and lateral basilar pons. FEF projections were even followed caudally to the nucleus prepositus hypoglossi, regarded as another preoculomotor structure.

These contrasting results will be discussed in terms of their possible explanation for the similarities and differences in the functions of the two cortical eye fields.
This study was supported by NSF Grant BNS 81-13387.

118.6 PHYSIOLOGY AND MORPHOLOGY OF AXOTOMIZED CAT TROCHLEAR MOTONEURONES. E.H. Murphy, R.F. Spencer, and R. Baker. Dept. Anat., Med. Coll. Pennsylvania, Philadelphia, PA 19129; Dept. Anat., Med. Coll. Virginia, Richmond, VA 23298; Dept. Physiol. Biophys., New York Univ. Med. Ctr., New York, NY 10016.

The nature and severity of the response of motoneurones (MNs) to axotomy varies both between species and between different nuclei within the same species. The most profound effects of central axotomy of the cat VIth nerve are the loss of ipsilateral vestibular inhibition and the withdrawal of synaptic endings from the somatic surface of abducens (Abd) MNs. By comparison, the present study has examined the short- and long-term effects of central axotomy of the IVth nerve on the physiology and morphology of cat trochlear (Troc) MNs and that of the superior oblique (SO) muscle.

Up to 14 days post-axotomy, Troc MNs could be antidromically activated by stimulation of the central stump of the transected IVth nerve. Both ipsilateral inhibitory (Vi) and contralateral excitatory (Vc) disynaptic profiles were elicited by stimulation of the vestibular nerve. In contrast to axotomized Abd MNs, neither the amplitude nor the time course of Vi IPSPs or Vc EPSPs in axotomized Troc MNs were qualitatively different from normal. The mode and pattern of synaptic connectivity of Vi and Vc axons, stained by intracellular injection of HRP, also appeared normal, despite ultrastructural evidence of widespread filamentous degeneration of dendrites in the neuropil of the Troc nucleus. The integrity and disposition of somatic cytoplasmic organelles and of axosomatic synaptic endings were typical. Myelinated axons distal to the transection displayed the usual features of Wallerian degeneration, and all fiber types of the SO muscle were atrophied.

Long-term survival periods resulted in a dramatic decrease in the number of neurones in the Troc nucleus contralateral to the axotomy. A small contingent of neurones remained in the dorsolateral portion of the nucleus. A few neurones, some of which were labelled by retrograde HRP from the normal ipsilateral SO muscle, also occupied the central region of the nucleus. Both Vi and Vc axons arborized within the dorsolateral and central regions of the Troc nucleus and established synaptic contacts predominantly with dendritic profiles. The SO muscle, however, apparently is reinnervated by an anomolous nerve of as yet unknown source with regeneration of all muscle fiber types.
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118.7 BRAIN STEM PROJECTIONS TO OCULOMOTOR PAUSE AREA. J. Ito*, C.H. Markham and I.S. Curthoys* Reed Neurological Research Center, UCLA Sch. of Med., Los Angeles, CA 90024.

The origins of the afferent connections to the eye movement-related pause neurons (PNs) in cats were examined using retrograde transport of horseradish peroxidase with wheat germ agglutinin (WGA-HRP). The major input sources to the PN area were nucleus prepositus hypoglossi, medial vestibular nucleus, gigantocellular reticular nucleus, parvocellular reticular nucleus, nucleus reticularis tegmentis pontis, nucleus reticularis pontis candalis, nucleus reticularis pontis oralis, and superior colliculus (SC). Less heavily labelled sources included the lateral and descending vestibular nuclei, the locus coeruleus and the dorsal raphe.

PNs are inhibitory and modulate quick eye movements by disinhibition. PNs themselves may be regulated by inhibiting pathways from the labyrinths and SC.

118.8 ANATOMICAL AND PHYSIOLOGICAL CHARACTERISTICS OF SACCADIC BURST NEURONS PROJECTING TO ABDUCENS NUCLEUS IN THE SQUIRREL MONKEY. A.Strassman*, S.M.Highstein, and R.A.McCrea Albert Einstein Col. of Med., N.Y., NY 10461, and Washington Univ. Sch. of Med., St. Louis, MO 63110.

Physiological and anatomical studies in the cat demonstrate separate populations of excitatory and inhibitory saccadic burst neurons (EBNs and IBNs) in the pontine and medullary reticular formation which project monosynaptically to abducens neurons. Physiology and extracellular tracer studies suggest a similar premotor organization in the monkey. In the present study saccadic burst neurons were studied in the alert squirrel monkey using intraaxonal recording and labelling with horseradish peroxidase (HRP). Two anatomically distinct populations of burst neurons with projections to abducens were found, analogous to EBNs and IBNs in the cat. The squirrel monkey EBNs(n=21) and IBNs(n=18) fire maximally during ipsilateral saccades, and are silent during periods of fixation. Most of the neurons also fire a lower-frequency, shorter-latency burst during vertical and contralateral saccades. IBNs are located in the medial reticular formation ventral and caudal to abducens, and have strictly contralateral axonal projections. EBNs are located in the medial reticular formation rostral to abducens, and have strictly ipsilateral projections. In addition to their terminations within abducens, both the EBNs and the IBNs have extensive collateral projections to the nucleus prepositus hypoglossi, the vestibular nuclei, and regions of the medullary and caudal pontine reticular formation caudal and rostral to abducens. EBNs have a heavier projection to prepositus than do IBNs, whereas IBNs have a more extensive projection to the vestibular nuclei. EBN terminations in the vestibular nuclei are confined to the medial vestibular nucleus, and are heavily concentrated in the medial part of the nucleus. Vestibular projections of IBNs are distributed throughout the medio-lateral extent of the medial vestibular nucleus, and also include regions of the superior, lateral, and descending vestibular nuclei. These findings demonstrate a premotor saccadic organization similar to that in the cat, and suggest that burst neurons, in addition to generating the saccadic activity in motoneurons, also contribute to the oculomotor activity in other classes of premotor neurons.

118.9 DISCHARGE CHARACTERISTICS OF INTERNUCLEAR NEURONS IN THE MONKEY. A. F. Fuchs, C. A. Scudder, and C. R. S. Kaneko. Regional Primate Center and Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195.

Internuclear neurons (INT) are neurons in the abducens nucleus that project to the contralateral oculomotor nucleus where they excite medial rectus motoneurons. In the cat, both abducens motoneurons (MN) and INT have qualitatively similar discharge patterns that result from apparently identical afferent inputs. Consequently, the INT serve to relay the same signal that impinges on abducens MN of one eye to medial rectus motoneurons of the other eye to facilitate conjugate horizontal gaze. However, in the cat, the INT have higher slopes in their relation between discharge rate and eye position and also have a higher velocity sensitivity than abducens MN. It has been suggested that their higher position and velocity sensitivities, allow INTs to compensate for the synaptic and conduction delays that they introduce.

To see if there are similar differences in the monkey, we compared the discharge characteristics of abducens MNs, identified by spike triggered averaging of lateral rectus EMG activity, with those of INT, identified by antidromic-activation and collision block following stimulation in the medial rectus subdivision of the oculomotor nucleus. Our preliminary results indicate any differences in INT and MN discharge are minor. The slopes of the discharge rate - eye position relation for 18 INT and 17 MNs were not significantly different (4.0 $\pm$ 0.85 vs. 5.6 $\pm$ 2.0 spikes/sec/deg, respectively). INT had higher firing rates at the primary position of gaze (109 $\pm$ 43 vs. 43 $\pm$ 36 spikes/sec) and they had lower thresholds (-17 vs. -7.7 deg) than MNs. During sinusoidal smooth pursuit at 0.5 Hz, the velocity gain (i.e., peak discharge/peak eye velocity) was again similar (1.51 $\pm$ 1.0 vs. 1.03 $\pm$ .42 spikes/sec/deg/sec) as were the phase leads (22.9 $\pm$ 3.1 for INT; 22.5 $\pm$ 4.4 deg for MN). Finally, comparisons of burst characteristics in a few INT and MNs (N=7 ea.) during 10 deg and 20 deg saccades showed no difference in peak discharge (20 deg: $p > .5$, 10 deg: $p > .1$).

Our preliminary data suggest that abducens MNs and INTs in the monkey have very similar disharge characteristics. In particular, there is no significant difference between either their velocity or position sensitivities. We conclude, therefore, that it is not necessary for MNs and INTs to have different discharge patterns to compensate for the small delays in the INT projection to the oculomotor nucleus. Other factors, such as stronger synaptic efficacy of this projection or the stronger medial rectus muscle could easily make up these differences.

118.10 ACTIVITY OF PURSUIT NEURONS AND EYE VELOCITY NEURONS IN PRIMATE BRAIN STEM DURING FOVEAL PURSUIT VERSUS OKN. R. Eckmiller and E. Bauswein. Division of Biocybernetics, University of Düsseldorf, D-4000 Düsseldorf, FRG.

Is the pre-motor eye velocity signal for the neural control of foveal pursuit versus the slow phase of OKN being carried to the final common pathway by only one or by several neural classes? This open question led to the following set of experiments in which single unit activity was recorded in the vicinity of the abducens nuclei in monkeys (Macaca fascicularis). Animals had been trained to pursue a visual target(8 min of arc) during three standard paradigms: a)Smooth Pursuit, b)VOR-light, and c)VOR-suppression; all at 10 deg movement amplitude and frequencies between 0.4 and 1.1 Hz. For elicitation of OKN a motor-driven endless loop of film material with vertical black stripes was combined with a slide projector and projected onto the screen as a square of about 40 deg by 40 deg. Recording sites were histologically verified by means of small electrolytic lesions.

Two main classes of pursuit-related neurons were found which seem to be important for the task of assuring foveal pursuit under the different paradigms: Pursuit Neurons(located ventral to and occasionally also at the dorsal border of the abducens nuclei) are modulated in phase with foveal pursuit velocity ipsilateral to the recording site only under a). Under b) impulse rate(IR) modulation is considerably reduced or even absent. Under c) max.IR typically exceeds the value under a) but occurs with 20 to 40 deg phase lead re: ipsilateral head velocity. The dynamic properties of Pursuit Neurons have clear similarities to those of 'Gaze Velocity' neurons in the flocculus(Miles et al.,J.Neurophysiol.,43:1437, 1980). During the slow phase of OKN the IR modulation of Pursuit Neurons is often drastically different from that during Smooth Pursuit. Eye Velocity Neurons(located at the dorsal border of the abducens nuclei) are modulated in phase with eye velocity under a) and b). No IR-modulation is present under c). No other neural class with similar dynamic properties has yet been described in the Cerebellum or Brain Stem.

These findings are compatible with the hypothesis that during Foveal Pursuit the Eye Velocity Neurons summate the eye velocity signals from both Pursuit Neurons and Vestibular Neurons and feed into the neural integration stage. Details of changes in IR-modulation occurring with a change from Foveal Pursuit to OKN will be discussed.

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118.11 VERTICAL OPTOKINETIC AFTERNYSTAGMUS IN THE ALERT CAT. J.H. Anderson, M. LeTaillanter*, B. Blakely*. Depts. of Otol. and Physiol., Univ. of Minn., Minneapolis, MN 55455.

Some of the dynamic characteristics of vestibular and optokinetic reflexes have been ascribed to a velocity storage mechanism which helps to maintain an eye velocity signal. The storage has been extensively characterized and modelled for horizontal eye movements (cf., Raphan et al, Exp Br Res, 35:229, 1979). Evidence also indicates that there is velocity storage for vertical eye movements which is not symmetrical, storage is greater for upward than for downward eye movements and is dependent upon the gravity bias acting on the otolith organs (Matsuo and Cohen, Exp Br Res, 53:197, 1984). The present work sought to quantitate the velocity storage for vertical eye movements and to develop a model which could account for the asymmetry and different time constants and describe the time domain over which the storage is acting.

Six cats were implanted with scleral search coils. For recording each animal was positioned on its side and placed inside an optokinetic drum. Constant velocity drum rotations from 2 to 40 deg/s in both directions were used. The upward optokinetic response (OKN) had a steady-state gain (slow phase eye vel/drum vel) which ranged from 1 at 2 deg/s to .4-.6 at 40 deg/s. The downward gains ranged from 1.0 to 0.1-0.3. After the light was turned off,during the optokinetic afternystagmus (OKAN), the eye velocity decayed to zero (OKAN I) and sometimes reversed direction (OKAN II). An OKAN II was frequently present after a downward OKN and the peak of the slow phase eye velocity during the OKAN II was about 50 to 70% of the velocity during the preceding OKN. Occasionally there was an OKAN II after an upward OKN with the higher drum velocities. Immediately after the lights were turned off, there was frequently a rapid decrease in eye velocity, followed by a more gradual decay. The initial, fast component was greatest for stimulus velocities at 40 deg/s, about 3-10 deg/s for upward OKN and2-3 deg/s for downward OKN. The subsequent slower decay component was considerably shorter after a downward OKN than after an upward OKN.

These results are in agreement with previous work in showing that there is velocity storage for vertical eye movements which is much greater for upward compared to downward movements. In addition the frequent presence of an OKAN II indicates that it is behaving as a second arder system. One realization of this might be the coupling of two leaky integrators, each representing part of the velocity storage for vertical eye movements.(Supported by NIH, ROl-NS-16567.)

118.12 EYE MOVEMENTS IN CATS REARED WITH CYCLODEVIATION. C.K. Peck, School of Optometry, University of Missouri-St. Louis, St. Louis, MO 63121 and R. Baker, Department of Physiology and Biophysics, NYU Medical Center, New York, NY 10016

Large cyclodeviations can be produced surgically in young visually-inexperienced kittens without impairing retinal function. When reared in visually enriched environments, such kittens generally show competent visual behavior, including visual pattern discriminations. However, quantitative visual testing has revealed significant losses in such functions as acuity and in the extent of the visual field of the deviated eye.

Although there are also some observations of oculomotor abnormalities in kittens reared with cyclodeviations, there have been few quantitative studies of these movements. We have analyzed both saccadic and optokinetic (OKN) movements using the magnetic search coil technique to record the positions of both eyes. The results reveal a surprisingly good degree of recovery of saccadic and quick-phase amplitude, including normal amplitude-velocity relationships thereby suggesting that eye muscle reattachment is functionally restored with mechanical efficiency near that normally observed.

More significantly, during binocular viewing in cats with monocular intorsions, both the normal and the rotated eye showed a strongly asymmetrical OKN with gain dependent on drum direction. Cats with monocular extorsion showed only a slight OKN asymmetry but in the opposite direction. When the rotated eye was tested alone, the pattern of asymmetry was the same as during binocular viewing. For the "normal" (unoperated) eye, OKN abnormalities were seen only during binocular viewing but not during monocular viewing. The asymmetrical reduction in gain is likely due to the direction of stimuli moving on the retina of the rotated eye and its interaction with temporal movements of stimuli in the unoperated eye. These results can be explained by some recent developments in understanding of the organization of the accessory optic system in mediating horizontal and vertical OKN. However, other neural mechanisms can also be envisioned for the oculomotor abnormalities.

SUBCORTICAL AUDITORY PATHWAY I

119.1 REPRESENTATION OF TONES IN NOISE IN THE DISCHARGE PATTERNS OF AUDITORY-NERVE FIBERS OF CATS. J.A. Costalupes and L.I. Hellstrom*. Dept. of Biomed. Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

We previously reported that the dynamic region of responsiveness of auditory-nerve fibers to best frequency (BF) tones shifts to higher tone intensities in the presence of broadband noise (Costalupes, et. al. J. Neurophysiol., 51: 1326, 1984). This shift extends the overall operating range of auditory-nerve fibers to BF tones in noise. In this report, we describe the representation of tones in noise in the discharge patterns of the array of auditory-nerve fibers in the cat.

We recorded responses of individual auditory-nerve fibers in anesthetized cats to a limited stimulus set consisting of 1 kHz or 8 kHz tone bursts in continuous noise. We selected signal-to-noise ratios (S/N's) that were near the mean behavioral threshold for detecting tones in noise by cats reported by Costalupes (J. Acoust. Soc. Am., 74: 1195, 1983). S/N's varying from 5 to 16 dB above mean behavioral detection thresholds and noise spectrum levels from -9 to 46 dB SPL were used.

Analyses of rate changes evoked by the test tone (computed as the discharge rate during the tone burst in noise minus the noise-evoked rate) reveal that fibers with BF's near the test tone frequency responded differentially to the test tone over a wide range of overall levels and S/N's. At each S/N tested, low and medium spontaneous rate fibers (SR $\leq$ 19 spikes/s) exhibited a larger tone-evoked rate change than high spontaneous rate fibers (SR > 19 spikes/s). At S/N's within 8 dB of mean behavioral detection threshold, differential rate changes to the test tone were apparently limited to the low and medium spontaneous rate group, especially at moderate to high overall sound levels. In addition, responses of fibers with BF's just above and below the test tone frequency were suppressed by the test tone, especially at high overall sound levels. These results support a coding scheme of tones in noise based on average discharge rates of auditory-nerve fibers.

Analyses of responses phase-locked to the 1 kHz test tone reveal strong synchrony at S/N's near behavioral detection threshold over the range of overall sound levels tested. Synchronized rate for 1 kHz tones in noise varies with S/N, is independent of overall level for fibers with BF's in the region of 1 kHz, and spreads to fibers with successively higher frequencies if overall level is raised.
(Work supported by grants and fellowships from NIH.)

119.2 EFFECTS OF COCB STIMULATION ON AUDITORY-NERVE FIBER RATE FUNCTIONS FOR TONES IN NOISE. Raimond L. Winslow* and Murray B. Sachs, Johns Hopkins Univ. School of Medicine, Dept. of Biomedical Engineering, Baltimore, Maryland 21205.

Wideband masking noise shifts the dynamic range of auditory-nerve fiber rate response for best frequency (BF) tones to higher sound pressure levels. This shift in dynamic range is accompanied by compression of the range of discharge rates available for coding intensity changes. This compression results from a noise induced increase in rate at low tone levels and a decrease in saturation rate caused by noise induced adaptation. Electrical stimulation of the Cross Olivocochlear Bundle (COCB) reduces tone evoked responses and produces shifts in dynamic range for tones in quiet. The data presented here show that stimulation of the COCB can compensate for both of the compressive effects of noise by reducing the noise driven response of auditory-nerve fibers.

Average discharge rate was measured as a function of stimulus level for BF tone bursts in quiet both with and without electrical stimulation of the COCB. These rate-level measurements were then repeated in the presence of wideband masking noise at a number of spectral levels.

For all units studied, rate to the background noise was reduced by COCB stimulation. For most units studied with moderate levels of noise, saturation rates also increased with COCB stimulation. Thus, for all units studied, COCB stimulation increased the range of rate changes available to code intensity changes in low and moderate noise levels. The range of tone levels over which these rate changes in noise occur can be shifted by COCB stimulation. At low noise levels, where noise induced dynamic range shifts were small, the additional shifts due to COCB stimulation could be large. As noise level increased, additional shift of dynamic range due to COCB stimulation decreased monotonically. In many cases, there was no additional dynamic range shift due to efferent stimulation. Thus, at moderate noise levels, range of rate change in noise could be restored by COCB stimulation without shifts of the operating point of auditory-nerve fibers to higher sound pressure levels. In many instances, tone evoked rate changes of high spontaneous fibers were almost completely eliminated by background noise. In almost all of these cases there is considerable restoration of range of available rate change; in some of these cases, the range of rate change was restored to that observed in quiet.

119.3 GAD-LIKE IMMUNOREACTIVITY IN THE VENTRAL COCHLEAR NUCLEUS. J.C. Adams. Dept. of Otolaryngology, Med. Univ. of S.C., Charleston, SC 29425 and E. Mugnaini. Dept. of Biobehavioral Science, Univ. of Conn., Storrs, CT 06268

Immunostaining in the ventral cochlear nucleus (VCN) using an antibody to glutamate decarboxylase (GAD) indicates that GABA plays a major role in neural communication in this nucleus. Virtually all cells receive immunoreactive terminals. Identification of characteristic staining patterns of various cells holds promise as a means of identifying functional cell classes and may be useful in cross species comparisons. Preliminary results indicate that GAD immunoreactivity in the human VCN is similar to that in the cat except for the granule cell domain. In the cat the granule cell layer contains extremely dense fine terminals. Spherical cells and globular cells share a very similar pattern of terminals that is characterized by a dense mixture of both coarse and fine processes covering the somata and proximal dendrites. Octopus cells receive a less dense but still substantial complement of somatic and dendritic terminals. These terminals are more uniform in size than those on spherical cells and are on the average smaller than the latter. Stellate cells and small cells have few, if any, terminals on cell bodies or on proximal dendrites. There are at least two classes of VCN cells whose somata also show GAD-like immunoreactivity: large cells of the same size and distribution as those that project to the contralateral cochlear nucleus; and smaller, multipolar cells resembling certain categories of local circuit and projection neurons. These two cell classes most likely provide some of the many GAD immunoreactive terminals in the VCN. Other GAD immunoreactive cells that may give rise to the VCN terminals are located in the dorsal cochlear nucleus, in the periolivary regions around the lateral superior olive, and in the ventral nucleus of the trapezoid body (VTB). The latter nucleus contains cells that are immunoreactive to enkephalins and appear to be the same as those that are GAD immunoreactive. Enkephalin positive terminals in the VCN are largely confined to the granule cell domain and we suggest that GAD and enkephalins may co-exist in terminals originating in the VTB and terminating in the granule cell domain. Interestingly, the human appears to lack the small cells that characterize the VTB in the cat. This absence may be related to the previously reported absence of a granule cell domain in the VCN of the human.

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119.4 **CLASSIFICATION OF PSTH RESPONSE PATTERNS IN BARN OWL COCHLEAR NUCLEUS: BEHAVIORAL RELEVANCE AND IMPLICATIONS FOR COMPARATIVE AUDITORY PHYSIOLOGY.** W. E. Sullivan (SPON: M. Konishi) Div. of Biology 216-76, Caltech, Pasadena, California 91125.

The two cochlear nuclei of the barn owl have different functional roles in sound localization. The owl uses differences in stimulus phase at the two ears to localize sounds along the horizon. The magnocellular cochlear nucleus contains neurons which preserve monaural phase information by responding to sinusoidal signals in a phase-locked fashion. Vertical asymmetries in the openings of the external ears enable interaural intensity differences to be related to positions along the vertical axis. The other cochlear nucleus, n. angularis, is involved in the coding of monaural intensity information (Sullivan and Konishi, J. Neurosci. in press; Takahashi et al. J. Neurosci. in press).

Comparison of post-stimulus time histograms (PSTHs) for units in the two nuclei shows that all magnocellular units have "primary-like" patterns, whereas the vast majority of angular units are classified as "transient choppers". This distinction between PSTH shape in conjunction with the previously defined functional differences between the nuclei suggest that a particular PSTH type is related to a particular acoustic function; or in other words to the coding of a particular behaviorally-relevant acoustic variable.

The classification of response types in the barn owl's cochlear nuclei allows for a better comparison with divisions or unit types in the mammalian cochlear nuclear complex. Units in the magnocellular nucleus are clearly analogous to the bushy or spherical cells of the anterior anteroventral cochlear nucleus. The majority of angularis units appear to be analogous to the stellate cell population of the anteroventral and posteroventral cochlear nuclei (Rhode et al. J. Comp. Neurol. 213:448-463 1983). Some onset units were seen in angularis and these are also found in the ventral cochlear nucleus of mammals. No units sharing the properties of mammalian dorsal cochlear nucleus units were isolated. This suggests that the separation of magnocellular and angular cochlear nuclei in barn owls is analogous to different cell types within the ventral cochlear nucleus and not to the segregation between the ventral and dorsal divisions of the mammalian cochlear nuclear complex.

119.5 PROJECTIONS OF THE PRINCIPAL CELLS IN THE MEDIAL NUCLEUS OF THE TRAPEZOID BODY OF THE CAT. K.M. Spangler*, W.B. Warr, C.K. Henkel. Boys Town National Institute for Communication Disorders in Children, Omaha, NE 68131 and Bowman Gray School of Medicine, Winston-Salem, NC 27103.

The principal cells (PC) of the medial nucleus of the trapezoid body (MNTB) give rise to a projection to the lateral superior olivary nucleus (LSO), providing the LSO with contralateral auditory input but it is unclear what role, if any, the PCs play in MNTB's projection to the ventral nucleus of the lateral nucleus (VNLL). Moreover, the topography of the PC projections, the course and diameter of their axons and their terminal synaptic relationships are known only in part. The projections of the MNTB, and in particular the PCs, were studied using anterograde and retrograde axonal tracing methods. Following injections of ^{3}H leucine into the MNTB, labeled axons reached the LSO by passing dorsal to, ventral to, or through the medial superior olivary nucleus. Autoradiographic experiments revealed that the medial and lateral portions of the MNTB project to corresponding parts of the LSO, as confirmed by retrograde HRP marking. Autoradiography was performed on plastic sections in one animal in order to determine the diameter of labeled fibers and to obtain good resolution of terminal synaptic relationships. Labeled fibers had a modal diameter of between 5 and 6 um. In the LSO, discrete aggregations of silver grains were found around fusiform cell bodies and their proximal dendrites. Collaterals of axons passing to the LSO terminated in the dorsomedial and ventromedial periolivary nuclei. Fibers projecting to the ventral nucleus of the lateral lemniscus ascended in the lateral portion of the lateral lemniscus, and terminated in a discontinuous "ladder rung" fashion. The results of HRP injections into the LSO and the nuclei of the lateral lemniscus showed that the PC was responsible for both of these projections, indicating the presence of an ascending collateral of the PC axon. Control HRP injections demonstrated that other projections of the MNTB arose from minor cell populations in that nucleus. The findings suggest a wider role for the MNTB in the ascending auditory system than has been previously supposed. Supported by NSF Grant BNS-8209987 to W.B Warr, NIH Postdoctoral Fellowship to K.M. Spangler, and NSF Grant BNS-7918832 to C.K. Henkel.

119.6 FINE STRUCTURAL FEATURES OF MEDIAL OLIVOCOCHLEAR NEURONS IN THE RAT. J. S. White, Dept. of Anatomy, Creighton Univ. Sch. of Med., Omaha, NE 68178.

Retrograde HRP studies have shown that one of the two groups of olivocochlear (OC) neurons that project to the cochlea consists of large multipolar neurons situated bilaterally in the medial part of the superior olivary complex. In the cat, these neurons have been shown to project to the region of outer hair cells and to the cochlear nucleus, yet little is known about the types or sources of their synaptic input. In the present study, the fine structure and synaptic organization of medial OC neurons in the rostral ventral nucleus of the trapezoid body was investigated in the albino rat following cochlear injections of HRP. Labeled OC neurons were detected in vibratome sections utilizing either DAB or TMB as chromogens, then osmicated, embedded in plastic, and resectioned for electron microscopy.

The medial OC neurons contained large, somewhat eccentric nuclei with highly indented nuclear membranes, an extensive Golgi apparatus, and abundant rough endoplasmic reticulum. The rough ER was usually distributed in numerous patches composed of a small number of cisternae surrounded by dense clusters of polysomes. Several types of bouton-like profiles were observed in synapse with medial OC neurons, in particular with long tapering dendrites, but infrequently with the cell body. Boutons containing large round or small round vesicles had a widespread distribution over the dendrites and were occasionally observed in synapse with the perikaryon. Other boutons containing mainly flat or elongate vesicles synapsed predominantly with proximal parts of dendrites. One intriguing finding was the observation of large myelinated axons giving rise to large presynaptic elements containing numerous neurofilaments and clusters of round vesicles scattered within the terminal and adjacent to punctate synaptic specializations. These terminals bear some resemblance to large presynaptic elements observed in the cochlear nucleus and in the medial nucleus of the trapezoid body. In view of evidence obtained in the cat, that lateral and medial OC neurons differentially innervate the inner and outer hair cell regions, respectively, and most likely comprise functionally separate systems, it is not surprising to find that the two kinds of OC neurons receive different types of synaptic input.

119.7 **BINAURAL AND LATERAL INHIBITION REVEALED IN THE INFERIOR COLLICULUS OF THE CAT BY THE 2-DEOXYGLUCOSE TECHNIQUE.** W.R. WEBSTER, R. MARTIN*, M. BROWN* AND J. SERVIERE, NEUROPSYCHOLOGY LABORATORY, PSYCHOLOGY DEPT. MONASH UNIVERSITY, CLAYTON, AUSTRALIA 3168.

The study of the auditory pathway using 2-deoxyglucose (2-DG) has revealed bands of labelling in the central nucleus of the inferior colliculus (ICC) after stimulation with pure tones. Single unit recordings have demonstrated that each band represents an iso-frequency contour. However, inhibitory effects have not been observed with 2-DG under stimulus conditions known to produce strong inhibition. We hypothesized that inhibition was not seen because the spontaneous activity in ICC might be too low. That is, the 2-DG method could not detect small reductions in a low level of activity. To increase background activity, white noise was delivered in conjunction with pure tones. There are two major types of inhibition seen in ICC: binaural and monaural (or lateral) inhibition. To test for binaural inhibition under 2-DG, tone bursts were given to one ear and white noise bursts to the other ear. To test for lateral inhibition under 2-DG, both tone and white noise bursts were given to the same ear.

In each experimental situation, compared with control animals, a band of inhibition or reduced 2-DG labelling was produced. Under binaural conditions, a band of reduced labelling was produced in the ICC ipsilateral to the ear receiving the tone bursts and an excitatory band was produced in the ICC contralateral to the ear receiving tone bursts. In the monaural condition, both an excitatory and an inhibitory band were produced in the ICC contralateral to the ear stimulated. Single unit recordings revealed that both the binaural and the lateral inhibitory bands were organized into iso-frequency contours. Binaural inhibitory contours became larger in more posterior parts of ICC, while the lateral inhibitory contours maintained a rather uniform width throughout ICC. Densitometric measurements showed that the inhibitory contours still contained more label than background levels. The lateral inhibitory contours suggest that high frequency inhibitory sidebands were most active under these conditions. These data show the power of the 2-DG method as it is difficult to see how any other technique could reveal the scale and the scope of these inhibitory effects. [Supported by a grant from the National Health and Medical Research Council of Australia.]

119.8 ABSENCE OF DORSAL TO VENTRAL REGISTER IN PROJECTIONS FROM THE VENTRAL NUCLEUS OF THE LATERAL LEMNISCUS TO THE INFERIOR COLLICULUS. C. K. Henkel, J. M. Whitley* and A. Shneiderman*, Department of Anatomy, Wake Forest University Medical Center, Winston-Salem, NC 27103.

Although the ventral nucleus of the lateral lemniscus (VNLL) is a source of afferents to the inferior colliculus on the same order of magnitude as the cochlear nuclei, the organization of VNLL and its connections remains comparatively obscure. This study was undertaken to elucidate the organization within this important auditory nucleus in the cat. Using the autoradiographic tracing method it was shown in a series of 12 cases that relatively small injections of tritiated leucine along the dorsal to ventral axis of VNLL labeled axons that diverged broadly as they ended throughout the central nucleus of the inferior colliculus. However, there was also an area of denser labeling in restricted regions of the inferior colliculus that varied with the position of the injection. For instance, small injections in the dorsal part of VNLL resulted in a heavy field of label in the dorsomedial part of the central nucleus of the inferior colliculus in contrast to heavy labeling in the dorsolateral part of the central nucleus after ventral injections in VNLL. Regardless of these topographical differences no pattern of a progression from dorsal to ventral in VNLL emerged from the autoradiographic data. In a second series of experiments, HRP or WGA-HRP was injected into the inferior colliculus. Depending upon the size of the injections, two patterns of labeled cells were found in VNLL. Large injections labeled at least in some areas horizontally arranged bands of labeled cells alternating with regions of unlabeled cells. Small injections of WGA-HRP, on the other hand, labeled clusters of cells. In one case with a small WGA-HRP injection in the dorsolateral part of the inferior colliculus, these clusters were found along the dorsal to ventral dimension of VNLL alternating at some points from its medial to lateral side. Both the divergence of its efferent connections and the occurrence of projections that arise from compartments of cells, organized in bands or clusters, indicates an order of complexity in VNLL that differs fundamentally from that of other tonotopically organized auditory nuclei. Supported by NIH Grant NS 18627.

119.9 A DIFFERENCE IN STIMULUS FOLLOWING ALONG THE FREQUENCY AXIS OF THE INFERIOR COLLICULUS. D. H. Sanes* and M. Constantine-Paton (SPON: R. Greenspan). Dept. of Biol., Princeton Univ., Princeton, NJ 08544.

Several studies have demonstrated that sensory neurons are able to follow more rapid stimulation rates with increasing age. In the present study, we examined the following response of 2 populations of neurons in the mouse inferior colliculus (IC), those driven by lower (i.e. 3-9 Khz) and higher (i.e. 8-17 Khz) frequency ranges. After first characterizing the frequencies that evoked a compound action potential (CAP), we began to present a regimen of click stimuli at incremental rates. CAPs were averaged at the beginning of each click rate and 5 mins later. All averaged responses were compared to a baseline value (i.e. 0.5/s at time=0).

The most dramatic finding was that the evoked response fatigued at lower repetition rates, and more severely, for low frequency regions of IC. For presentation rates of 5/s and above, the CAP amplitude in low frequency regions declined 20-40% below the level in higher frequency regions. This was found for animals aged 14-60 days postnatal. Repetitive tone pips of 5-6 Khz or 12 Khz, presented at increasing rates, gave results very similar to those observed with click stimuli. The response decrement of single units with relatively sharp frequency tuning, characteristic of the central nucleus of IC, were also examined with repetitive clicks. Units with a best frequency below 9 Khz generally exhibited a more pronounced reduction in response when the stimulation rate was increased from 0.5/s to 20/s, and to stimulation over time. Both the tonal probes, which recruit a more defined population of units, and the single unit recordings, indicate the frequency difference can not be exclusively attributed to the rapidly habituating units in the adjacent subnuclei of IC.

The present results are intriguing in light of a recent study describing a differential projection of the high and low frequency regions of the lateral superior olivary nucleus to IC (Glendenning and Masterton, J. Neurosci., 3: 1521, 1983). These authors suggested that interaural time and intensity cues, normally processed in different frequency ranges, are differentially parcelled at the level of the auditory midbrain. At present we can only speculate that low frequency habituation in the mouse IC reflects such a functional organization.

Supported by the Deafness Research Foundation.

119.10 ELECTROPHYSIOLOGICAL STUDY OF SALICYLATE EFFECT IN INFERIOR COLLICULUS OF GUINEA PIGS. P.J. Jastreboff, and C.T. Sasaki. (SPON: P.E. Pedersen) Sect. of Otolaryngology, Yale University School of Medicine, New Haven, CT 06510.

Tinnitus, the conscious experience of sound that originates in the head, is a widespread otologic symptom for which no effective therapy exists and which in a majority of cases arises from anomalies in the auditory nervous system. Previous works from our laboratory, attempting to create an animal model of tinnitus with the use of 2-deoxyglucose (2DG), have shown an increase in 2DG uptake in the cochlear nucleus and inferior colliculus (IC) of guinea pigs after manipulation which, in humans elicits tinnitus. To overcome certain limitations of 2DG methodology we decided to use an electrophysiological approach.

The experiments were done on guinea pigs anesthetized with pentobarbital (50 mg/kg). Left inferior colliculus was exposed by aspirating the overlying cerebral cortex of an animal fixed in a stereotaxic frame. Two tungsten microelectrodes separated by a few hundred microns were driven through IC. To prevent pulsation and drying, the brainstem was covered with agar. Spontaneous activity of single cells was recorded on magnetic tape for further more detailed analysis, while simultaneously constructing interval histograms. At the end of the tract sodium salicylate (400 mg/kg), which is known to evoke tinnitus in humans, was injected i.p. Two hours after salicylate administration the spontaneous activity of single cells was again recorded from the same tracts during withdrawal of the electrodes. At the end of experiment microlesions were made for later histological verification of electrode position.

Comparison of spontaneous activity recorded from the same tract before and after salicylate administration has revealed statistically significant differences. Before salicylate injection the majority of the cells had a typical frequency below 60 Hz and a significant proportion of these units had activity below 20 Hz. After salicylate administration the majority of the cells exhibited activity above 60 Hz and units with low frequencies became sparse. Changes in the temporal patterns of the discharges were observed as well. Part of the cells had a tendency to fire in a more regular manner, as revealed by smaller dispersions of the interval histograms. Control experiments, in which salicylate was replaced by saline injection, revealed no statistically significant differences in cell discharges. The observed changes in single unit activity due to salicylate administration may be related to tinnitus-like phenomena. (Supported by NIH Grant #NS16288).

119.11 REPRESENTATION OF PERIODICITY INFORMATION IN THE INFERIOR COLLICULUS OF THE CAT. Chr. Schreiner* and G. Langner. Coleman Laboratory, University of California at San Francisco San Francisco, CA 94143 and Zool. Inst. der TH-Darmstadt, 61 Darmstadt, FRG.

The pitches of harmonic complexes (e.g., in speech and music) are related to the periods of their envelopes. AM signals (amplitude modulated sine waves) constitute simple models of complex periodic signals. The questions advanced in the present study were: Is there a temporal representation of AM periodicities at the level of the midbrain in the cat, as described for the Guinea fowl (Langner, G., Exp. Brain Res. 52:333-355, 1983)? How are neurons with different sensitivities to these complex, periodic stimuli topographically arrayed?

Single and multiple unit responses to AM signals have been studied in the IC (inferior colliculus, mainly central nucleus) of anesthetized cats. Post stimulus time and interval histograms were utilized to determine the temporal structures of neural responses, and to define the most effective modulation frequency. This "best modulation frequency" (BMF) was defined using combined rate and synchronization measures. Results included: a) Temporal information about the envelope period of AM signals is represented in the IC for modulation frequencies up to about 1000 Hz. b) For given neurons, temporal patterns of AM-generated neural responses were systematically influenced by the carrier frequency. c) The BMF increased with penetration depth along a dorsoventral axis of the IC. d) Within isofrequency laminae, a systematic topographic representation of BMFs could be demonstrated. Maximal BMFs were in the lateral parts of the central nucleus. On a given isofrequency lamina, units with similar BMFs were arranged on concentric lines around the zone of maximal BMFs. e) Other response parameters (Q 10, latency, binaural type, threshold) seemed to be systematically related to this topographically ordered periodicity representation.

These results indicate that the extraction and topographical representation of periodicity information is an important accomplishment of the auditory system in the projection to and at the level of the inferior colliculus in the cat. The present results are in line with the evidence that the perception of pitch is based on periodicity mechanisms which are similar for birds and mammals (Langner, G., Exp. Brain Res. 44:450-454, 1981).

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119.12 THE INFLUENCE OF NOISE ON THE SELECTIVITY OF NEURONS IN THE INFERIOR COLLICULUS OF THE RAT FOR AMPLITUDE-MODULATED SOUNDS. A. Rees* and A.R. Møller. Dept. of Neurological Surgery, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15213.

We have investigated the responses of over two hundred inferior collicular neurons to tones and noise that were amplitude-modulated with pseudorandom noise. Units were recorded from urethane-anesthetized rats and the sound stimuli were presented contralaterally through a closed acoustic system. Period histograms of the response to amplitude-modulated sounds (modulation depth of 15% RMS)were cross-correlated with one period of the pseudorandom noise used as the modulating waveform. Modulation transfer functions (MTFs) were obtained by Fourier transforming these cross correlograms.

When amplitude-modulated tones were used the MTFs for the majority of the identified central nucleus units changed from lowpass to bandpass (maximum response at 50 to 150 Hz) and then to inhibitory bandpass as the mean intensity of the sound was increased over a range of about 40 dB above the unit's pure tone threshold. In contrast, when the same units were stimulated with a modulated broadband noise many of them showed lowpass characteristics over the range of intensities which produced bandpass tuning with a modulated tone. Addition of an unmodulated broadband noise to a pseudorandom noise-modulated tone, set at an intensity which produced a bandpass response, offset the effect of intensity on a neuron's MTF; as the intensity of the unmodulated noise approached and exceeded that of the tone the unit's response to amplitude modulation rate became increasingly lowpass.

It is suggested that these responses are representative of neuronal mechanisms specifically adapted for coding variations in sound amplitude, and it would appear, in keeping with the properties of other sensory systems, that their resolution changes according to the nature of the stimulus. (A. Rees is supported by a Harkness Fellowship.)

119.13 FREQUENCY SENSITIVITIES OF AUDITORY NEURONS IN THE CEREBELLUM OF THE CAT. R. Burkard and C. Huang. Department of Neurophysiology and The Waisman Center for Mental Retardation and Human Devolopment, University of Wisconsin, Madison, Wisconsin 53705

Threshold tuning curves were obtained from neurons in the cerebellar auditory area of the cat in which the threshold of the auditory brainstem evoked response was also measured as a function of sound frequency. Auditory neurons in the cerebellar cortex of the cat responded to sound stimuli with little or no discrimination for the frequency of the sound. Tuning curves for cerebellar neurons from different animals were similar. Within each individual animal, the tuning curves of single cerebellar neurons were superimposable onto each other and onto the threshold curve of the brainstem evoked response as a function of sound frequency. The values of $Q_{10\ dB}$ for most neurons were less than 2. There was no statistical difference or bimodal distribution in the sharpness of tuning for neurons in the various layers of the cerebellar auditory area. Similarly, frequency selectivity of neurons did not appear to vary as a function of the location of the neuron within the cerebellar auditory area which extends from lobule VI to VII of Larsell. Broad tuning was also demonstrated by short-latency neurons which responded to ipsilateral ear stimulation only. Equally broad tuning was observed in long-latency neurons which responded to binaural sound stimulus. Electrophysiological mapping over the entire cerebellar auditory area did not reveal any evidence for tonotopic organization. These data suggest that each auditory neuron in the posterior vermis may receive inputs that involved convergence from the entire length of the cochlea. The pattern of this convergence was apparently the same for all the cerebellar neurons that were investigated. Under these circumstances, tonotopic organization in the cerebellar auditory area is unlikely.

120.1 "SELFISH" AND "SELFLESS" INFORMATION-MATCHING IN SIMPLE, HEBBIAN NEURAL NETS. Rolf Martin, Biochem. Lab., Chemistry Dept., Brooklyn College, Brooklyn, NY 11210.

Four types of matches can be performed by neural nets governed by three learning rules (Hebb's[1] and two others) that together give rise to and regulate the plant-like branching behavior of neurons within the network. This explanation for matching is of interest because experiments[2] on neuronal interaction in the rat hippocampus have recently provided evidence that lend support to two of the three learning rules and because these and three additional rules have recently been shown to provide an initial basis for risk benefit assessment, representation of concept hierarchies, learning by simile, construction of cognitive maps, game-playing and certain other information-management operations, as well as associative and sequential recall.[3] Consider as an example the use of these networks to match job descriptions prepared by employers and prospective employees, so that each job applicant can receive a list of the positions that best match his or her interests and capabilities. Four types of matches can be obtained: 1) based only on the number of attributes specified as present or important (i.e. given non-zero values) by both employer and job applicant, with no weight given to the number of mismatched attributes; 2) based on the number of mutually important attributes with negative weight given only to attributes of importance to the applicant that are not matched by the employer job description; 3) based on mutually important attributes with negative weight given only to attributes required by the employer that the applicant does not possess; and 4) based on mutually important attributes taking into account both kinds of mismatches. Because type 2 assessments tell applicants which jobs best meet their preferences without regard for employer requirements, and type 3 matches indicate jobs for which applicants most closely match employer requirements with no consideration for the applicant's preferences, types 2 and 3 can be referred to as selfish and selfless, respectively.

1) Hebb, D.O., The Organization of Behavior, Wiley, New York, 1949.

2) Levy, W.B. and O. Steward, Neuroscience 8, 791, 1983.

3) Martin, R., Lukton, A. and S.N. Salthe, to appear in the proceedings of the 1984 Summer Computer Simulation Conference, Society for Computer Simulation, Boston.

120.2 A COMPARISON OF THE ESCAPE BEHAVIOR OF THE COCKROACHES BLABERUS CRANIIFER AND PERIPLANETA AMERICANA. B. Simpson* and R.E. Ritzmann (Spon: M. Forte). Dept. of Biology, Case Western Reserve University, Cleveland, OH 44106.

Comparative studies have been critical in elucidating the evolutionary forces that have shaped the behavior of animals. Our study represents a comparative investigation of the escape responses of two species of cockroaches, Blaberus craniifer and Periplaneta americana. Although the morphology of the nervous systems of B. craniifer and P. americana appear to be similar (Zilber-Gachelin and Chartier, J. Exp. Biol. 59:359), our study shows that their escape stratagies are very different.

We tested both species behaviorally using a wind machine and arena similar to that used by Camhi and Tom (J. Comp. Physiol. 128:193) to describe the escape movements of P. americana. In contrast to the rapid, oriented escape behavior of P. americana, B. craniifer never moved more than a few centimeters and usually made no running movements at all. Moreover, when tested with a natural predator, the toad Bufo marinus, B. craniifer made no escape movement and was, therefore, eaten on each trial. We also compared the response of the two species to tactile stimuli with a blount probe. These experiments were carried on in a dirt floor arena. P. americana again simply ran away from the probe. However, B. craniifer exhibited a rapid digging behavior and was, within seconds, obscured from view. This digging behavior is consistent with the ecology of B. craniifer which is normally found under leaf litter.

A considerable amount of evidence has suggested that the giant interneurons (GIs) play an important role in the wind-mediated escape response of P. americana. We, therefore, wanted to varify that the GIs of B. craniifer were morphpologically similar to those of P. americana. We filled the GIs intracellularly and studied them both in wholemounts of the terminal ganglion and in cross-sections of the abdominal connectives. The morphology of both ventral and dorsal GIs were very similar in the terminal ganglion. However, although they were also similar in the connectives, the size of the ventral GIs relative to the dorsal GIs was not as great in B. craniifer as in P. americana. Indeed there was no significant difference in the diameter of ventral GIs and that of dorsal GIs. In contrast in P. americana the diameter of ventral GIs may be 2 times that of dorsal GIs.

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120.3 CRAWLING IN THE LEECH. W. Stern-Tomlinson, M.P. Nusbaum, and W.B. Kristan, Jr., Dept. of Biology, UCSD, La Jolla, CA 92093.

We are studying the neurophysiology and behavior of crawling in the leech, Hirudo medicinalis. Of the two possible leech locomotory behaviors, crawling and swimming, crawling is far less stereotypic. A given crawling episode can incorporate features, to various degrees, of two distinct but related types of crawl: vermiform crawling and inchworm crawling (also called "looping"). By making measurements from videotapes of behaving leeches, we have characterized these two behaviors analytically.

A cycle of vermiform crawling can be divided into two active and two quiescent phases: 1) the anterior sucker is lifted, followed by rostrocaudal extension of the body and reattachment of the anterior sucker; 2) a brief pause, during which the animal is stretched between the fixed suckers; 3) a rostrocaudal contraction, during which the posterior sucker is lifted, and following which the posterior sucker is reattached; 4) a variable pause, after which another cycle can begin. Inchworm crawling includes the first three phases, but has additional terminal elements: relifting the back sucker, folding the tail under the body, and symmetrical arching of the body.

We are studying various quantitative aspects of crawling; e.g., duration of each of the four parts of the cycle vs. cycle period duration. Analysis is being done both upon intact animals, and upon animals which have been denervated in ways necessary for tethering and neurophysiological recording. By comparing the behavior of intact animals to that of denervated animals and tethered, dissected preparations, we can estimate the normality of the neurophysiological data.

We have recorded identified motor neurons extracellularly from semi-intact behaving preparations, and initially it appears that neural activity is consistent with the behavior. Excitors to the circular muscles are activated upon behavioral extension, and excitors to the dorsal and ventral longitudinal muscles are activated with behavioral contraction.

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120.4 THE STRIKE OF STOMATOPOD CRUSTACEA: MOVEMENT, PATTERNS AND MOTOR INNERVATION. Brent LaMon* (SPON: J. Miller). Dept. of Zoology, University of California, Berkeley, CA 94720.

A distinctive feature of the behavioral repertoire of Mantis shrimps is the ability to inflict a powerful blow with the highly modified second thoracic appendages. Based on the morphology of these raptorial appendages two types of stomatopods may be distinguished: the "spearers" and the "smashers". For the spearing-type appendage, the distal segment (dactyl) is elongate with sharp spines. The smasher appendage is equipped with a dactyl which has the exteroproximal portion greatly enlarged in a bulbous, armored "heel". Using high-speed cinematography the strike movements of two species, Pseudosquilla ciliata (spearer) and Odontodactylus scylarus (smasher), were analyzed for various behaviors. For all stomatopods the strike movement involves a rostral extension of the three distal segments (carpus, propodus and dactyl) from a folded position beneath the merus. Strike movements are produced by operation of a 'click-joint' mechanism releasing stored tension (Burrows, 1969). The majority of the movement is accomplished by extension of the merus-carpus joint. When inflicting a smashing blow, the dactyl is maintained in a folded position and the target is struck with the heel of the dactyl. A spearing strike is performed by extending the dactyl prior to release of the strike, exposing the pointed dactyl tip. Both stomatopod species use both forms of strike, but under opposite conditions. For prey capture, Pseudosquilla struck with both appendages simultaneously and dactyls extended. Agonistic strikes were typically performed with the dactyls folded. Odontodactylus delivered smash-type blows for prey capture. Processing hard-shelled prey always involved a smashing blow by a single appendage. In agonistic contexts both forms of strike were used, but during intense combat, spearing strikes increased.

The distal segments of the raptorial appendage contain 9 muscles innervated by 16 efferent neurons (Govind & Atwood, 1982). In both species the two peripheral nerves that pass into the merus were backfilled with CoCl. This yielded variable numbers of stained cell bodies on the ipsilateral side of the second lobe of the subesophageal ganglion, but in no single preparation was a full compliment of 16 somata observed. Most somata were clustered near the ventral surface in an anteriolateral region around the insertion of the first lateral root. Several relatively larger cell bodies were also stained in a posteriomedial position.

(Supported by NIH-NRSA NS06979 and NIMH grant MH 37846.)

120.5 BIOLUMINESCENCE AND COMMUNICATION IN THE TERRESTRIAL SNAIL DYAKIA (QUANTULA) STRIATA. Jonathan Copeland and Maryellen Maneri*, Department of Zoology, University of Wisconsin-Milwaukee, Milwaukee, WI 53201.

Dyakia striata, a common land snail of Singapore and the Malay Peninsula, is the world's only known bioluminescent terrestrial mollusk. The bioluminescence is produced by a discreet photogenic organ found in the head-foot. The behavioral function and neural control of bioluminescence in Dyakia is unknown.

Bioluminescence was found in 87% of the juvenile snails and 29% of the adult snails collected (N=60). Flashes and glows (0.5 - 4.5 sec at 26°C), pale yellow-green in color, were both symmetrical and asymmetrical when viewed via photomultiplier transduction. Some flashes had multiple peaks.

Flashing in these nocturnally active animals occurred almost always during locomotion, about half the time while feeding, and never while resting. Flashing occurred in bouts, with some bouts lasting for more than two hours. Flash patterns during a bout were variable, but a tendency for the clustering of flashes into slow bursts was seen.

Flashing could not be triggered by tactile stimulation or by treatment with drugs (acetylcholine, 5-hydroxytryptamine, or synephrine injected into the haemocoel or superfused on the photogenic organ in situ). However, photic stimulation of the intact animal could sometimes trigger single flashes (5 - 6 sec delays at 26°C) or bouts of activity.

The effects of photic stimulation on the flash behavior of flashing individuals was viewed. Photic stimulation was provided by using either a conspecific flashing snail or a counterfeit flashing snail (electric torch). The presence of the stimulus significantly increased the spontaneous flash rate of the animals tested.

When two flashing snails were introduced into separate ends of a large test chamber, their flash rate increased as they crawled (directly) toward each other. As they neared, antiphonal flashing occurred and, eventually, when they came to rest side-by-side, flashing ceased.

Results such as these are consistent with the notion that bioluminescence in Dyakia is involved in intraspecific communication.

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120.6 HEAD ORIENTATION IN PIGEONS: POSTURAL, LOCOMOTOR AND VISUAL DETERMINANTS. William Hodos, Jonathan T. Erichsen, Brenda B. Bessette* and Sally J. Phillips*. Dept. of Psychology & Dept. of Physical Education, Univ. of Maryland, College Park, Maryland 20742; Department of Neurobiology & Behavior, SUNY at Stony Brook, NY 11794

Because of the restricted range of its eye movements, the orientation of the pigeon's head is a reliable measure of the orientation of the eye and its visual field. We have determined the pigeon's head orientation for two postures and two locomotor activities that do not involve a specific visual stimulus. Under these conditions the head orientation is relatively constant, allowing us to define the normal horizon of the visual field and thus the horizontal meridian of the retina.

Using a high speed cine camera, we filmed four pigeons (Columba livia) while: (1) flying down a long corridor, (2) walking, (3) perching and (4) standing on a flat surface. A cross with scale markings was mounted directly behind the bird to provide a horizontal and vertical reference in all frames. Using a computer-assisted digitizer, the pigeon's head orientation was determined frame by frame for all four types of postural and locomotor sequences. Head orientation was measured as the angle made by the horizon with a line connecting the center of the pupil and the bill tip.

In each type of sequence, all the birds maintained a head orientation well below the horizon. The mean head angle for all four birds was 34.5°, with a total range of only 10° for the four types of sequences (i.e., 28°-38°). The birds were also filmed as they were presented with hand held seeds at a distance of 5-10 cm and at a variety of heights. In contrast to their relatively stable head posture during locomotion, the pigeons consistently altered their head orientation to fixate seed targets with a small portion of the visual field around the bill tip (see Goodale, 1983). In the head orientation used in the Karten & Hodos (1967) stereotaxic atlas of the pigeon brain, the line between the center of the pupil and the bill tip is 72° below the horizon. We conclude that the downward orientation of the head reported here (approx. 35°) is the normal head posture of the pigeon and should be used in future studies to define the horizontal meridian of the visual field (i.e., the stereotaxic instrument should be tilted up accordingly).

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120.7 THE REFRACTIVE STATE OF THE PIGEON EYE. V. M. Rao* and Jonathan T. Erichsen (SPON: D. H. Cohen). Dept. of Physiology and Animal Behaviour Research Group, Dept. of Zoology, Univ. of Oxford, Oxford, England

Numerous studies of visually guided behavior in the pigeon have suggested that the pigeon eye is heterogeneous with respect to its refractive characteristics. In particular, Catania (1964) first proposed that pigeons were farsighted in the lateral visual field and myopic in the frontal field. Although some refraction measurements have been reported for the visual axis (i.e., the lateral field) of the pigeon eye, no studies using a mydriatic have been carried out in other parts of the visual field.

After the pigeon (Columba livia) was deeply anesthetized, its head was placed in a stereotaxic instrument and centered in a visual perimeter (0.33 m diam.). One eye was sutured open, and a mydriatic solution containing curare (Campell & Smith, 1962) was dripped into the eye to paralyze the ciliary body (i.e., the accommodative muscle) and dilate the pupil. Using retinoscopy, the refractive state of the eye was determined at 15° intervals along the stereotaxic horizontal (i.e, 0°-90°) and at least 30° above and below. The amount of astigmatism was also measured at each position in the visual field. The head orientation varied somewhat in each experiment, but the location of the pecten in the visual field was determined with an ophthalmoscope to provide a constant reference for the refraction measurements. In all, data for eight eyes were obtained.

Correcting for the artifact of retinoscopy (Glickstein & Millodot, 1970), all pigeons were emmetropic throughout the horizontal meridian of the field (including the frontal field well above the bill) and over most of the upper field as well. No significant astigmatism was found. In a localized region of the lower frontal field near the tip of the bill, a myopia of at least 3-4 diopters was clearly evident. This region was shown to correspond well with the high receptor density region of the Red Area mapped by Clarke & Whitteridge (1976). Again, no astigmatism was apparent in this portion of the visual field.

These data suggest that the pigeon eye has a wide-angle, panoramic view along the horizon that is emmetropic and non-astigmatic. In contrast, the lower region of the frontal visual field, which has been reported to be used for close inspection, is relatively myopic. Supported by the Danforth Foundation (JTE).

120.8 THE CONTRIBUTION OF OLFACTORY AND TACTILE STIMULI TO THE PERFORMANCE OF THE NIPPLE-SEARCH BEHAVIOR OF NEWBORN RABBITS. R. Hudson* and H. Distel* (SPON: A. Hofbauer). Inst. Med. Psychol., Univ. München, D-8000 München, Germany.

As rabbit pups are only nursed for about 3 minutes once a day they must be able to find nipples within seconds. In doing so they are totally dependent on a short-ranging pheromone present on the mother's belly which releases a stereotyped search behavior. By combining lateral head-sweeps with rapid, vertical head movements (3-5 per sec) pups appear to follow an odor gradient increasing in strength towards nipples. The high odor concentration at nipples, or possibly a second odor, is then essential for attachement (Hudson & Distel, Behaviour 85:260,1983).

Nevertheless, tactile cues are presumably necessary, at least for nipple grasping. To differentiate the relative contribution of tactile and olfactory input to the search behavior, bilateral and unilateral olfactory bulbectomies and transsections of the sub-ophthalmic branch of the trigeminal nerve were performed on day 2. Bilaterally lesioned animals failed completely to obtain milk during normal nursing, while animals with unilateral trigeminal lesions received 54% and those with unilateral bulbectomies 29% less milk than controls.

Nipple-search performance was tested on day 4 under standardized conditions by placing pups individually in an arena enclosing the belly of an upturned mother (Distel & Hudson, Anim.Behav.32:501,1984). Bilaterally bulbectomized pups failed to show any search behavior, and the tactile input from nipples was insufficient to elicit grasping even when pups were directly held on them. The bilateral trigeminal pups searched vigorously but showed only erratic lateral head movements, and neither nipple attachement nor the repeated mouth-opening characteristic of normal searching. Unilateral trigeminal pups were able to grasp nipples, but only from the intact side. Interestingly, these pups inclined their heads towards the lesioned side during searching and sucking but turned toward the intact side when releasing nipples. The pattern of nipple-search behavior of unilateral bulbectomized pups appeared unaltered, although they were slower to locate nipples than controls. This suggests that nipple location is not dependent on bilateral input from the olfactory bulbs, and mouth-opening and lateral head movements in response to tactile stimulation to be facilitated by the action of the pheromone.

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120.9 TRIGEMINAL DEAFFERENTATION AND CONTROL OF INGESTIVE AND GROOMING SEQUENCES IN RATS. K.C. Berridge* and J.C. Fentress. Dept. Psychology, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada.

Infusions of solutions into the mouths of rats via chronic oral cannulae elicit ingestive/aversive stereotyped actions and grooming sequences. Precisely which actions are elicited is determined by the palatability of the infused solution. The purpose of this study was to determine 1) the role of nongustatory information in the assessment of solution palatability, and 2) the role of tactile sensory feedback in the production of ingestive and grooming movements and sequences.

Male Sprague-Dawley rats (n=14) were implanted under anesthesia (ketamine and acepromazine) with chronic oral cannulae. After recovery, rats were given 1 ml oral infusions of taste solutions over 1 min; one infusion was presented each day and the order of presentation was balanced. Taste stimuli were: 0.3 and 1.0 M sucrose, 0.01 and 0.1 M HCl, and 3 $X10^{-5}$ and $3X10^{-4}$ M quinine hydrochloride. Behavior was videotaped for subsequent computer-assisted analysis. The rats were then subjected to either hemilateral (n=5) or bilateral (n=9) trigeminal deafferentation using a modified procedure of Jacquin & Zeigler (1983). This procedure (transection of the lingual, inferior alveolar, and auriculotemporal nerves of the mandibular branch, and of the infraorbital and anterior superior alveolar nerves of the maxillary branch of the trigeminal) eliminates tactile sensation from the face and mouth while sparing gustatory and motor function. Following deafferentation, rats were again presented with infusions of all taste solutions.

All individual action components persist following trigeminal deafferentation, supporting the hypothesis of central pattern generating mechanisms. Subtle changes appear in the form of certain actions (e.g. tongue protrusions), however, and the number of most taste-elicited actions is reduced. This reduction applies to aversive as well as to ingestive actions, suggesting that trigeminal input multiplies the effectiveness of gustatory stimuli rather than contributing a constant additive component to the assessment of palatability. The effects of deafferentation upon the temporal structure of grooming and ingestive sequences are examined using analyses of sequential dependency.

Supported by the Canadian Medical Research Council and by the Killam Foundation.

120.10 BRAIN TEMPERATURE RISES DURING MOTHER-YOUNG CONTACT IN NORWAY RATS. L. Adels*, R. Coopersmith* and M. Leon. (SPON: R. S. Bridges), Department of Psychobiology, University of California, Irvine, CA 92717.

The duration of contact bouts between mother Norway rats and their young is limited by acute maternal hyperthermia (Crosskerry et.al., 1978, Leon et.al., 1978). We wanted to determine the site of the thermal cue that induced contact bout termination. We rejected the hypotheses that rises in core or skin temperature induced bout termination. While both skin and core temperatures increase near contact bout termination, experimental heating of these areas induces bout termination only after long delays (Woodside et al., 1980). When the preoptic area is diathermically heated, bouts are terminated rapidly (Woodside et al., 1980). We now report that maternal brain temperature reliably rises prior to bout termination.

Brain temperature and mother-young contact were continuously monitored in 10 lactating females for 72 hr beginning on day 10 postpartum. In 79% of the 279 contact bouts analyzed, there was a characteristic rise in brain temperature which began 2-5 min prior to bout termination and continued to rise well into the interbout interval. Brain temperature began to fall several minutes before the dam initiated her next bout and continued to fall during the first few minutes of mother-young contact.

Mother rats do not appear to be responding to either a critical temperature, or a critical rate of temperature rise. Indeed, bout termination temperature was not the highest temperature experienced during 59% of the bouts. Moreover, there were other brian temperature increases in which the rate of rise was equal to those at bout termination. Of these intrabout rises, 66.5% occurred during the second half of contact bouts, suggesting that mothers may be responding to a gradually increasing heat load over the course of a contact bout that makes them increasingly vulnerable to a prolonged brain temperature rise. The prolonged preoptic area temperature rise may then force them to interrupt pup contact. This research was supported by NSF grant BNS 80-23107 and Research Scientist Development Award MH 00371 from NIMH to M.L.

120.11 PERIAQUEDUCTAL GRAY GABAERGIC MEDIATION OF DEFENSIVE BEHAVIORS IN THE RAT. A. Depaulis * and M. Vergnes * (SPON: M.E. Jarvik). Laboratoire de Neurophysiologie, Centre de Neurochimie du CNRS, 5 rue Blaise Pascal, 67084 Strasbourg Cedex, France.

Enhancement of defensive behaviors toward a conspecific has been recently observed following intracerebroventricular injections of a GABA antagonist in male rats and indicates that central GABAergic mechanisms are involved in the control of these behaviors. Several lines of evidence suggest that these mechanisms are, at least in part, located within the periaqueductal gray matter (PAG): 1) this structure has long been implicated as a critical locus for the control of defensive behaviors (Adams D.B., Brain Behav Sci, 2:201-241, 1979), and midbrain neurons mediating defensive reactions have been revealed in our laboratory by microinjections of excitatory amino acids in the rat; 2) GABA-accumulating neurons have been localized in this structure (Belin M.F. et al., Brain Res, 170:279-298, 1979); 3) escape-like behaviors have been elicited by microinjections of GABA antagonists into the PAG (Brandao M.L. et al, Pharmac Biochem Behav, 16:397-402, 1979; Di Scala G. et al, Brain Res, in press). In order to test this hypothesis, the behavioral effects of unilateral microinjections into the PAG of picrotoxin, a GABA antagonist, were investigated in male rats confronted with an untreated conspecific introduced into their cage ("resident-intruder" paradigm). The behavioral analysis of the encounters was performed using a microcomputer-based method (Depaulis A., Pharmac Biochem Behav, 19:729-732, 1983), the respective location of the partner being taken into account.

Microinjections of picrotoxin (25 and 50 ng) into the PAG were found to increase the occurrence of defensive postures such as defensive uprights, defensive sideways and avoidance, whereas offensive behaviors (attacks, offensive uprights, offensive sideways) were suppressed. Enhancement of defensive behaviors was observed when the partners were located on the side of the body contralateral to the site of the injection. These data support the hypothesis that GABAergic neurons located in the PAG are involved in the mediation of defensive behaviors toward a conspecific. The observed lateralization further suggests that these behavioral effects may be partly mediated by a modification of sensorimotor responsiveness.

120.12 A Vibration-Evoked Startle Response in Larval Lampreys (Petromyzon marinus). Scott Currie, Dept. Human Physiology, U.C.Davis, Davis, CA. 95616

EMG's were recorded from intact larvae, laying in a small pan of water. Three cycles of 300 hz. vibration, produced by a speaker beneath the pan, evokes a brief muscular response of the head, trunk and tail which is lost after labyrinthectomy.

EMG's obtained from both sides of midbody reveal a response latency of 15-30 msec and a duration of 10-20 msec. Lampreys contract both sides of their bodies simultaneously. The relative force of contraction on the two sides, as indicated by EMG amplitudes and observed movement, depends on the animal's resting position. Lateral body bends contract more forcefully on the side of inward curvature, so that the animal flexes to that side.

When perfectly straight, startled larvae exhibit very little actual movement. The body briefly stiffens as a slight ripple passes posteriorly. EMG's obtained from three ipsilateral sites show a caudally directed wave of contraction, propigating between 5-12 M/sec in different individuals. The wave is blocked at the site of an acute spinal transection.

Responses also involve a strong contraction of various head structures, including the gill basket and oral hood. Gill region EMG's show a latency and duration similar to those seen at midbody. The gill response persists after severing the spinal cord near the brain.

A semi-intact preparation has been developed which makes a cellular analysis of this behavior possible. A rigid probe (0.5mm D) is used to deliver controlled vibration trains to an auditory capsule. The brain and rostral spinal cord are dorsally exposed to allow extracellular and intracellular recording while the remaining body is left intact. Both head and body responses have been elicited from this preparation. Recordings made with fine suction electrodes, and confirmed intracellularly, show activity in several reticulospinal somata during the response, including both Mauthner and both B_1 Muller cells. Curarized preparations also exhibit large time-locked bursts in cranial nerves V, 1X and X.

121.1 HEARING AND VOCALISATION IN THE AUSTRALIAN GHOST BAT *MACRODERMA GIGAS*. A. Guppy*, R. Coles* and J.D. Pettigrew. Department of Behavioural Biology, Research School of Biological Sciences, Australian National University, Canberra, ACT, Australia, 2601.

Biophysical and neurophysiological studies on the auditory system of the Ghost bat show a well developed low frequency sensitivity. The Ghost bat has remarkably large ears for which biophysical measurements show that the acoustic gain rises sharply above 3 kHz, reaching a peak amplification of 28 dB around 5-8 kHz. Significant acoustic gain occurs up to 100 kHz. Recording from auditory neurons in the inferior colliculus show response thresholds near -18 dB SPL for frequencies as low as 10 kHz, consistent with the high amplification at low frequencies seen at the external ear. An acoustic axis of the pinna can be defined for frequencies between 3 kHz and 100 kHz. Acoustic axes are head-referenced since the pinnae are fused at the mid-line and are essentially immobile. The direction of the acoustic axis is frequency dependent, varying from 80° lateral at low frequencies to mid-line azimuths at ultrasonic frequencies. In addition, the acoustical axis moves about 60° in elevation over this frequency range. Spatial receptive fields (axial) which can be recorded from mid-brain auditory neurons shows similar frequency shifts in their position.

In the Ghost bat, extended low frequency sensitivity is clearly related to the use of vocalisations which are audible, in sharp contrast to the ultrasonic sonar. Sound recordings and analyses of the "Chirp", "Squabble" and "Twitter" show a main energy band around 12 kHz. The "Chirp", for example, has highly a stereotyped song-like structure, with discrete audible and ultrasonic elements. The sonar pulses of the Ghost bat are extremely short duration (<1 msec), frequency-modulated and contain 3-4 harmonics. Most of the energy of the sonar pulses is distributed between the second and third harmonics with the fundamental (20-15 kHz) considerably suppressed. Ultrasonic communications, distinct from sonar, have also been recorded.

The sensitive low-frequency hearing of the Ghost bat may also enable prey capture by passive listening. These bats prey extensively on small vertebrates as well as insects, and are readily attracted in the field to sounds in the audible frequency range (2-5 kHz).

121.2 PERCEPTUAL FUNCTIONS OF CORTICAL NEURAL MAPS OF TARGET RANGE IN ECHOLOCATING BATS. J.A. Simmons. Institute of Neuroscience, University of Oregon, Eugene, Oregon 97403.

The distance to sonar targets, or target range, is represented in the auditory cortex of echolocating bats by neurons which respond selectively to echoes at different delays (O'Neill, W.E. and Suga, N., J. Neurosci., 2:17, 1982; Sullivan, W.E., J. Neurophysiol., 48:1011, 1982). These range-tuned neurons are topographically arranged to display range along one axis of a neural map on the cortical surface. The width of range tuning-curves (receptive fields for depth) is roughly proportional to the magnitude of the neuron's "best" range, being tens of centimeters wide for best ranges of 50-100 cm. At their tips they might conceivably provide the bat with range resolution of several centimeters. The bat's range acuity actually is about 2 orders of magnitude better--a fraction of a millimeter at 50 cm. The bat's range image of a target (the half-wave-rectified crosscorrelation function of emissions and echoes), with its fine phase structure (Simmons, J.A., Science, 204:1336, 1979) cannot be obtained through simple averaging or interpolation of cortical neural activity patterns represented by known range tuning-curves. Some mechanism apart from, rather than working through, the cortical range map must provide fine range acuity. Range tuning-curves and the map may mediate a different aspect of target perception, however. One target interferes with detection of another if they fall within a span of range comparable in width to range tuning-curves. Clutter interference may thus occur if two targets evoke activity in the same neurons on the map, and be rejected if they excite different neurons. The map allows the bat to assign a range to each target and may be the basis for perceptual isolation of single targets through spatial segregation of neural activity representing different targets. The circumstance that the bat perceives the equivalent of the phase of echoes relative to emissions for fine range resolution, and that this cannot be obtained from the relatively broader range tuning-curves by collective neural computations similar to interpolation, suggests that neural maps are not the basis for all aspects of spatial perception.

121.3 ECHOLOCATION SOUNDS ELICITED FROM THE MUSTACHED BAT BY ELECTRICAL STIMULATION OF A SUPRACALLOSAL REGION OF THE BRAIN. D.M. Gooler and W.E. O'Neill. Center for Brain Research, University of Rochester Medical Center, Rochester, NY 14642.

Auditory feedback not only plays a significant role in the ongoing control of vocalization in the mature organism, but is especially important in the development of species-specific vocal patterns, including those of human speech. Studies are now underway to determine the relations between the auditory and vocalization systems of the mustached bat, Pteronotus parnellii. The mustached bat emits stereotyped orientation sounds consisting of a long constant-frequency component followed by a short downward-sweeping frequency-modulated sound. During target-oriented flight, the bat can alter the frequency as well as the temporal pattern of the echolocation sounds. The midbrain periaqueductal gray is the most rostral vocal control area identified in previous studies (Suga, N. et al., J. Acoust. Soc. Am., 54:793, 1973; Suga, N. et al., J. Exp. Biol., 61:379, 1974). The aim of the present study is to further define the vocal control system and clarify its role in the production of echolocation sounds.

Suspected vocal control areas anterior and dorsal to the corpus callosum were stimulated electrically using glass-insulated platinum-iridium microelectrodes. Electrical stimuli consisted of trains of constant current (10-90 μamp), cathodal pulses. The waveform envelope and frequency (zero-crossing period meter) of the elicited vocalizations were measured. The dominant frequencies (maximum energy) of the vocalizations were generally in the range of the second harmonic of the naturally occurring echolocation sounds. The shape of the envelope and duration of the vocalizations resemble those measured during spontaneous emission of echolocation sounds. Accompanying motor patterns such as movement of the pinnae, cone-shaped mouth and protruded lips, and strong respiratory activity were observed and appeared similar to those seen in bats that are echolocating. No other gross body movements were observed. Evidence is now available to suggest that a supracallosal area of the brain may be involved in the control of biosonar vocalizations. The horseradish peroxidase technique for anatomical localization is being used to further characterize the vocal control system.

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121.4 NEUROETHOLOGY OF SOUND COMMUNICATION IN THE AFRICAN RUNNING FROG KASSINA SENEGALENSIS. R.R. CAPRANICA, G.D. HARNED* and N.I. PASSMORE*. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853 and Department of Zoology, University of Witwatersrand, Johannesburg 2001, South Africa.

During the breeding season male African Running Frogs broadcast intense (102 dB SPL at 50 cm), very distinct frequency-modulated mating calls that sweep upward from 750 Hz to 2250 Hz in about 140 msecs. Field studies of phonotaxis involving two-choice discrimination trials with natural and synthetic calls verify that the female's response is very selective and that the sweep direction, rate, and frequency range in the male's call are important cues for species-specific recognition. Electrophysiological recordings from single auditory fibers in the eighth nerve reveal typical "V" shaped tuning curves with best excitatory frequencies distributed over the range 100-1500 Hz, thresholds between 40-90 dB SPL; spike-rate functions increase monotonically over a dynamic intensity range of 30-50 dB. Thus auditory nerve fibers are well designed to process the signal characteristics in the male's call over a wide broadcast distance, but there is no evidence of specialization for FM detection in the periphery. On the other hand, individual cells in the auditory midbrain exhibit selectivity to these FM features. African Running Frogs represent an excellent neurobehavioral model for studies of FM signaling and neural encoding.

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121.5 HORMONAL ACTIVATION OF VOCALIZATION AND CONCOMMITANT THRESHOLD CHANGES IN THE CENTRAL AUDITORY SYSTEM OF THE GREEN TREEFROG. M. Penna* and R.R. Capranica (SPON: M.C. Nelson). Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853

Male and female green treefrogs (Hyla cinerea) can be induced to emit mating calls following treatment with testosterone and vasotocin. (Watson and Capranica, in preparation). It is conceivable that these hormones also affect the auditory nervous system, which shows selectivity for detection and analysis of species-specific signals. To explore this question, we have conducted evoked calling experiments together with simultaneous electrophysiological recordings in the auditory nervous system of 13 intact H. cinerea females before and after hormonal treatment. A group of 24 castrated males is still in progress. Each animal's vocal responses to a ten-minute presentation of conspecific mating calls was tested periodically during the hormonal schedule. Following each test, neural audiograms (in response to tones) were recorded in the torus semicircularis using insulated tungsten electrodes (50-70 µm tip exposure). These recordings were conducted before hormone administration and repeated 3 to 4 weeks after implantation of a 5 mg androgen pellet (control animals received a sham implant operation), and then again 2 days later following a 25 µg arginine vasotocin (AVT) injection.

Six of 9 testosterone implanted females produced male-like mating calls during the evoked calling test following AVT injection. Each of the 4 control females failed to vocalize during every test. The midbrain neural audiograms of green treefrogs generally exhibit two regions of good sensitivity: between 400 and 1000 Hz, and around 3000 Hz. Following hormonal treatment, several of the experimental animals showed enhanced sensitivity in these regions: in some cases thresholds shifted by as much as 30 dB. Such concommitant modification of vocal behavior and auditory sensitivity by hormonal stimulation may constitute an important coupling between the two systems to enable effective communication during the reproductive season.

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121.6 TECTAL AND CEREBRAL INFLUENCES ON THE BEHAVIOR OF LARVAL AND JUVENILE BULLFROGS. D.J. STEHOUWER. Dept. Psychology and the Center for Neurobiol. Sci., Univ. Florida, Gainesville, FL 32611.

Stepping behavior of the larval bullfrog normally commences at about Stage XVII, the final stage prior to the onset of metamorphic climax. However, the neural and muscular prerequisites of hindlimb stepping are present and functional no later than Stage XII, suggesting that the ontogenetic transition from larval swimming to locomotion via the hindlimbs is dependent on factors other than maturation of the spinal cord and peripheral tissues. One possibility is that there is a functional change in descending influences from the brain, which could also explain the precipitous decline of spontaneous "fictive locomotion" of the isolated CNS that begins with the onset of metamorphic climax.

This study examined the effects of decerebration, tectotomy, and combined decerebration and tectotomy on posture, withdrawal response thresholds, vestibular responses, gross locomotor activity and the optomotor response of larval and juvenile frogs.

Results showed that intact juveniles are much less active than intact larvae. Decerebration and tectotomy each reduced spontaneous locomotion of the larvae by 50%, and combined lesions virtually eliminated spontaneous locomotion. None of the lesions had any detectable effect on posture, vestibular responses, withdrawal response thresholds, or the optomotor response of larvae. In contrast, neither decerebration nor tectotomy altered spontaneous locomotion of juveniles. The threshold for limb withdrawal, normally an order of magnitude higher in juveniles than in larvae, was reduced to larval levels by either decerebration or tectotomy. Decerebration increased forelimb extension of juveniles at rest on land but did not affect vestibular responses, whereas tectotomy affected neither. Intact juveniles displayed only postural responses to the optomotor stimulus; only after decerebration and/or tectotomy did they pursue the stimulus. These results suggest that, in the larva, the tectum and cerebrum enhance spontaneous locomotion but exert little influence on responses elicited by sensory input. In the juvenile, those structures appear to have little effect on the level of spontaneous locomotion, but suppress limb withdrawal and the optomotor response.

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121.7 MASKED AUDITORY THRESHOLDS IN THE GREEN TREEFROG, (HYLA CINEREA). C. F. Moss and A. L. Megela, Dept. of Psychology, Brown University, Providence, RI 02912

When green treefrogs (Hyla cinerea) communicate in their natural habitat, they face the task of detecting salient acoustic signals against a background of environmental noise. This task requires that the auditory system perform a spectral filtering operation by which the frequency components of the signal are extracted from those of the noise. In order to study the characteristics of this filtering operation in the green treefrog, we measured critical ratios for detection of pure tones in broadband background noise.

Our basic technique involves reflex modification, as originally described by Yerkes and recently developed in our laboratory as a psychophysical procedure for examining auditory detection and perception in anurans. In our procedure, a brief electrical stimulus elicits a muscle twitch from the frog. When a prestimulus (200 msec pure tone; frequencies between 300 and 4,000 Hz) precedes the reflex-eliciting stimulus, the amplitude of the muscle twitch response is reduced. The amplitude of this reflex modification varies systematically with the sound pressure level (SPL) of the prestimulus tone. We define pure tone threshold as the SPL at which the reflex modification effect disappears relative to control trials (no prestimulus tone). From each pure tone threshold, we calculated a critical ratio (CR), the difference between the SPL of the tone at threshold and the spectrum level of the noise.

At 900 Hz, a major frequency component of the green treefrog's mating call, the CR is lowest of all frequencies tested. Based on data from 3 frogs at 2 noise levels, we estimate that the CR at 900 Hz is approximately 15 dB SPL. This indicates remarkable resolving power of the green treefrog's ear at this frequency. With the exception of 300 Hz where the CR is about 30 dB SPL, CR increases monotonically with increasing tonal frequency. This pattern resembles that reported for many mammals, but differs from that previously reported for treefrogs based on a selective phonotaxis technique.

121.8 BEHAVIORAL AUDIOGRAMS FOR ANURA. A. L. Megela, K. M. Daniel*, and D. J. Uhlrich. Dept. of Psychology, Brown University, Providence RI 02912.

The study of the neuroethology of sound communication in anurans has been hampered by the lack of precise, quantitative data on the sensitivity and limits of the hearing of these animals. We now report the first successful determination of behavioral audiograms for sound detection in anurans. Our data have important implications for the hypothesis of anuran communication as a "matched filter" system, and our procedures provide a fast, sensitive and reliable technique for measuring a variety of auditory perceptual phenomena in these animals.

Our basic technique (reflex modification) is a variant of one introduced by Yerkes in 1905 and currently used for psychophysical determination of sensory function in many animals. In this paradigm, pure tones presented 200-400 msec before a mild electrical stimulus inhibit (e.g., decrease the amplitude of) the reflex elicited by this electrical stimulus. The degree of inhibition of the reflex response depends on the intensity of the preceding tones; on this basis, psychometric functions relating the degree of reflex inhibition to the intensity of the tones can be generated and used for estimating thresholds. Threshold is defined as being at those tone intensities at which reflex inhibition effects disappear.

This technique is providing useful threshold data at many different sound frequencies for two species of anurans, the bullfrog (R. catesbeiana) and the green treefrog (H. cinerea). Our data show that bullfrogs can detect pure tones of frequencies up to 3 kHz. They are most sensitive (thresholds of about 20 dB SPL) around 800 Hz, a frequency not present in their species-specific vocalizations. Thresholds to frequencies which are present in their species-specific vocalizations (around 300 Hz and around 1500 Hz) are higher, lying between 40-60 dB SPL. Green treefrogs are sensitive to sounds up to at least 5 kHz. They are most sensitive (thresholds about 20 dB SPL) to frequencies around 900 Hz, a frequency which is present in the vocalizations of this species. Thresholds to frequencies around 3000 Hz, which are also present in their vocalizations, rise to about 50 dB SPL. At 300 Hz, thresholds are about 60 dB SPL. For both species, the behavioral audiograms measured by our technique approximate in shape and sensitivity neural audiograms recorded from the midbrain auditory nuclei of these animals.

121.9 MALE COURTSHIP VOCALIZATION AND THE NORADRENERGIC SYSTEM. S.R.Barclay*,A.L.Johnson*, and M.F.Cheng. Institute of Animal Behavior, Rutgers University,Newark,NJ 07102.

The purpose of this study was to examine a possible role of the noradrenergic system in the breeding cycle of the male ring dove. Various noradrenergic agonists, such as phenylephrine, and antagonists, such as phenoxybenzamine and prazosin, were used. Each drug was tested initially in intact males and a dose response curve was established; if the effect was significant, it was replicated in castrated males primed with testosterone-filled Silastic capsules. All drugs were administered (i.m.) to the male doves in isolation, then after one to three hours the males were paired with a receptive female and several measures of male courtship behavior were recorded for fifteen minutes.

One hour after treatment with phenylephrine, intact males paired with a female showed significant decrease in the incidence of bow-cooing (an aggressive component of courtship), while they maintained normal levels of activity on other measures of courtship, such as nest-soliciting. Since bow-cooing is known to be androgen dependent and we had previously shown that serum LH levels decrease after phenylephrine treatment, the supression of bow-cooing may be accounted for by the decrease in LH resulting in a decrease in circulating testosterone. However, the castrated testosterone-treated group did not support this conclusion: phenylephrine treatment still resulted in a decrease in the amount of bow-coos in these doves. The noradrenergic antagonist, phenoxybenzamine, had no effects on male courtship behavior at any of the doses administered. In addition, at the highest dose approximately fifty percent of the animals showed symptoms of distress. Another antagonist, prazosin, resulted in a significant increase in courtship behavior without any symptoms of distress. Based on the results in testosterone-treated animals,it can be concluded that the effects of these drugs are not mediated by changes in androgen levels.

These results suggest that the normal induction of courtship in the breeding cycle may depend on changes in the level of endogenous catecholamines. Since courtship occurs in the context of the social interaction between the male and the female, these proposed changes of endogenous catecholamines may depend on that interaction. (Supported by NSF-BNS-8121495 and RSD-KO2-MH-7089 to M.F.Cheng)

121.10 AUDITORY NUCLEI IN THE BUDGERIGAR. S. E. Brauth and R. J. Dooling, Department of Psychology, University of Maryland, College Park, Maryland 20742.

Structural and functional properties of telencephalic auditory structures were investigated in the budgerigar using 2-deoxy-D-glucose (2DG) autoradiography, histological pathway tracing techniques (horseradish peroxidase histochemistry and amino acid autoradiography) and behavioral testing.

2DG autoradiography indicates that the principal telencephalic auditory nucleus, Field "L", is excited by all acoustic stimuli audible to the animal. Two higher order auditory nuclei also show increased activity with acoustic stimulation; these are located in the dorsolateral and ventrolateral portions of the neostriatum intermedium (NIDL and NIVL). The NIDL consists of a large neuronal field appearing immediately caudal to the ectostriatum and extending caudally, dorsally and laterally up to the level of the rostral Field "L". This field overlaps the regions described by Patton et al (J. Neurosci., 1:1279-1288, 1981) as nuclei interface and neostriatalis anterior magnocellularis and appears to correspond to areas P and Q in the guinea fowl (Scheich et al, Cell and Tissue Res., 204:17-27,1979). In the budgerigar NIDL is particularly responsive to species-specific calls. The NIVL overlaps the region identified as 'HVc' by Patton et al (1981) and is less responsive to direct auditory stimulation. Stimulation with long warbles, vocalizations which function to coordinate reproductive behavior, also increased activity within a limbic system structure, nucleus taeniae.

The anatomical connections of Field "L" and immediately surrounding neostriatum were also studied. Afferent projections are derived from the auditory thalamic nucleus ovoidalis, from the nuclei annularis and raphe dorsalis in the midbrain and from the hyperstriatum ventrale in the telencephalon. Efferent projections terminate within the paleostriatum augmentatum, NIDL and NIVL as well as within the nucleus taeniae. There are thus many pathways by which auditory stimulation can reach higher order sensory and limbic structures in this species.

Behavioral conditioning tests demonstrate that the budgerigar is adept at discriminating and remembering complex, species-specific acoustic signals. The functional pathways described above reveal an intricate neural network within the budgerigar telencephalon for the support of such complex behaviors.

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121.11 DEVELOPMENT OF THE NEURAL NETWORK CONTROLLING SONG BEHAVIOR IN ZEBRA FINCHES. S.W. Bottjer, S.L. Glaessner* & A.P. Arnold. Dept. of Psychology, UCLA, CA 90024.

The primary purpose of this study was to examine the normal ontogeny of the total volume of 3 brain nuclei that have been directly implicated in song learning and behavior in male zebra finches. In addition, the corresponding nuclei of age-matched females were examined. 18 male and 12 female zebra finches were overdosed with anesthetic and perfused with saline-formalin. Brains were frozen-sectioned transversely at 25 um and stained with thionin. Alternate sections were examined using a microprojector. HVc (caudal nucleus of the ventral hyperstriatum), RA (robust nucleus of the archistriatum) and MAN (magnocellular nucleus of the anterior neostriatum) were outlined in order to calculate the total volume of each nucleus. As a control, the average cross-sectional area of the telencephalon at the level of the anterior commissure was measured. All birds were divided into 3 age groups with means of 12, 25 and 53 days. These 3 ages correspond to the time (a) prior to production of any song, (b) when song sounds are first produced, and (c) when the final song pattern begins to form. Female zebra finches do not sing at any age.

The major findings are as follows: The volumes of HVc and RA were smaller in females than males at all ages studied. Between 12 and 25 days the volumes of female HVc and RA increased by 23 and 15%, respectively, but the telencephalic control area increased by 32%. Female HVc and RA decreased by 21 and 43%, respectively, between 25 and 53 days, whereas the control area did not change. The volume of male HVc and RA increased by 147 and 46%, respectively, between 12 and 25 days, whereas the telencephalic control area increased by only 27%. Between 25 and 53 days, male HVc and RA increased by 28 and 78%, respectively, while the control area decreased by 3%. Even more striking was that MAN had a very large volume at 25 days, which decreased (by 59%) by 53 days. This interval corresponds to a restricted period of development when MAN lesions disrupt song learning (Bottjer et al, Sci. '84).

These findings suggest the following: (1) the sexual dimorphism in the song-control system is evident at 12 days but increases markedly thereafter, primarily due to the growth of male HVc and RA; (2) the increase in volume in male HVc appears to lead that in RA, suggesting that hormones may act directly on HVc to trigger growth, and that HVc may then exert a trophic influence on RA; (3) MAN is large when birds are learning to produce song, and decreases markedly around the time when the motor pattern of song begins to stabilize.

121.12 AFFERENT INPUT TO A FOREBRAIN NUCLEUS INVOLVED IN SONG LEARNING IN ZEBRA FINCHES. E.A. Miesner, S.W. Bottjer and A.P. Arnold. Dept. of Psychology, UCLA, CA 90024.

Lesions of the magnocellular nucleus of the anterior neostriatum (MAN) severely disrupt song development in juvenile male zebra finches, but have no effect on production of already-learned song by adult birds (Bottjer et al, Sci., '84). MAN makes monosynaptic efferent connections to the hyperstriatum ventrale, pars caudale (HVc) and the nucleus robustus archistriatalis (RA), two other telencephalic nuclei known to be involved in control of adult song behavior (Nottebohm et al, JCN, '83). The purpose of this study was to determine sources of afferent input to MAN in male zebra finches.

A 20% solution of horseradish peroxidase (HRP) in polyacrylamide absorbent gel was injected through a cannula into MAN of two juvenile zebra finches (40 days) and three adults (90 days). After 24 hours the birds were overdosed with anesthetic and perfused with paraformaldehyde-glutaraldehyde in a cacodylate buffer. Brains were sectioned at 40 to 50 um and reacted using tetramethyl benzidine; sections were counter-stained with neutral red or thionin and examined under bright-field illumination. Labeled cells were found in all birds in an area tentatively identified as nucleus dorsointermedius posterior thalami (DIP). Fibers leave DIP and travel through the tractus thalamo-frontalis et frontalis-thalamicus medialis (TFM) to MAN.

HRP was also injected into DIP of juvenile and adult birds. Labeled cells were found in nucleus tegmenti pedunculo-pontinus, pars compacta (TPc) in adult birds after survival times of 24 or 48 hours. In contrast, no labeled cells were seen in TPc in juvenile birds following survival times of 24 hours. (We have not yet examined the brains of juvenile birds after a survival time of 48 hours to determine whether retrograde transport to TPc requires more time than in adults.) A small group of cells within TFM, just anterior to DIP, was also labeled in all birds.

Developmental studies have shown that MAN decreases in size between 25 and 53 days in zebra finches (see adjoining abstract). This size change correlates directly with the time when MAN lesions disrupt song development - that is, MAN lesions become ineffective shortly after 50 days. It is possible that the decreased size of MAN is accompanied by changes in input to or output from MAN. Further developmental studies of MAN and its afferent and efferent connections will examine this hypothesis.

121.13 COMPARISON OF MALE AND FEMALE VOCAL CONTROL REGIONS IN DUETTING BIRDS. E.A. Brenowitz, A.P. Arnold, and R.N. Levin*. Dept. Psychology, UCLA, Los Angeles, CA, 90024 and Section of Neurobiology and Behavior, Cornell University, Ithaca, NY, 14853.

In species of song birds such as the zebra finch and canary, there are large sex differences in vocal behavior and in morphology of brain regions controlling song. In these species, males sing much more than females, and males possess brain vocal control regions (VCRs) which are larger in volume. In zebra finches, males have more and larger vocal control neurons which are packed less densely than VCRs in females. A greater percentage of VCR neurons accumulate androgens in male zebra finches.

We compared attributes of VCRs in two Panamanian wrens, the bay wren (Thryothorus nigricapillus) and the buff-breasted wren (T. leucotis). In both of these species, females possess vocal repertoires comparable in size to males, with whom they participate in complex antiphonal song duets. Wrens were captured in nets in Panama, anesthetized, perfused and their brains were post-fixed in formalin. Sections cut from frozen brains were stained with thionin. We used standard morphometric techniques to measure neuron size, number, and density, as well as total volumes of various VCRs. Other bay wrens were gonadectomized, and injected with tritiated testosterone for steroid autoradiography.

In the duetting birds, the volume of telencephalic VCRs are 1.2 - 1.6 times larger in males than females, in contrast to zebra finches and canaries, in which these ratios are 2.7 - 5.5. In the robust nucleus of the archistriatum (RA), the male:female ratios are 1.1 - 1.5 for neuronal number, (2.4 in zebra finches); 1.0 - 1.1 for RA neuron soma size (2.2 for zebra finches); and 0.9 - 1.0 for neuronal density (0.2 for zebra finches). In telencephalic VCRs such as the caudal nucleus of the hyperstriatum (HVc), steroid concentrating cells are found in both males and females, with much less sexual dimorphism in the number of steroid labeled cells, compared with zebra finches. Thus, in all attributes measured, the two duetting wren species show much less sexual dimorphism in VCRs than zebra finches and canaries. Coupled with data reported previously for the white-browed robin chat (Cossypha heuglini, Brenowitz and Arnold, 1983), the present results suggest that the extent of sexual dimorphism observed in VCR volume of different species correlates with the degree of behavioral sexual dimorphism in song repertoire size. Neuronal soma size in RA is identical in males and females of all three duetting species and may thus relate more directly to the basic motor ability to produce song. (Supported by NIH grant NS 19645).

121.14 SEXUALLY DIMORPHIC BRAIN AREAS IN THE RED-WINGED BLACKBIRD. J. Kirn, R. P. Clower, M. Ascenzi* and T. J. DeVoogd. Dept. of Psychology, Cornell University, Ithaca, NY 14853.

In many bird species, song is a sexually dichotomous behavior: males sing and females sing little if at all. Nottebohm and associates have described several nuclei which are necessary for song production or learning in zebra finches and canaries: hyperstriatum ventrale, pars caudale (HVc), robustus archistriatalis (RA), magnocellular nucleus of the anterior neostriatum (MAN), area X, and the hypoglossal nucleus (nXII). These nuclei are much larger in males than in females (reviewed by DeVoogd, Prog. in Brain Res., in press). However little study has focused on the neurobiology of cell groups corresponding to song control nuclei in wild birds. We now report preliminary findings on song control nuclei in the red-winged blackbird (Agelais phoeniceus).

Redwings were collected in central New York during Oct.-Nov. 1983. In cresylecht violet stained 30 μ brain sections, cell aggregates were discovered with staining characteristics and locations similar to all of the major canary and finch song control nuclei: HVc, RA, MAN, X, and nXII. Preliminary data on 4 male and 4 female redwings indicate a robust sex difference in the volumes of RA and HVc. Male RA volume in mm3 ($\bar{x}\pm s$=0.776±0.08) was 3 to 4 times larger than female RA volume (0.218±0.02). Male HVc (1.393±0.32) was 5 times larger than female HVc (0.276±0.12 n=2). In contrast, the volume of n. spiriformis medialis, a nucleus not directly involved in song control, was 0.525±0.04 in males and 0.461±0.02 in females, representing a much smaller sex difference parallel with the slightly greater overall brain weight of males ($\bar{x}\pm s$=1.89±0.15 gms. for males and 1.65±0.04 for females).

These data extend the anatomical findings from canaries and finches to a wild songbird and suggest that the redwing is a suitable subject for further research on song and its neural correlates.

Supported by NIH R3HD177146 and the Sloan Foundation.

NEUROETHOLOGY III

122.1 A ROLE FOR NON-MAUTHNER ESCAPE CIRCUITS IN LARVAL ZEBRAFISH: IMPLICATIONS FOR FUNCTIONAL SUBSTITUTION IN LESIONED AND NORMAL ANIMALS. J. Nissanov*, R. C. Eaton and C. M. Wieland* Dept. of Biol., E.P.O., Univ. of Colorado, Boulder, CO 80309

The Mauthner (M) cell triggers a complex startle or escape movement when the zebrafish, Brachydanio rerio, is given a sudden vibrational stimulus to the head. However, as previously shown, when the M-cell is missing or fails to fire, the behavior is still executed. These are called non-Mauthner (non-M) responses and are mediated by escape circuits that do not include the M-cell. Is the functional role of the non-M circuits only to serve as a back-up to the M-cell?

We show here that in intact animals non-M circuits have sensory inputs which only partly overlap with the M-cell and are thus not functionally identical to the M-cell. Both types of circuits were activated by vibration of the body surface, but were differentially sensitive depending on stimulus site. The non-M responses were readily elicited by stimulation of the tail.

To characterize sensory inputs for the M-initiated and non-M responses, we made electrophysiological comparisons of response thresholds and latencies to vibrational stimuli applied to the head and the tail. The stimulus was produced by a 94-Hz axial excursion of a fine glass probe placed against the body surface. In 77% of the trials, M-initiated responses occurred at lower stimulus intensities than the responses initiated by non-M circuits when the vibrational stimulus was applied to the head. But, when the tail was stimulated, there was no apparent difference in stimulus intensity required to elicit M-initiated and non-M responses. The M-initiated responses were always shorter in latency (by an average of 15 or 21 ms) than the non-M responses when stimulating either the head or tail.

The differences in sensory input suggest different behavioral roles for the M-cell and non-M circuits in larval zebrafish. The M-cell mediates short-latency responses primarily to head stimuli whereas the non-M circuits mediate long-latency responses, primarily to tail stimuli. The response regions are not strictly segregated: M-initiated responses occur to tail stimulation and the non-M circuits account for a low proportion of responses to stimulation of the head. Because of this sensory overlap between the M-cell and non-M circuits, we propose that the latter can functionally substitute for the M-cell when it fails to fire.

Supported by NSF grant BNS81-12423.

122.2 EVIDENCE FOR MAUTHNER-DERIVED INHIBITION OF NON-MAUTHNER ESCAPE RESPONSES IN GOLDFISH. C. M. Wieland* and R. C. Eaton (SPON:J.CALDWELL). Dept. of Biol., E.P.O., Univ. of Colorado, Boulder, CO 80309.

We previously showed that in goldfish the Mauthner (M) cell initiates a stereotyped escape response away from the side of a ball dropped into the water above the fish. After lesioning the M-cells, a mechanically identical escape response can still be obtained to this stimulus. This demonstrates the existence of an alternate, non-M, circuit for the escape response. Present work is directed to understand the functional significance of the non-M circuit and to characterize its interaction with the M-cell.

Goldfish with single M-cell lesions were given a vertical displacement stimulus while behavioral and electrophysiological responses were recorded. The stimulus lacks any horizontal component and is non-directional for the escape responses. Stimuli were given at threshold levels until the animal habituated. The majority (92%) of escape responses were to the side opposite the remaining M-cell and used the M-circuit. In most animals (6 of 8), no non-M responses were seen opposite the lesioned M-cell.

In lesioned animals the non-M circuits were functional because all animals readily gave non-M escape responses away from the side of a directional visual stimulus. Also, animals with bilateral M-cell lesions gave escape responses in both directions to the displacement stimulus. Response probabilities and thresholds were the same as M-initiated responses, but the non-M response latencies were consistently 10 ms longer than the M-initiated responses.

The results show that the M-cell can inhibit the contralateral non-M circuit. Otherwise we would have seen an equal number of left and right responses in single-lesion fish. This inhibition is not totally effective in these because of the presence of a small number of non-M responses. The M-cells must also inhibit the ipsilateral non-M circuits, as only M-initiated responses were observed in single-lesion animals with chronically implanted recording electrodes.

Response latency and mechanical performance are thought to be crucial factors in executing a successful escape response in the presence of an attacking predator. The proposed M-derived inhibition may enhance these factors by preventing activation of conflicting motor commands by circuits that receive some of the same sensory input as the M-cell but activate escape responses that occur at longer latency.

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122.3 EFFERENT CONNECTIONS OF THE ELECTROSENSORY LATERAL LINE LOBE: AN INTRACELLULAR HRP AND LUCIFER STUDY
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The electrosensory lateral line lobe (ELLL) of gymnotoid electric fish is a hindbrain structure with 8 laminae and 11 cell types (Maler et al., J.Comp.Neurol.195:87-139,1981). The ELLL receives information about phase, i.e. the timing of zerocrossings of the sinusoidal electric signal, and amplitude via the primary afferents of T and P-type tuberous electroreceptors, respectively. Amplitude information is coded in the ELLL by 2 types of cells: E units, which are excited by an increase in amplitude, and I units, which are inhibited by an increase in amplitude. These units have recently been shown to correspond to the anatomically identified basilar and nonbasilar pyramidal cells (Saunders and Bastian, J.Comp. Physiol.154:199-210,1984). Heiligenberg and Dye (J.Comp.Physiol.148:287-296,1982) discovered that the ELLL is somatotopically organized into 4 maps of the fish's body surface - 3 tuberous and 1 ampullary. The tuberous maps are interesting in that they receive identical afferent information: each P and T afferent terminates in all 3 maps. A major question concerns a possible functional specialization of these maps.

The efferent connections of the ELLL are to two midbrain structures, the nucleus praeminentialis and the torus semicircularis. The 4 maps of the ELLL project 1:1 to 4 maps in the n. praeminentialis but coalesce into a single map in the torus. Phase information from the ELLL is received in lamina VI of the torus, whereas amplitude information is received in laminae III,V,VII,VIIIB and D, and IX (Carr et al.,J.Comp.Neurol.203:649-670,1981). Since amplitude information from the ELLL projects to so many different laminae of the torus, it seems plausible that a functional differentiation of the 3 tuberous maps is reflected in differences in their efferent projections to the torus. This possibility is being examined by intracellular labelling of the basilar and nonbasilar pyramidal cells of the ELLL. Preliminary results indicate a large variation in the toral projection patterns of pyramidal cells which appears to be correlated with their origin.

122.4 NEURAL CORRELATES OF THE JAMMING AVOIDANCE RESPONSE: DF-SIGN-SPECIFIC NEURONS IN THE OPTIC TECTUM OF THE ELECTRIC FISH EIGENMANNIA.
G. Rose and W. Heiligenberg, Scripps Inst. of Oceanogr., UCSD, La Jolla, Ca. 92093.

Behavioral studies have demonstrated that Eigenmannia is able to discriminate whether the electric organ discharges (EODs) of a neighbor are of lower or higher frequency than its own: In its famous Jamming Avoidance Response (JAR), the animal shifts its own EOD frequency away from interfering signal frequencies. When the EOD of one fish is similar in frequency to that of a neighbor the two EODs sum to form a sinusoidal signal which "beats" at the difference frequency (Df) of the interfering EODs. This beat is characterized by a periodic modulation of the amplitude and of the phase (the timing of positive zerocrossings) of the signal. While the animal can determine the magnitude of Df from the rate of the amplitude modulation alone, discrimination of the sign of Df, i.e, whether the neighbor's frequency is higher or lower, requires additional evaluation of the phase modulation. Specifically, with the neighbor's EOD having a lower frequency, the area of the animal's body surface with the largest signal modulation will experience a rise in amplitude during a phase lead and a fall in amplitude during a phase lag of this signal in reference to the signal in some other part of the body surface. The reverse association holds if the neighbor's EOD frequency is higher.

Extensive extra- and intracellular recordings in the electrosensory lateral line lobe (ELLL) of the hindbrain and the torus semicircularis of the midbrain, which receives afferents from the ELLL, have failed to find cells with a Df-sign-specific firing rate. Toral cells, however, have been found (see abstract by Heiligenberg and Rose), whose firing pattern reflects the sign of Df, and the joint evaluation of their responses by higher-order neurons should yield unambiguous information about the sign of Df. These toral cells project to the optic tectum, and tectal cells within the lamina of their projection have now been discovered which obviously perform this computation. These cells fire only under a stimulus regime which elicits JARs and only for one particular sign of Df. They are silent for the opposite sign of Df. The details of their response properties, morphology and projections will be presented.

122.5 NEURAL CORRELATES OF THE JAMMING AVOIDANCE RESPONSE: THE TORUS OF THE ELECTRIC FISH EIGENMANNIA PROCESSES AMPLITUDE AND PHASE INFORMATION.
W.Heiligenberg and G.Rose, Scripps Inst. of Oceanogr., UCSD, La Jolla, CA 92093.

The Jamming Avoidance Response (JAR) is a shift of the animal's own electric organ discharge (EOD) frequency away from a similar EOD frequency of a neighbor. This behavior is controlled by modulations of amplitude and differential phase, i.e. local differences in the timing of zerocrossings, which characterize the sinusoidal signal which results from the interference of the two EODs. In order to explore neuronal mechanisms of amplitude and phase evaluation we recorded intracellularly in the torus semicircularis, a richly laminated midbrain structure which receives somatotopically ordered electrosensory afferents from the electrosensory lateral line lobe (ELLL) of the hindbrain. Fish, with their own EOD silenced by curarization, were placed in a two-compartment chamber such that electrically separate sinewave stimuli could be applied to the head and tail region of the body respectively. In this manner, the phase difference between the stimuli applied to the two regions of the body could be modulated without concurrent modulations in stimulus amplitude. Units insensitive to pure amplitude modulations but strongly driven by small phase differences between the stimuli in the head and tail compartments were found almost exclusively in lamina 6. This phase sensitivity results from an inhibitory mechanism, since removal of the reference (unmodulated) stimulus in the other compartment causes the cell to fire tonically; return of the reference signal leads to a complete suppression of activity during either a small phase lead or a small phase lag. The phase sensitivity of these cells was expressed regardless of whether stationary phase values or modulations were used.

Phase-sensitive neurons were also found in the deeper laminae (notably 8b,c). In addition, most of these neurons could also be driven by pure amplitude modulations: E-type units responded to a rise in stimulus amplitude, I-type units responded to a fall in stimulus amplitude. These units could also be classified in terms of their preference for either a phase lead or a phase lag of the signal in the region of the body most sensitive to amplitude modulations. This led to the classification of E-advance, E-delay, I-advance and I-delay units. Reconstruction of Lucifer-labelled units indicate that all four cell types as well as cells which respond to amplitude modulations exclusively, project to the tectum opticum.

122.6 NEURAL CORRELATES OF THE JAMMING AVOIDANCE RESPONSE:THE CELL TYPES OF THE TORUS OF THE WEAKLY ELECTRIC FISH,EIGENMANNIA.
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Eigenmannia produces a high frequency electric organ discharge(EOD) which it uses for electrolocation and social interactions. These fish possess a jamming avoidance response (JAR) whereby they are able to raise or lower the frequency of of their EOD so as to avoid being jammed by a conspecific of a similar frequency. The JAR requires that the fish be able to evaluate the simultaneous changes in amplitude and phase which occur when its signal is contaminated with that of a neighbour's, and then that it produce an appropriate shift in its EOD frequency.

Behavioral,physiological and anatomical studies have demonstrated that the combination of phase and amplitude information necessary for the evaluation of the JAR must occur in the midbrain dorsal torus semicircularis(torus). The torus is a large laminated midbrain nucleus which is analagous to the inferior colliculus. It receives phase and amplitude information through two separate channels from the electrosensory lateral line lobe in the medulla.Phase-coding afferents terminate in the contralateral lamina VI ofthetorus, while amplitude-coding afferents project to laminae IX,VIIID and B,VII,V and III. The fish must combine this phase and amplitude information in order to perform the computations for the JAR. This can only occur in the torus,as phase and amplitude information are segregated up to this level, and phase input is restricted to lamina VI which has no efferent projections. A number of different cell types have been found in the torus which respond to these stimuli (Heiligenberg and Rose, abstract this volume),particularly combinations of amplitude and phase.

The torus has twelve laminae, and fifty-three cell types as determined by Golgi methods. There are three major orientations to the dendritic fields of the torus neurons: (1)purely horizontal with dendrites confined to a single lamina, (2) multipolar cells whose dendrites often do not respect lamina boundaries and (3) vertical cells which have dendrites that travel in the vertical bundles or columns of dendrites and axons which pierce the torus at regular intervals. These cells are able to receive and combine input from a number of different laminae. The neurons of the torus will be described and,where possible, their structure correlated with function. In particular the types involved in processing amplitude and phase information for the JAR will be compared with those identified by intracellular dye injection.

122.7 THE EFFECTS OF STIMULUS MODALITY, STIMULUS INTENSITY, AND SUBJECT SPECIES ON THE NOVELTY RESPONSE OF PULSE-TYPE GYMNOTOID FISH. Harold J. Grau (SPON: L.Devenport). Dept.of Zool.,Univ. of Oklahoma, Norman, OK 73019.

Pulse-type weakly electric fish generate Electric Organ Discharges (EOD's) at relatively regular frequencies ranging from 4-25 Hz. Gymnotoid pulse species will momentarily increase their EOD frequency when presented with a novel stimulus. This novelty response (NR) can be elicited by electroreceptive, visual and acoustico-lateral stimuli. The magnitude of the NR will habituate.

54 Hypopomus (4 species) and 15 Gymnotus carapo were tested under a paradigm designed to examine the effects of stimulus modality, stimulus intensity, and subject species on the magnitude of the NR and on the rate and amount of NR habituation. Subjects were presented with a series of 32 electric, visual, or acoustic stimuli, and the magnitudes of the sequentially evoked NR's were estimated. I plotted these responses as a function of time and found the exponential curve that best fit the data. The exponent of this curve was used as a measure of habituation rate. The amount of habituation was estimated as the ratio of the habituated to maximum response magnitudes. Main treatment effects were tested by a 3-way ANOVA.

Stimulus intensity had no significant effect on habituation rate; however, higher stimulus intensities significantly increased the amount of habituation ($P<.019$), and the initial ($P<.024$), maximum ($P<.008$), and habituated response magnitudes ($P<.009$). Stimulus modality had no significant effect on response magnitudes. However, in both genera habituation rates to acoustic stimuli differed from those due to electric or visual stimuli. The latter did not differ from each other. G.carapo habituated to acoustic stimuli more slowly than to electrical or visual stimuli ($P<.05$), whereas Hypopomus habituated to acoustic stimuli significantly faster than to electrical or visual stimuli ($P<.0001$). The habituation rate to acoustic stimuli was slower in G. carapo than in Hypopomus ($P<.0001$). Species did not differ in the rate or amount of habituation to electrical or visual stimuli. Species did differ significantly in all response magnitude measures ($P<.0001$). There was no correlation between the rate and amount of habituation, or between the rate of habituation and response magnitude.

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122.8 PROPERTIES OF DESCENDING INPUTS TO A PRIMARY SENSORY PROCESSING AREA, THE ELECTROSENSORY LATERAL LINE LOBE OF WEAKLY ELECTRIC FISH. J. Bastian. Dept. of Zoology, University of Oklahoma, Norman, OK 73019.

The Electrosensory Lateral Line Lobe (ELLL) of Gymnotiform electric fish receives afferent electrosensory information via the anterior Lateral line nerve as well as descending inputs from electrosensory regions of the midbrain. The neuroanatomy of the ELLL as well as that of the sources of the descending input has been previously described as have the physiological responses of the major efferent cell types of the ELLL. The relative simplicity of this structure coupled with the detailed information about its anatomy and physiology make it a good candidate for studies of the integration of ascending and descending inputs.

Microinjection of the local anesthetic Xylocaine was used to reversibly block the electroreceptor afferents or the descending input to the ELLL from the midbrain N. Praeeminentialis. Two categories of the ELLL neurons, E-cells (basilar pyramidal cells) and I-cells (non-basilar pyramidal cells), were studied. The typical E-cell response to a 100 ms, 2mV/cm stepwise increase in electrosensory stimulation consists of a brief, approx. 25 ms, very high frequency burst which rapidly adapts to an intermediate frequency that is sustained for the duration of the stimulus. Removal of the descending input results in E-cell responses becoming "receptor-like". The response becomes very slowly adapting and persists for the duration of the stimulus. Removal of the receptor afferents eliminates all but the early 25 ms high frequency burst of activity and the cell is silent for the rest of the stimulus period. The E-cell responses are made up of two components, the ascending component which is due to electroreceptor afferents from the ipsilateral side of the body and the descending component, ultimately due to electroreceptor activity from the contralateral and ipsilateral sides of the body. Similar results are obtained for I-cells. The effects of descending inputs on the ELLL cells' frequency response characteristics and on their responses to more natural, moving object, stimulation will be presented.

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122.9

WITHDRAWN

122.10 SEX-COLOR CHANGES EVOKED BY BRAIN STIMULATION IN FISHES. L.S.Demski, J.G. Dulka* and P.J. Hornby*. School of Biological Sciences, University of Kentucky, Lexington, KY 40506 and Gulf Coast Research Laboratory, Ocean Springs, MS 39564.

Serranus subligarius is a small sea bass plentiful along the Gulf Coast. The fish is unique in being a synchronous hermaphrodite with several distinct male and female color patterns which can change within seconds (Demski and Dulka, Amer. Zool. 23:881). We have studied Serranus using brain stimulation to identify areas controlling the sex-related color changes.

Electrical stimulation of the brain was carried out in fish anesthetized in 2% urethane and then maintained with seawater perfusion. Techniques previously described (Bauer and Demski, J. Exp. Biol. 84:149) were used to evoke color changes with 50 Hz stimulation up to 150 μA. Sex-related color changes were elicited on 45 dorsoventrally directed electrode tracts in 25 fish. Seventeen sites (12 animals) with thresholds at or below 100 μA were identified using the Prussian blue method. The male banding pattern was most readily elicited while the typical female dark phase was observed in only a few cases e.g. after prostaglandin treatment and in fish with freshly ovulated eggs. A reverse V pattern, typical of the "female" just before spawning climax, occurred during stimulation but more frequently was an after-response. All three patterns resulted from stimulation in the thalamus (9 sites). Banding was evoked from isolated points in the preoptic area, forebrain bundles, tuberal region and tegmentum while darkening resulted from stimulation of single points in the area ventralis telencephali pars supracommissuralis (Vs) and optic fibers near the preoptic area. The results suggest that the thalamus is a major controller of sex-color change. Visual pathways are likely to be important in this regard since the color patterns probably function as signals and the thalamus of teleosts receives a strong projection from both retina and tectum (see Braford and Northcutt, Fish Neurobiology, Vol. 2, Univ. of Michigan Press, 1983, p. 117). Other regions known to be involved in sex behavior in fishes e.g. preoptic area and Vs are apparently also involved in modulating the color changes.

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123.1 ALTERATION IN ^{3}H-NITRENDIPINE BINDING IN BRAIN FOLLOWING AMYGDALA KINDLING IN JUVENILE RATS.

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As part of an ongoing study on kindled seizures in juvenile rats, calcium channel antagonist binding was studied. Rats were implanted with bipolar electrodes in the right amygdala on postnatal day 23 and randomly assigned to a kindled or a control non-kindled group. Kindling stimulations were started on day 28, on an hourly basis, with 10-12 stimulations per day. Stimulations were continued until two consecutive stage 5 convulsions were achieved, followed by no further stimulations. On postnatal day 48, rats were sacrificed and brains rapidly frozen immediately at -40°C.

Binding studies were performed using crude synaptic membrane fractions from 10% brain homogenates incubated with 0.2 nM ^{3}H-Nitrendipine (a specific calcium antagonist) in a final volume of 1 ml of 50 mM Tris buffer (pH 7.4). Nifedipine (1 uM) was used to define nonspecific binding. Samples were incubated at room temperature in the dark for 90 min.,followed by filtration through GF/C glass fiber filters and 3 washes of 3 ml of cold 50 mM Tris buffer.

The results indicate a significant increase in ^{3}H-Nitrendipine binding in kindled brains compared to age-matched unkindled controls. Binding values (fmoles/mg protein ± SEM) were 30.3 ± 3.7 (N=5) and 54.9 ± 5.5 (N=6) for brains obtained from control and kindled animals, respectively. Saturation curves from kindled and non-kindled animals, using 0.05-1.0 nM ^{3}H-Nitrendipine, showed Bmax (fmoles/mg protein) of 89.92 and 35.49 and Kd (nM) of 0.191 and 0.104, respectively.

These results demonstrate that the specific binding of ^{3}H-Nitrendipine is altered by amygdala kindling in juvenile rats and suggest that changes in calcium channels may be associated with the acquisition and retention of kindled seizures.

123.2 AMYGDALA KINDLING AND ALTERATIONS IN BETA ADRENERGIC RECEPTORS IN JUVENILE RATS TREATED WITH 6-HYDROXYDOPAMINE AS NEONATES.

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Accelerated amygdala kindling following catecholamine (CA) depletion is established in adult rats. We investigated the role of central CAs in kindled seizures in developing rats by administering intracisternal 6-hydroxydopamine (6-OHDA) or saline on postnatal (PN) day 1 (100 ug) and 2 (50 ug). On PN day 23, the rats were implanted with a bipolar electrode in the right amygdala. Kindling stimulations were initiated on PN day 28, administered each hour, 10-12 per day, through two consecutive stage 5 convulsions after which rats received no further stimulations. On PN day 48, kindled and non-kindled rats were killed, brains rapidly removed, and the forebrains hemisected and frozen at -40° C for beta receptor analysis. The hemisections were assayed for ^{3}H-DHA (3 nM) binding using 2 uM l-propranolol for nonspecific binding. Saturation curves (.25-12 nM) on 3-4 hemisections pooled from rats from different litters were performed.

Control rats (N=6) required 26.2±1.4 afterdischarges (AD) to kindle and accumulated 42% of total AD seconds to kindle during the early stages of kindling (stages 0-II). By contrast, the 6-OHDA treated rats (N=7) required significantly fewer AD's to kindle (64% of control) and accumulated only 13% of total AD seconds during the early stages. The first AD in the treated rats was significantly longer (56.9±9.7 vs. 19.2±3.3 sec) and was often associated with stage 3 or 4 convulsions.

Receptor analysis revealed a 35% increase in ^{3}H-DHA binding in 6-OHDA treated rats in both hemispheres compared to saline controls (98.3±12.9 vs. 73.1±5.2 fmoles/mg protein). Kindling resulted in a significant 41% increase in binding in the stimulated hemisphere of saline treated rats, but no change in 6-OHDA rats compared to their non-kindled controls. No significant kindling-related changes in binding were found in the non-stimulated hemispheres of saline or 6-OHDA treated animals. The increased binding changes were related to increases in Bmax with no change in affinity.

Although the facilitation of kindling in juvenile rats following neonatal 6-OHDA parallels results in adult rats, the long-term decrease in beta receptors found in adult rats (McIntyre and Roberts, Exp.Neurol. 82:17-24,1983) was not present; in fact, a significant increase was found. These results suggest that neurochemical adaptations associated with kindling in the developing brain may be different from those in the adult brain. (PHS-BRSG 2 S07 RR0577-05)

123.3 EFFECTS OF KETAMINE, PHENCYCLIDINE AND SELECTED ANTICONVULSANT DRUGS ON HIPPOCAMPAL KINDLED SEIZURES.

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Ketamine has been reported to both suppress and precipitate seizures in experimental animals and man. Since ketamine excites neuronal activity within the limbic system, it may be more likely to provoke seizures with a limbic origin. Male Sprague-Dawley rats were implanted with bipolar depth electrodes in the dorsal hippocampus and 3.5x threshold current (1 sec, 60 Hz, 1.0 msec biphasic pulses) was repeatedly delivered until generalized clonic motor convulsions developed. Ketamine, phencyclidine and several anticonvulsant drugs were evaluated against the kindled seizure threshold, the duration of recorded electrical spike activity and the severity of convulsive motor symptoms.

Ketamine caused a dose-dependent elevation of the kindled seizure threshold and a dose-dependent decrease in seizure duration and severity; an anesthetic dose (80 mg/kg) increased the threshold 9-fold, shortened seizures by 50% with minimal motor symptoms. Thus, ketamine appears to decrease rather than increase susceptibility to hippocampal seizures. At cataleptic doses (15-20 mg/kg), phencyclidine produced similar non-selective anticonvulsant actions although it was less effective than ketamine.

Each anticonvulsant drug produced a different profile of effects against hippocampal kindled seizures. Carbamazepine at 25 mg/kg increased the threshold by 7 fold without producing observable effects on behavior, but did not decrease seizure duration. Diazepam at a dose that produced mild sedation (2 mg/kg), decreased seizure duration by 75% and totally suppressed motor convulsions; however, diazepam had no effect on seizure threshold even at higher doses. Phenobarbital (50 mg/kg) and valproic acid (150 mg/kg) elevated the threshold to a lesser degree than carbamazepine and also shortened seizure duration and minimized clonic motor symptoms. Phenobarbital was more effective than valproic acid in raising the threshold but valproic acid provided more protection against convulsive symptoms and seizure duration. The hippocampal seizure model may have predictive value since the anticonvulsant drugs produced effects against hippocampal kindled seizures consistent with their use in the treatment of epileptic disorders.

123.4 THE FACILITATING EFFECT OF FLUROTHYL-INDUCED GENERALIZED SEIZURES ON KINDLING IN ADULT RATS.

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In a previous study (Devel. Brain Res., 1984), we found that generalized seizures in infancy do not increase the susceptibility to kindled seizures later in life. To evaluate whether this effect is an age-related phenomenon, we studied the effects of flurothyl induced generalized seizures on kindling in adult rats.

Thirteen Sprague Dawley adult male rats were exposed to a total of 3 flurothyl induced generalized seizures at 8-day intervals. Subsequently, the experimental group(N=13) and naive controls(N=11) were implanted with a bipolar electrode in the right amygdala. After a 5-day recovery period, each rat was stimulated with increments of 50μA, 60Hz, 1 sec current until an afterdischarge (AD) was elicited and then reduced once by 25μA. The lowest current that elicited an AD was defined as the AD threshold and considered to be an index of the local epiletogenicity of the amygdala. The rats then were kindled with hourly stimulations (400μA, 60Hz, 1sec) and the kindling rate was determined, defined as the number of AD producing stimulations required for the development of the first generalized seizure.

There were no significant difference in the AD thresholds of the amygdala between the two groups (mean±SD, 108±40 and 179±103 uA for experimental and control groups, respectively). On the other hand, the kindling rate was significantly facilitated in the rats previously subjected to generalized seizures (experimental group=5.9±2.0, control= 11.3±2.8, $p<0.01$).

These results indicate that in adulthood flurothyl induced generalized seizures produce a bimodal effect on kindling. Although they do not alter the local epileptogenicity of the amygdala, they markedly enhance the development of bilateral kindled seizures perhaps by remodelling specific neurocircuits responsible for the control of seizure propagation. The data suggest that the neuronal mechanisms accounting for the development of a permanent seizure disorder differ with age.

123.5 LINDANE EXPOSURE INCREASES DENTATE GYRUS EXCITABILILITY TO PERFORANT PATH STIMULATION IN THE RAT. R. M. Joy and T. E. Albertson. Health Sciences Neurotoxicology Unit, Schools of Medicine and Veterinary Medicine, University of California, Davis, CA 95616.

Lindane markedly enhances the rate of acquisition of kindled seizures in rats. This enhancement has been demonstrated for kindling produced by amygdaloid and hippocampal stimulation. While these findings indicate that lindane can significantly alter the reaction of the limbic system to induced, repetitive afterdischarge, little is known of the direct effects of lindane on cellular excitability at these loci. We report here an analysis on the response of dentate gyrus cells to perforant path stimulation.

Rats were anesthetized with Chloropent. Electrodes were positioned to stimulate the perforant path and to record the monosynaptic-induced response in dentate gyrus. Stimulus intensities were varied to determine: 1) the threshold for generation of the population EPSP, 2) the amplitude of the EPSP at a fixed voltage which did not elicit cell discharge, as indicated by the lack of a population spike, 3) the threshold for appearance of the population spike, and 4) the maximal amplitude of the spike. Pairs of pulses 30 msec apart were used to evaluate the inhibitory effect induced by the first pulse on the response to the second. Effects of lindane were compared to those of solvent administration at equivalent time periods after anesthesia.

Lindane (10 mg/kg, ip, in DMSO) did not change the population EPSP threshold (100% ± 1.9% [$\bar{X}$ ± S. E.]) nor its amplitude (111% ± 11.7%). In contrast the population spike threshold was decreased (90% ± 3.5%) and the maximal population spike amplitude was increased substantially (262% ± 40.8%). Under anesthesia the inhibition induced by the first of the paired stimuli blocked completely the population spike when the second stimulus was applied 30 msec later. This blockade of the population spike was not reduced by lindane, suggesting no major deterioration of recurrent collateral inhibition.

These data indicate that lindane increases dentate pyramidal cell excitability to activation via the perforant path in anesthetized subjects. The lack of effect on EPSP threshold and amplitude support a postsynaptic locus of effect. A reduction in recurrent collateral inhibition does not seem to play a major role in the effects observed. (Supported by NIH Grant Nos. BRS 2S07-RR5457 and 2S07-RR05684, and by the Health Sciences Neurotoxicology Unit.)

123.6 Chlorinated Hydrocarbon Pesticides and Amygdaloid Kindling. T.E. Albertson, R.M. Joy and L.G. Stark. Health Sciences Neurotoxicology Unit, Schools of Medicine and Veterinary Medicine, University of California, Davis, CA 95616.

Previous studies have shown that exposure to either lindane or dieldrin enhanced the rate of acquisition of kindled amygdaloid seizures (KAS) in rats. (Joy, et al., Neurobehav. Tox. Terat. 2:117,1980 and 4:347,1982). To examine whether this enhancement generalizes to all CNS active chlorinated hydrocarbon pesticides, the effect of daily administration of DDT or a single exposure of chlorodecone (kepone) on the acquisition of the KAS was examined. After 4 days of pretreatment, daily oil or DDT (5,10 or 20 mg/kg) exposures continued 60 min. before daily kindling stimulations until all animals had kindled (Rank 5) seizures. Additional animals were then treated with one dose p.o. oil or kepone (50 mg/kg). Daily amygdaloid kindling stimulations began 24 hrs. later until all animals were kindled. Kepone treated animals demonstrated pronounced tremors 6-9 days post exposure. High dose DDT treated animals showed moderate tremors during treatment. Table 1 compares the results of DDT, kepone, lindane and dieldrin on the acquisition of KAS.

Table 1.

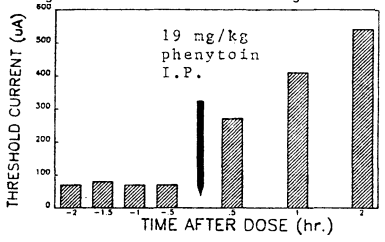
Class/Drug	Dose (mg/kg)	N	Days to Kindle (% Cont.)	Length of First Kindled A.D. (% Cont.)
Hexachlorocyclohexanes				
Lindane	1	13	86%	111%
	3	7	56%**	105%
	10	14	43%**	103%
Cyclodienes				
Dieldrin	2.5	7	60%*	96%
	5	10	63%*	94%
Dichlorodiphenylethanes				
DDT	5	8	86%	95%
	10	9	90%	53%
	20	9	82%	103%
Complex				
Kepone	50	10	100%	89%

*$P \leq .05$; **$P \leq .01$ compared to control.

These data demonstrates that not all CNS active chlorinated hydrocarbon pesticide exposures result in enhancement of KAS acquisition. The KAS model of epilepsy appears to differentiate between the neurotoxic consequences of chlorinated hydrocarbon pesticides that cause myoclonus and seizures (e.g. lindane and dieldrin) and those that primarily cause tremors (DDT and kepone). (BRS2S07 RR05457 and RR05684).

123.7 EFFECTS OF ANTICONVULSANT AGENTS UPON AFTERDISCHARGE THRESHOLD IN KINDLED RATS. M. G. Vartanian*, C. P. Taylor Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI. 48105.

The kindling phenomenon as first described by Goodard et al, (Exp Neurol 25: 295, 1969) is characterized by a progressive development of seizures to a repeated, local electrical brain stimulation. At first, only focal afterdischarges occured in response to hippocampal stimulation, but with repetition (500 μA biphasic pulses, 60 stimuli/day for 2 days) the focal discharges spread to become fully generalized behavioral seizures. The amount of electrical stimulus current required to elicit an afterdischarge became stable and low over time. These changes appeared to be permanent. Effects of seven anticonvulsant agents (carbamazepine, phenytoin, sodium valproate, phenobarbital, ethosuximide, clonazepam, and primidone) were examined in rats that were previously kindled by hippocampal stimuli. Threshold measurements from a single kindled animal before and after phenytoin are shown below. All drugs elevated the threshold electrical current for producing focal afterdischarges. Median ataxic doses of the same drugs were determined in a separate population of animals in order to compare central side effects. Carbamazepine and phenytoin were the most effective in elevating the threshold for afterdischarges without causing ataxia, while valproate and phenobarbital were somewhat less effective. Clonazepam and primidone were relatively ineffective at the doses tested; ethosuximide increased AD threshold, but only at ataxic doses. These results indicate that the drugs of choice for complex-partial seizures in humans are also the most effective in elevating hippocampal afterdischarge threshold without causing ataxia in rats.



123.8 SUPPRESSION OF PENCILLIN INDUCED EPILEPTIFORM ACTIVITY BY LOCUS COERULEUS (LC) OR NOXIOUS STIMULATION. R.S. Neuman, Fac. of Medicine, Memorial University, St. John's, Nfld., Canada, A1B 3V6.

There is now considerable evidence that norepinephrine (NE) has a role in suppressing or reducing the severity of seizures in several animal models of epilepsy although the mechanism underlying this suppression remains unknown. To further explore the role of NE, the effects of LC stimulation and noxious stimulation (known to activate LC neurones) were investigated on epileptiform activity induced by focal application of pencillin G (PG).

Rats, anaesthetized with 1.25 g/kg urethane i.p., were mounted in a sterotaxic frame. Low frequency electrical activity (0.5 to 75 Hz) was recorded from the cortex (1mm below dural surface) using the centre barrel of a 5 barrel micropipette. PG was applied from another barrel of the pipette by iontophoresis or pressure ejection. A Rhodes bipolar stimulating electrode was aimed at either the LC (pos. 1.5 lambda, 1.1 lat, 6.5 ver) or dorsal noradrenergic bundle (DB) (ant. 1.5, 0.5 lat, 6.5 ver.).

Application of PG (150-200 nA or 0.6-1.4 kg/cm^2) for 5 to 30 min. resulted in the appearance of continuous epileptiform activity consisting of large amplitude spikes and high frequency activity which could be recorded for several hours while PG application continued. Stimulation in or near the LC or DB (0.047-0.1 mA, 10-100 Hz) suppressed the epileptiform activity within seconds. The suppression outlasting the period of stimulation. The duration of suppression following stimulus offset was related to stimulus parameters (amplitude, freq. and stimulus duration) and the "severity" of the epileptiform activity. When PG was ejected with larger pressures (1.6-2.2 kg/cm^2) very large "interictal" spikes resulted on which LC or DB stimulation were only marginally effective.

Noxious somatic stimulation (mini-gator clip) applied to the tail or noxious odor (ammonia hydroxide or acetic acid) also suppressed epileptiform activity. The suppression continued for the duration of stimulus application and outlasted stimulus offset. As with LC stimulation, interictal spikes were effected to a smaller extent than other less "syncronized" activity.

Supported by the MRC(C).

123.9 POSSIBLE INVOLVEMENT OF GLYCINE IN UREA-INDUCED MYOCLONUS. M.H. Van Woert, E. Chung and F. Yocca*. Departments of Neurology and Pharmacology, Mount Sinai School of Medicine, New York, N.Y. 10029.

In clinical uremia, neurological symptomatology is prominent and includes generalized myoclonus, asterixis, and seizures. Intravenous injection of urea into cats has produced similar abnormal movements, which correlated with spike and sharp wave electrical discharges in the lower brain stem reticular formation, mostly in nucleus gigantocellularis (NRG) (Arch. Neurol. 27, 14, 1972).

We have found that unilateral local infusions of p,p'-DDT or strychnine into the NRG of the rat medulla induce bilateral stimulus-sensitive myoclonus (Expt. Neurol., in press). DDT is known to cause repetitive neuronal discharges in response to a single stimulus and we believe DDT produces myoclonus by this mechanism. Strychnine is a glycine receptor antagonist and may produce secondary hyperexcitability in reticular neurons by blocking inhibitory glycinergic input to this area. We have therefore, investigated whether urea might also block glycinergic neurotransmission in the rat medulla.

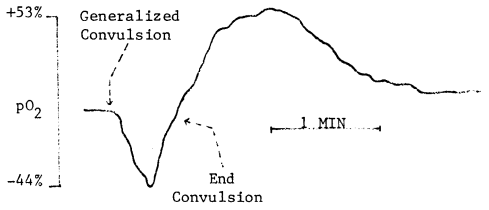
Male Sprague-Dawley rats weighing 125-150 g were injected with urea 2 g/kg i.p. every 15 min. After the 4th dose, all rats showed spontaneous myoclonus. At the time of maximum intensity of myoclonus, brain urea concentrations of these rats were 6.8 x 10^{-2}M, which is sevenfold greater than controls. Brain glycine levels did not change after urea injections. We next examined the effect of various concentrations of urea (10^{-4}M to 10^{-1}M) on glycine receptor binding using ^{3}H-strychnine as ligand. 10^{-2} and 10^{-1}M urea significantly decreased ^{3}H-strychnine binding by 30% and 43% respectively. Urea inhibition of ^{3}H-strychnine binding was reversible and binding kinetics revealed that 10^{-1}M urea decreased Bmax by 65% with no effect on affinity.

On the other hand, 10^{-4}-10^{-1}M urea had no effect on ^{3}H-GABA, ^{3}H-glutamate and ^{3}H-QNB receptor binding, indicating that interference with glycinergic neurotransmission may be a relatively specific action. The role of disinhibition of glycinergic neurotransmission by urea in clinical uremia deserves further investigation. (Supported by USPHS grants NS 12341, NS 17258 and The Gateposts Foundation).

123.10 IN VIVO HIPPOCAMPAL pO_2 TRANSIENTS DURING BICYCLIC ORGANOPHOSPHATE INDUCED SEIZURES IN THE RAT. T.J. Lynch, D.D. Walczak and J.L. Meyerhoff (SPON:V. Bates). Neurochemistry and Neuroendocrinology Branch, Walter Reed Army Institute of Research, Washington, DC 20307

Axon degeneration following status epilepticus may be the result of brain tissue hypoxia incurred during seizures. A particular vulnerability to anoxia of certain areas of the brain is evidenced by the gliomesodermal reaction appearing selectively in layers 3,5 and 6 of the neocortex, ammons horn and in cerebellar purkinje fibers following anoxic insult. Recently, Yan et al. (1982) reported a decrease in hippocampal blood flow during bicuculline seizures in rats, despite a concurrent increase in hippocampal 2-DG uptake. They cite this mismatch as a possible cause of hippocampal sclerosis in human temporal lobe epileptics, with the implication that hippocampal hypoxia may be the root cause.

Using the bicyclic organophosphate and GABA antagonist, ethyl phosphatrioxabicyclo octane (EPTBO), we monitored seizure-induced, relative pO_2 transients polarographically in dorsal and temporal pole hippocampus of free-ranging rats. In response to a convulsant dose of EPTBO (0.77mg/Kg, I.P.), hippocampal pO_2 was found to decrease by as much as 44% of baseline during the early tonic stages, but then to reverse and overshoot pre-ictal pO_2 by as much as 91% during the remaining tonic-clonic and postictal stages. Decreases in pO_2 after EPTBO exposure began with seizure generalization, while little or no change in pO_2 occurred during isolated myoclonic jerks. That transient hypoxia may occur often in this model is in contrast to the findings of Lynch and Jackson (1982) using the kindling model of epilepsy, in which the predominant ictal response in the amygdala was a significant pO_2 increase, even in the earliest stages of kindling. Thus the nature of the ictal pO_2 transient may be specific for both the site of measurement and the mode of seizure induction.



123.11 EVIDENCE FOR PROCONVULSANT EFFECT OF SOMATOSTATIN IN LIMBIC SEIZURES. E.W. Lothman, J.B. Perlin*, W.A. Geary. Dept. Neurology, Univ. of Virginia, Charlottesville, VA 22908.

As part of our laboratory's effort to define mechanisms involved in the regulation and spread of hippocampal seizures, the effects of systemic cysteamine were studied. Administration of this drug decreases somatostatin throughout the brain without changing levels of other neuropeptides. The mechanism by which this occurs is unknown, but the time course has been defined. Following a single injection of cysteamine, brain somatostatin is decreased at 2-4 hours, reaches its lowest levels at 24-48 hours and returns to normal after a week. Recurrent limbic seizures were elicited with electrical stimuli through bipolar electrodes implanted in the hippocampus of albino rats according to a stimulation paradigm previously described (Soc. Neurosci. Abstr. 8:1018, 1982). The severity of individual seizures was followed with a multi-stage behavioral seizure scoring (BSS) system and afterdischarge durations (ADD) measured at the site of stimulation. In animals that did not receive electrical stimuli, dose-response experiments showed that cysteamine 200 mg/kg i.p. did not cause seizures. However, for 2-4 hours after the same dosage ADD of electrically provoked seizures were prolonged and the associated behavioral convulsions were enhanced. Mean ADD and BSS were lowered from 1-3 days following the injection, but returned to pre-drug, baseline levels 3-4 days later. The suppression of limbic seizures seen at intermediate time points is similar to that shown by Higuchi et al. (Brain Res. 288:359, 1983) for epileptiform responses elicited from the amygdala. This result can be attributed to depletion of somatostatin which sustains limbic seizures once they have been triggered. To account for the early enhancement of seizures we suggest that cysteamine acutely releases somatostatin which then exerts a proconvulsant action.

123.12 ANTICONVULSANT AND PROPHYLACTIC EFFECTS OF A NEW THYROTROPIN RELEASING HORMONE(TRH) ANALOG:DN-1417 ON AMYGDALOID KINDLING IN RATS. M. Sato, M. Okamoto, T. Moriwake* and N. Ogawa*. Depts. of Neuropsychiatry and Neurochemistry of Institute for Neurobiology, Okayama Univ. Medical School, Okayama, Japan, 700.

TRH has been demonstrated to have several central actions independent of the pituitary-thyroid axis. In this study, intraventricular administration of DN-1417(γ-butyrolactone-γ-carbonyl-L-histidyl-L-prolinamide citrate) or saline was made to evaluate both anticonvulsant and prophylactic properties in kindling model of epilepsy. Thirty six male Sprague-Dauley rats(360-380 g) were used. These rats were stereotaxically implanted bilaterally in the amygdalae. Guid canulas were placed directedly toward the left lateral ventricle. One week later, stimulation was applied to the left amygdala once daily in 1-sec trains of 60 Hz sine wave until the stimulus elicited a generalized convulsion on 5 consecutive days(kindling sessions).

Firstly, the effects of intraventricular injection of saline(2 μL) or DN-1417(10, 20, 40 and 80 μg/2 μL of saline) on kindled convulsions were determined 30 min after the injection(n=7). To examine duration of the effect, the left amygdala was stimulated 10, 30, 60 min and 1 to 6 days after 40 μg of DN-1417 administration(n=7). Secondly, prophylactic effect of DN-1417 on kindling was tested. Two groups of rats(n=14, 7) were given saline or DN-1417(20 μg/2 μL) 5 min prior to each daily stimulus during kindling sessions. Kindling rates were compared between these two groups. Thirdly, non-kindled rats were decapitated 30 min after saline(n=4) or DN-1417(40 μg; n=4) administration. The frontal cortex, striatum, septum, thalamus, hypothalamus, amygdala plus pyriform cortex, hippocampus and brain stem were dissected for determination of TRH by RIA method.

The results obtained were: 1) DN-1417 has a potent, dosely related anticonvulsant effect on kindled amygdaloid seizures. Fourty μg of DN-1417 suppressed the seizure completely. 2) DN-1417 treatment retarded the development of kindling($p<0.01$). 3) Behavioral stereotypy, hyperemotionality and piloerection were observed after 40 μg of DN-1417 injection. 4) TRH plus DN-1417 level in rats treated with DN-1417 was elevated only in the frontal cortex(3.4-fold), as compared with control.

We conclude that DN-1417 has anticonvulsant and prophylactic properties in the kindling model of epilepsy.

123.13 EFFECTS OF REPEATED TREATMENT OF PENTYLENETETRAZOLE ON MOTOR ACTIVITY AND OPIATE RECEPTORS IN RAT. Y. Watanabe[1]*, T. Shibuya[1,2]*, S. Khatami[1]* and B. Salafsky[1] (SPON: E. Anderson). [1]Dept. of Biomedical Sci., University of Illinois College of Medicine at Rockford, Rockford, IL 61107-1897, [2]Dept. of Pharmacol., Tokyo Medical College, Tokyo, 160, Japan.

The effects of repeated administration of a convulsant dose of pentylenetetrazole (PTZ) on spontaneous motor activity and the changes in benzodiazepine and opiate receptor binding densities in cerebral cortex were investigated in the rat. PTZ (45 mg/kg) was injected I.P. daily for 7 days. On the first day of PTZ treatment all treated rats showed the tonic-clonic convulsion between 1.5 and 2.0 min after drug administration. The onset time for convulsive activity increased with each successive day of treatment up to day 6. During this period all animals convulsed from day 1 to day 4, but by day 5 and 6 relatively few of the animals convulsed. At day 7 no animals convulsed. Concommittantly, over-all motor activity was assessed in these same animals prior to injection of PTZ and for a period of 60 min after injection. Independent of convulsions, animals on days 1-3 tended to demonstrate hypoactivity whereas from day 3 to day 6 the animals were increasingly hyperactive. We also noted that body weight of these PTZ injected animals changed over the course of 7 days compared to controls. Significant lower body weight was found on day 2, whereas significant higher body weight was observed on day 8. The change in body weight paralled motor activity. Binding characteristics of [^{3}H] diazepam (DZP), [^{3}H] RX783006 (DAGO) and [^{3}H] D-ALa^2, D-Leu^5-enkephalin (DADLE) in rat cortex were compared in animals treated after 7 days (day 8) to control (untreated). Kd values were similar. However, Bmax measurements showed significant ($P<0.05$) differences in day 8 animals compared to control. In particular, the Bmax for [^{3}H] DADLE and [^{3}H] DAGO were decreased by 48% and 15%, respectively. In contrast, the Bmax for [^{3}H] DZP was increased by 15%. These results suggest that at least two different opiate systems in the cortex may play an important role in the mechanism of PTZ-induced convulsions.

123.14 SEIZURE-SPECIFIC, DOSE- AND TIME-DEPENDENT ANTICONVULSANT PROFILE FOR U50,488, A NOVEL κ OPIOID AGONIST, IN RATS. F.C. Tortella, L. Robles*, and J.W. Holaday Neuropharmacology Branch, Division of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, D.C. 20307.

The recently synthesized κ agonist, U50,488 {trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide}, displays opioid receptor-mediated actions in vivo, and selectively interacts with κ sites in binding studies (Lahti et. al., Life Sci. 31:2257,1982; VonVoightlander et. al., JPET 224:7, 1983). In view of the numerous studies demonstrating anticonvulsant profiles for several opioid agonists in various models of experimental seizures, and the potential non-addictive nature of U50,488, we sought to determine whether U50,488 protects against chemically- or electrically-induced seizures.

Male S.D. rats (250-300 g; n=4-15) were injected either i.c.v. (50-400 μg) or s.c. (5-80 mg/kg) with U50,488 and tested at 0.25-8 hr postinjection. Electrical seizures were produced via transauricular maximal electroshock (MES; 2 sec at 60 Hz, 50 mA). The duration of tonic forelimb extension (TFE) and the motor seizure pattern were recorded as measures of seizure severity. Chemical seizures were produced using flurothyl, a volatile convulsant. The latency to a clonic convulsion was recorded as the seizure threshold.

Injections of U50,488 resulted in a dose- and time-dependent protection against MES seizures. Anticonvulsant ED50s (95% confidence intervals) for s.c. and i.c.v. U50,488 were 8.58 (5.92-12.45) mg/kg and 103.78 (58.5-184.1) μg, respectively. Maximal effect following either route of injection occurred within 15-30 min. While the duration of action for i.c.v. U50,488 was short-lived (approximately 1 hr), the anticonvulsant effect of s.c. U50,488 was still evident 8 hr postinjection. Naloxone (1-10 mg/kg,s.c.) only partially antagonized the anticonvulsant effect of U50,488.

In contrast to the results obtained with MES, regardless of route of administration, dose, or time following injection, U50,488 had no clear effect on the threshold to flurothyl convulsions.

We conclude that U50,488 is an efficacious, long-acting anticonvulsant against MES seizures in rats. The results with naloxone suggest that this action is mediated by non-μ (probably κ) binding sites. More importantly, this structurally novel opiate may represent a new class of therapeutically effective anti-grand mal agents.

U50,488 generously supplied by P.F. VonVoightlander from Upjohn.

123.15 INTRACEREBROVENTRICULAR MORPHINE PRODUCES CONVULSIONS IN GENETICALLY EPILEPSY-PRONE RATS. J.J. Stewart, J.D. Rinaudo*, C.E. Reigel*, P.C. Jobe and J.W. Dailey. Departments of Pharmacology and Psychiatry, Louisiana State University School of Medicine, Shreveport, LA 71130.

Seizures in the genetically epilepsy-prone rat (GEPR) have consistently been demonstrated to be related to deficits in central norepinephrine (NE). GEPRs with the most severe seizures (GEPR-9s) have the most pronounced deficits in NE. GEPRs with moderate seizures (GEPR-3s) have lower NE levels than normal rats. Any manipulation which decreases synaptic NE in the GEPR increases seizure severity whereas manipulations which increase synaptic NE decrease seizure severity.

In addition to the apparent primary role of NE in the etiology of the epilepsy in the GEPR, these animals are also more susceptible to some other convulsant manipulations such as pentylenetetrazole and barbiturate withdrawal which may affect non-noradrenergic pathways. We have observed GEPRs to be uniquely susceptible to convulsions produced by the intracerebroventricular (i.c.v.) administration of morphine.

The i.c.v. administration of morphine has been reported to produce electrographic seizures in non-epileptic rats when given at high doses (Frenk, H., et al., Brain Res., 147: 327, 1978). The only behavioral correlates of these seizures were wet-dog shakes and, more rarely, myoclonic twitches of the trunk, head or limbs. In contrast, i.c.v. morphine produced a dose-related continuum of increasingly severe convulsive responses in the GEPR. Wet-dog shakes were the initial response regardless of dose. Responses to higher doses progressed to rearing accompanied by myoclonic twitches of the face, trunk or forelimbs. With still higher doses, sustained forelimb clonus preceded full tonic extention, which was the most severe convulsive response observed. In addition to increases in severity, latencies to a particular convulsive response decreased with increasing dosage. GEPR-9s were more sensitive to i.c.v. morphine convulsions than GEPR-3s which were far more susceptible than non-epileptic rats. (Supported in part by NIH Grant 16829).

123.16 A GABA-ERGIC CONVULSANT PROFILE IN THE GENETICALLY EPILEPSY-PRONE RAT. C.E. Reigel*, P.C. Jobe, T.W. Woods* and J.W. Dailey (SPON: K.W. Barron). Departments of Pharmacology and Psychiatry, Louisiana State University School of Medicine, Shreveport, LA 71130.

The seizure-prone state in the genetically epilepsy-prone rat (GEPR) is believed to be due to deficits in central norepinephrine (NE). Manipulations that decrease synaptic NE increase seizure severity whereas manipulations that increase synaptic NE decrease seizure severity. Consistent with this, GEPRs with severe seizures (GEPR-9s) have lower NE levels than GEPRs with moderate seizures (GEPR-3s) which have lower NE levels than seizure resistant control rats. Because of this, there is a tendency to view the difference in seizure severity between GEPR-3s and GEPR-9s as being due solely to the magnitude of noradrenergic deficits.

The results of a recently completed anticonvulsant profile in the GEPR suggests that GEPR-3s and GEPR-9s also differ in terms of non-noradrenergic systems. Consistent with the noradrenergic hypothesis, GEPR-9s were more sensitive to the anticonvulsive effects of agents capable of producing NE uptake blockade. However, GEPR-3s were either equal or more sensitive than GEPR-9s to the anticonvulsive effects of agents reported to work through GABAergic mechanisms.

These results suggested that GEPR-3s and GEPR-9s might also differ in GABAergic responsiveness. GABAergic inhibition can be augmented by agonist binding at three distinct, but functionally related sites: benzodiazepine receptors, GABA receptors and GABA chloride ionophores. Similarity, various agents are capable of producing convulsions through binding at these sites. In the present experiments, the convulsive thresholds of agents putitively acting at these GABAergic sites were compared in GEPR-9s, GEPR-3s and seizure resistant controls. Our goal was to provide further evidence that GEPR-3s and GEPR-9s also differ in GABAergic sensitivities.

All seizure thresholds were determined by continuous I.V. infusion to the onset of sustained seizure spiking on the EEG of restrained animals. As compared to control, GEPR-3s and GEPR-9s were resistant to bicuculline; GEPR-3s were resistant to flurazepam; GEPR-9s were more sensitive to pentylenetetrazole; and GEPR-3s and GEPR-9s were equally sensitive to picrotoxin. (Supported in part by NIH Grant 16829).

123.17 SEROTONIN AND 5-HYDROXYINDOLE ACETIC ACID CONCENTRATIONS IN THE HIPPOCAMPUS, STRIATUM AND REMAINING TELENCEPHALON OF THE GENETICALLY EPILEPSY-PRONE RAT. J.W. Dailey, T.W. Woods and P.C. Jobe. Departments of Pharmacology and Psychiatry, LSU School of Medicine, Shreveport, LA 71130.

We previously observed that deficits in CNS serotonergic activity may be partially responsible for the seizure-prone state of the genetically epilepsy-prone rat (GEPR). We found that serotonin concentration in the telencephalon is abnormally low in two types of GEPRs: those with moderate seizures and those with severe seizures. This is consistent with a hypothesis that a deficit in serotonergic activity in the telencephalon may be one neurochemical deficit responsible for seizure susceptibility in the GEPR. However, serotonergic deficits in this structure would not be a determinant of seizure severity since the decrement in serotonin levels was not greater in severe seizure subjects than in moderate seizure subjects. The purpose of this investigation was to further trace serotonergic deficiencies to more specific areas of the telencephalon and to initiate studies on the serotonin metabolite, 5-hydroxyindole acetic acid (5-HIAA). As an initial step, we separated the telencephalon into three parts: hippocampus, striatum and remaining telencephalon. Although the hippocampus of moderate seizure GEPRs had a deficit in serotonin and 5-HIAA levels, no such abnormalities were present in severe seizure subjects. The striatum was characterized by a deficit in serotonin content in severe seizure rats but not in moderate seizure GEPRs. 5-HIAA levels were abnormally low in striatum of both types of GEPRs. In the remaining telencephalon, serotonin levels were abnormally low in both moderate and severe seizure GEPRs. However, serotonin levels in these two types of epileptic rats were not significantly different from each other. 5-HIAA was abnormally low only in the telencephalon of moderate seizure subjects. These observations indicate that a serotonergic deficit in the striatum could regulate both seizure intensity and susceptibility in the GEPR. Serotonergic deficits in telencephalon may regulate seizure susceptibility but not severity. The data do not strongly support a role for serotonergic abnormalities in the hippocampus as determinates of either seizure susceptibility or severity in the GEPR. (Supported in part by NIH Grant 16829).

123.18 CATECHOLAMINE CONCENTRATIONS IN THE HIPPOCAMPUS, STRIATUM, AND THE REMAINING TELENCEPHALON OF THE GENETICALLY EPILEPSY-PRONE RAT. P.C. Jobe, T.W. Woods*, and J.W. Dailey, Departments of Pharmacology and Psychiatry, LSU School of Medicine, Shreveport, LA 71130.

We previously reported that deficits in central nervous system noradrenergic activity are important determinents of the seizure-prone state of the genetically epilepsy-prone rat (GEPR). Dopaminergic activity appears unrelated to seizures in these epileptic rats.

As part of previous investigations, we observed that telencephalon norepinephrine concentration was highest in nonepileptic control rats, intermediate in moderate seizure GEPRs and lowest in severe seizure GEPRs. The purpose of this investigation was to trace noradrenergic deficiencies to more specific telencephalon areas. As an initial step we separated the telencephalon into three parts: hippocampus, striatum and remaining telencephalon. Our results show progressive noradrenergic deficits only in the remaining telencephalon. In this brain part, norepinephrine concentration was highest in nonepileptic animals, intermediate in moderate seizure animals and lowest in severe seizure animals. In contrast, hippocampal norepinephrine decrements were not progressive across these three types of animals, although hippocampal norepinephrine levels of both types of epileptic rats were significantly lower than controls. In the striatum, norepinephrine concentration was normal in both the moderate and severe seizure subjects. No abnormalities in dopamine concentration in the striatum and the remaining telencephalon were detected in either type of epileptic rat. These results indicate that progressive decrements previously detected in the whole telencephalon are retricted to the structures remaining after the hippocampus and the striatum are removed. Therefore, noradrenergic decrements in this brain part may be determinants of both seizure susceptibility and intensity in the GEPR. Noradrenergic decrements in the hippocampus may be determinants of seizure susceptibility but they do not appear to regulate seizure intensity. Norepinephrine in striatum and dopamine in striatum and the remaining telencephalon do not appear to participate in seizure regulation in the GEPR. (Supported in part by NIH Grant 16829).

123.19 2-DG UPTAKE PATTERNS IN THE CNS OF GENETICALLY EPILEPSY PRONE RATS: A COMPUTER FACILITATED ANALYSIS. D.L. McEachron*, P.C. Jobe, W.K. Smith, D. Schlusselberg, D.J. Woodward and B.D. Waterhouse (SPON: R.M. Stewart). Dept. of Cell Biology, U. TX Health Sci. Ctr., Dallas, TX 75235 and Dept. of Pharmacology, LSU Med. Ctr., Shreveport, LA 71130.

The ^{14}C-2-deoxyglucose (2-DG) method was used to examine glucose utilization patterns in brain regions of control and genetically epilepsy-prone rats (GEPRs). Control animals and GEPRs were divided into sound stimulated (SS) and unstimulated (US) groups. All rats received an i.p. injection of 2-DG (14mCi/100 gr. in normal saline) and were placed in a plexiglass enclosure for 60 min. prior to sacrifice. Following 2-DG administration, SS animals received periodic auditory stimulation (5 sec. ring of a warehouse bell at 18 min. intervals) but only SS GEPRs responded with seizures as described previously (Jobe et al., J.P.E.T. 184:1-10) followed by postictal depression (10-15 minutes). Autoradiographs were prepared from brain sections according to standard procedures (Sokoloff, et al., J. Neurochem. 28:897-916) and then video digitized, linearized and color enhanced by a computer-based imaging system (Schlusselberg et al., Neurosci. Abst. 9:352). Visual examination of autoradiographs indicated that US GEPRs (n=2) had increased 2-DG uptake relative to US controls (n=3) in: 1) cerebellum; post hemispheres and deep nuclei, 2) lateral reticular areas; and 3) a band of neocortex corresponding to layer IV. The paraflocculi in US GEPRs also showed an increased uptake in granule cell layers compared to controls. SS GEPRs (n=3) exhibited increased glucose uptake relative to US GEPRs in all cerebellar areas, whereas SS controls (n=2) showed a more moderate increase compared to US controls. 2-DG uptake in lateral reticular and neocortical areas of SS GEPRs was less than that observed in US GEPRs. Moreover, thalamo-cortical 2-DG uptake ratios were increased in SS GEPRs relative to all other groups. SS GEPRs also displayed a prominent right-left asymmetry of glucose uptake in the inf. colliculi, auditory cortex, and possibly med. geniculate nuclei. These studies reveal a pattern of glucose utilization in resting GEPRs which suggests neuronal hyperactivity in brainstem, cerebellum and neocortex that contrasts with depressed activity in cortex and brainstem following seizures. Moreover, the data suggest a dysfunction within the GEPR auditory pathway. Overall, these experiments identify specific areas of the GEPR brain which maintain abnormal levels of glucose metabolism and may be involved in the seizure generating process. (NIDA DA02338 to DJW, NINCDS NS18081 and a Klingenstein Award to BDW.)

123.20 EFFECTS OF NOREPINEPHRINE AND BENZODIAZEPINE ON AMINO ACID-INDUCED RESPONSES OF CEREBELLAR PURKINJE NEURONS RECORDED FROM THE GENETICALLY EPILEPSY PRONE RAT. B.D. Waterhouse and P.C. Jobe, Dept. of Cell Biology, Univ. TX. Health Sci. Ctr, Dallas, TX 75235 and Dept. of Pharmacology, LSU Med. Ctr., Shreveport, LA 71130.

Previous studies in Long-Evans hooded or Sprague-Dawley albino rats have shown that microiontophoretically applied norepinephrine (NE) or the benzodiazepine flurazepam (FLUR) routinely potentiate GABA-induced depression of cerebellar Purkinje cell spontaneous discharge. In addition, local administration of NE facilitates glutamate (GLUT)-evoked excitation of Purkinje neurons relative to suppression of spontaneous firing rate. In the present study, we have examined the interactions of NE and FLUR with amino acid-induced responses of Purkinje cells recorded from genetically epilepsy prone rats (GEPRs). Multibarrel micropipets were used to deposit drug and record extracellular activity of Purkinje neurons in halothane-anesthetized GEPRs. Responses to microiontophoretic pulses of GABA or GLUT were examined before, during and after NE (0.5M) or FLUR (0.1M) iontophoresis. Peri-event histograms were used to quantitate effects of NE or FLUR on spontaneous activity and amino acid-induced responses. The spontaneous firing rate of Purkinje cells recorded from GEPRs (29.5Hz, n=25) was not significantly different from that of normal Purkinje neurons. Moreover, doses of GABA ($\bar{x}$=15.6nA) sufficient to suppress Purkinje cell activity by 30% in normal rats produced a comparable depression ($\bar{x}$=16.0nA, 32.5%) of firing rate in 22 Purkinje neurons recorded from seizure susceptible animals. However, in 72% (n=18) of GEPR Purkinje cells tested, responses to GABA were either unchanged (9 cells) or reduced (4 cells) during NE iontophoresis (1-50nA) despite suppression of spontaneous discharge. Augmentation of GABA by NE was observed in only 5 cases (28%), whereas FLUR (1-27nA) potentiated GABA inhibition in all 9 cells tested. By contrast, NE produced the expected enhancement of GLUT-evoked excitation in 3 of 4 Purkinje neurons recorded from seizure susceptible animals. In summary, the experiments conducted to date indicate that in genetically seizure prone rats, NE is ineffective in routinely augmenting GABA-mediated inhibition, whereas other electrophysiological indices of GABAergic, noradrenergic and benzodiazepine function appear normal. Such reduced efficacy of NE/GABA interactions in local neuronal circuits of GEPR brains could contribute to the increased seizure susceptibility of these animals. (Supported by NINCDS NS-18081 and a Klingenstein Award to B.D.W.).

123.21 INCREASED NUMBERS OF GABAergic NEURONS IN THE INFERIOR COLLICULUS OF THE GENETICALLY EPILEPSY PRONE RAT. R.C. Roberts*, C.E. Ribak, G.M. Peterson and W.H. Oertel (SPON: R.H. Blanks). Dept. of Anatomy, Univ. of Calif. Irvine, CA 92717.

The genetically epilepsy prone rat (GEPR-9) always exhibits severe generalized motor seizures in response to loud auditory stimuli. The inferior colliculus (IC) is suspected to be an important site of epileptogenesis for audiogenic seizures based on lesion, physiology and pharmacology studies. Previous studies have shown a defect in the GABAergic system in focal models of epilepsy. This study was undertaken to determine if differences in the number of GABAergic neurons in the IC occurs between the GEPR-9 and the non-epileptic, Sprague-Dawley strain of rat. Both types of animals received colchicine injections 24 hours prior to perfusion to enhance somal staining. GABAergic neurons in the IC were identified by immunocytochemical localization of glutamate decarboxylase (GAD). Four to eight sections throughout the rostral caudal extent of the IC were analyzed quantitatively at the light microscopic level. A dramatic increase in the number of GAD+ neurons is seen in GEPR-9 as compared to the Sprague-Dawley. This increase is most evident in the central region of the rostral-caudal axis of the IC where it can be as much as 200-300%. In contrast, the number of GAD+ neurons remains relatively constant throughout the rostral-caudal axis in the Sprague-Dawley rat. GAD+ cell types include large cells in the ventrolateral and dorsomedial region that have bitufted, fusiform and multipolar shapes. The increase in the number of GABAergic neurons in the GEPR-9 seems to be due to a selective increase in the small and medium-sized subpopulation of these neurons. Pharmacological evidence suggests that a deficit in GABA receptor sensitivity may occur in the IC of audiogenic seizure prone rats. These results might be consistent with our morphological observations if excessive amounts of GABA are available. Another possibility is that the increased number of GABAergic neurons are inhibiting other GABAergic neurons, resulting in disinhibition of the output cells. Then, a hyperexcitable state may be the result. In any case, these data demonstrate a difference in GAD+ neuronal populations within the inferior colliculus of the GEPR-9 as compared to the non-epileptic Sprague-Dawley.

(Supported by a Klingenstein Fellowship awarded to CER and NIH Grant NS-15669.)

123.22 INCREASED NUMBER OF GABAergic NEURONS IN THE HIPPOCAMPAL FORMATION OF SEIZURE - SENSITIVE GERBILS. G. M. Peterson, C.E. Ribak and W.H. Oertel. Dept. of Anatomy, Univ. of Calif., Irvine, CA 92717.

The GABAergic neuronal system was investigated in the hippocampal formation of two strains of Mongolian gerbil: a seizure-sensitive (SS) and a seizure-resistant (SR) strain. These animals exhibit seizures when exposed to a novel environment, the intensity of which is consistent over many testings. Therefore, it is possible to correlate a known history of seizure intensity with morphological observations. Since SS gerbils do not show seizure activity until approximately 50 days of age, it is also possible to compare the seizure-predisposed (SP) brains of young SS progeny with the brains of SS and SR to determine if any differences which may exist between SS and SR brains are present prior to seizure onset.

Immunocytochemical localization of glutamic acid decarboxylase (GAD) was used to identify the GABAergic neurons. Light microscopic counts were made of the GAD+ neurons in the dentate gyrus and hippocampus proper. In addition, cell size and terminal density were determined. In both the hippocampus proper and dentate gyrus more GAD+ neurons were found in SS brains than in SR. The number of these cells in SR brains was similar to that reported in normal rat. Within the hippocampus the increased number of GAD+ somata was seen mainly in regio inferior. The apical dendritic zone showed a 50% increase and strata pyramidale and oriens each showed a 20% increase. In the dentate gyrus of SS brains the suprapyramidal blade of stratum granulosum showed a striking increase in the number of GAD+ cells compared to the same region in SR brains. This increase was most marked in the septal half of the dentate gyrus where it amounted to nearly a 100% increase in selected brains. A similar increase in GAD+ neurons was also found in stratum moleculare. The SP brains displayed similar increases in these two strata. In all regions analyzed there was some variability in the number of GAD+ neurons between animals, but this variability appeared to correlate with seizure intensity. GAD+ neurons in stratum granulosum of SS brains were, on average, smaller than those in SR brains. In addition, GAD+ terminal density was also different between the two strains, with an increased density in the infrapyramidal blade of stratum granulosum in the SS animals. The increased number of GABAergic neurons in the dentate gyrus and regio inferior of SS animals, as well as in their immature offspring (SP) which had not exhibited seizure activity, suggests a genetic aberration which may be functionally related to seizure sensitivity. (Supported by a Klingenstein Fellowship (CER) and NIH grant NS-15669.)

123.23 REDUCED 2-DEOXYGLUCOSE UPTAKE IN AUDIOGENIC SEIZURE PRONE MICE. R.A. Schreiber, J. Serviere and A.G. Lehmann.* Lab. de Psychophysiologie Sensorielle, Universite Pierre et Marie Curie, 75230 Paris, France.

Mice susceptible to audiogenic seizures (AGS) might have some difficulty in brain glucose uptake, or storage, or utilization which results in a disruption in brain energy flux during the first few seconds of response to an intense acoustic stimulus. As a result, excitatory and inhibitory firing patterns may become unbalanced, and populations of neurons might then suddenly begin the paroxysmal discharges characteristic of an epileptic seizure on excessive acoustic stimulation.

Rb-1 and Rb-3 mice were selected from a common Swiss Webster parent stock, and manifest respectively, tonic seizures, and no seizure response on acoustic stimulation. Rb-1 mice become susceptible to AGS at 12-13 days of age. There is then a transition to full susceptibility by 18 days, which continues for up to three months. These mice are useful for AGS research in that subline differences are more likely to correlate with AGS, while strain differences between DBA and C57 mice, for example, may be solely fortuitous.

Uptake of ^{14}C-2-deoxyglucose (2-DG) into brain was determined in an attempt to find differences in uptake (K_1) and/or phosphorylation (K_3) rates between Rb-1 and Rb-3 mice. K_2 (transport from tissue to plasma) is negligible due to the low level of glucose-6-phosphatase in brain tissue. Animals received 100 µCi/kg of 2-DG intraperitoneally. Mice were sacrificed 45 min later, and brains were prepared for radioautography. All mice were experimentally naive. There were 4 Rb-1 and 4 Rb-3 male mice, 60 days of age. In every case Rb-1 mice showed significantly less 2-DG uptake than Rb-3 mice, and there are no evident regional differences.

This 2-DG experiment can not differentiate between K_1 and K_3. However, there does not seem to be any uptake problem in Rb-1 mice, as indicated by a rapid increase in cortical glucose after an intraperitoneal glucose injection. This will be subjected to further study.

Should these data generalize to other AGS-prone strains of mice, then susceptibility to AGS may be due to a reduced capacity to use sufficient glucose during the first few seconds of an intense acoustic stimulus to provide energy to maintain transiently heightened balanced firing patterns.

This work was supported by D.G.R.S.T. 81-E-0564 to J.S. This work was performed while R.A.S. was a 1982-83 Fogarty International fellow, with support from the NATO/Minna-James-Heinemann Stifftung Foundation.

123.24 PHOSPHORYLATION OF ALPHA AND BETA SUBUNITS OF BRAIN (Na+K+)-ATPase IN DBA/2J AUDIOGENIC MICE. D. Guillaume*, T. Grisar*, A.V. Delgado-Escueta. Molecular Neuroscience Lab, UCLA and Neurology & Research Serv., VA Wadsworth Med.Ctr., Los Angeles, CA 90073.

Conflicting results have been reported on brain (Na+K+)-ATPase activity in audiogenic (DBA/2J) mice compared to normal (C57BL). Furthermore, effects of phenytoin on the (Na+K+)ATPase still remain controversial. Hence, we investigated the effects of Na+, K+, and phenytoin on the phosphorylation of (Na+K+)ATPase subunits partially purified from C57 and DBA brain microsomes.

Partially purified (Na+K+)ATPase (modified from Jorgensen's methods with specific activities as high as 1000 µ moles of Pi/mg prot./hr) was incubated at 20°C with 3-5 µM [γ^{32}P] ATP with various ionic and phenytoin concentrations. Solubilized samples were subjected to SDS polyacrylamide gel electrophoresis (acrylamide concentration from 5-10%). In a 140 mM Na+ and K+ free medium, autoradiographs showed similar patterns in the 2 mice strains, namely, two main phosphorylated bands, at 95K [α-subunit appearing as 2 close bands: α(+) and α(-)] and at 50 K [β-subunit]. In both mice strains, 3 mM K+ decreased the phosphorylation of the α-subunit by about 85% with no effect on the β-subunit. On the other hand, phenytoin, in a dose dependent manner, decreased phosphorylation of α-subunit (by 60% at 10^{-4} M) and β-subunit (by 40% at 10^{-4} M) only in C57 mice. In DBA mice, phenytoin at the same concentrations had no effect on the catalytic α-subunit, while it decreased β-subunit phosphorylation (by 40% at 10^{-4} M). These effects of phenytoin on the catalytic α-subunit of the (Na+K+)ATPase in C57 mice suggest that phenytoin at least acts on the Na+,K+ pump. The absence of similar effects of phenytoin in DBA/2J mice suggests that the molecular structure of the enzyme is probably different in C57 and DBA mice.

This work was supported by grant N01-NS-0-2332 and the Belgian National Fund of Scientific Research for D.G.(FNRS).

123.25 CLONIDINE EXACERBATES ABSENCE SEIZURES IN THE MUTANT MOUSE TOTTERING, Allen H. Heller, Dept. of Neuroscience, Children's Hospital, Boston, MA 02115

Tottering mice, (tg, autosomal recessive) exhibit frequent (~50/hr) behavioral absence seizures associated with stereotyped spike-wave or polyspike electrocorticographic (EEG) discharges. Homozygotes feature an abnormal increased density of noradrenergic terminal fibers selective to neurons of the locus ceruleus with a 100-200 % increase of norepinephrine (NE) levels in terminal fields (Levitt and Noebels, PNAS 78:4630,1981). Consistent with an abnormal increase of noradrenergic activity, the concentration of the NE metabolite MHPG is increased in the tg CNS (Cumiskey, Ferrari, Haubrich, and Heller, unpublished).

To probe the role of the abnormal NE system in the pathophysiology of the tg seizure disorder, drugs known to affect central NE activity were tested for an effect on tg absence seizures. Absence seizures were monitored by continuous EEG recording following a single intraperitoneal injection of the test drug. Clonidine in a dose-related fashion (0.05-0.25 mg/kg) increased the mean duration of absence seizure episodes and the total intraictal time interval without appreciable effect on the number of seizure episodes. During the 1 hr period after clonidine 0.25 mg/kg, mean seizure episode duration increased to 13.2 $\pm$ 0.8 sec vs 2.6 $\pm$ 0.3 sec for saline control, ($p < 0.001$). This effect was entirely abolished by pretreatment with yohimbine 2.5 mg/kg, while yohimbine 2.5 mg/kg administered alone had no appreciable effect on absence seizure incidence or duration. Pretreatment with phenylephrine 32 mg/kg, reduced mean seizure episode prolongation due to clonidine: 12.7 $\pm$ 1.6 sec to 3.7 $\pm$ 0.4 sec ($p<0.01$). The effect of clonidine was not antagonized by pretreatment with prazocin 4 mg/kg, propranalol 32 mg/kg, or methysergide 16 mg/kg.

These data demonstrate that seizures in the tg model (in common with other seizure models) are modified by drugs which act at central alpha-2 adrenoceptors. While the data do not prove whether clonidine prolongs tg absence seizures through an effect on presynaptic or postsynaptic alpha-2 adrenoceptors (or by an indirect peripheral effect), the data are consistent with independent evidence that clonidine at the doses used acts presynaptically to reduce NE release. This interpretation would suggest that increased NE activity in tg mice may function to limit seizure activity possibly through an action on alpha-1 postsynaptic receptors.

Supported by the Epilepsy Foundation of America and NIH grant NS00871.

BASAL GANGLIA: BEHAVIOR AND PHARMACOLOGY

124.1 IS INFORMATION PROCESSED BY CAUDATE NEURONS RELATED TO MOVEMENT COGNITION? H. Conde, M. Amalric, J.F. Dormont, D. Farin, and A. Schmied. (SPON: E.E. Fetz) Lab Neurobiologie du Dev, Universite de Paris 11, 91405, Orsay, France.

Cooling and unit recording were combined to reveal the behavioral involvement and the information processed in the Caudate Nucleus, during a reaction time task. Five cats were trained to depress a lever through a variable foreperiod and to release it at the occurrence of an auditory conditioned stimulus (CS); responses made in less than 300 ms were reinforced with food pellets.

For the first 2-5 minutes, Caudate cooling had no effect on onset latency of force changes after the CS, although a slight increase of force change duration was observed. [In contrast, cooling of Ventrolateral Thalamic Nucleus (VL) or Red Nucleus (RN) immediately delayed onset of force change during the same task (1,2).] After several minutes of Caudate cooling, cats stopped performing the task, although they were still able to move normally and readily ate free food pellets. A slowing of response rate always preceded the arrest and was present without arrest for moderate cooling. These deficits suggest that Caudate is not involved in this task as a direct motor-command structure although it plays a crucial role in performance.

41 of 80 cells recorded in Caudate Nucleus showed the same activity patterns as VL or RN cells (3,4); the firing rates changed at comparable short latencies after the CS (20-100 ms) and showed frequencies correlated with movement latency.

Given the lack of immediate effect of Caudate cooling on movement onset, we suggest that the recorded Caudate activities do not represent command programs but copies of them, providing information on movements performed to obtain reinforcement. Disruption in this information processing would explain the apparent extinction of conditioned responses occurring after few minutes of Caudate cooling.

References:
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(2) AMALRIC et al, Exp.Brain Res. suppl.7 (1983) 204
(3) SCHMIED et al, Exp.Brain Res. 36 (1979) 285
(4) AMALRIC et al, Exp.Brain Res. 52 (1983) 210.

124.2 REGIONAL CAUDATE-PUTAMEN DOPAMINERGIC INFLUENCES ON SENSORIMOTOR PERFORMANCE IN RATS? P.C. Fairley* and J.F. Marshall. Dept. of Psychobiology, Univ. of Calif., Irvine, CA 92717.

Although the dopaminergic (DA) innervation is quite dense throughout the neostriatum, its contribution to behavior may differ from one subregion to another. Marshall et al. (JCPP 94:833, 1980) suggested that the impairments in localizing somatosensory stimuli result from damaging the DA inputs to the caudate-putamen (CP); and Dunnett and Iversen (Brain Res. 248:121, 1982) further suggested that 6-hydroxydopamine (6-OHDA) injections into the ventrolateral CP are particularly effective. Because of the importance of this issue to the growing evidence for striatal heterogeneities, the present study reexamined the contribution of several CP regions to rats' orientation abilities.

Unilateral 6-OHDA injections were aimed at four sites (N=61): the anterior dorsal and ventral (AD and AV) and the posterior dorsal and ventral (PD and PV) CP. Orientation to somatosensory stimuli was tested for one week postoperatively, after which coronal sections were examined for DA histofluorescence. The entire zone of fluorescence loss throughout the CP of each animal was reconstructed.

The rats of the AD, AV, and PD groups showed virtually no impairments in postoperative somatosensory orienation. The animals with PV 6-OHDA injections had contralateral impairments, the magnitude of which depended upon the extent of CP DA loss. After injections into the lateral PV CP, the zone of DA loss was moderate and the localization impairment slight. PV CP injections adjacent to the globus pallidus (GP) produced larger depletions and moderate sensorimotor impairments. Injections into GP resulted in the most extensive fluorescence losses and orientation deficits.

The postoperative orientation scores were correlated with the pattern of DA fluorescence loss measured in each animal. The behavioral impairment correlated highly ($r=.79$) with the volume of the CP denervated of its DA fluorescence. However, correlations between the sensorimotor scores and the fluorescence losses within particular CP subregions did not indicate the overriding importance of any single subregion.

The results suggest that any differences between the contributions of CP subregions to somatosensory orientation are subtle and are masked by the principle of mass action, in which localized depletions of DA are apparently compensated for by DA activity in the remainder of the CP. The experiment emphasizes the importance of conducting thorough histochemical examinations of each animal when determining the influences of localized brain injury.

124.3 WITHDRAWN

124.4 DOPAMINE IN CAUDATE AND NUCLEUS ACCUMBENS CORRELATES WITH THE POSTURE AND SPEED OF RATS RUNNING ON CIRCULAR AND STRAIGHT TREADMILLS. Bryan K. Yamamoto and Curt R. Freed. Div. Clin. Pharm., Univ. Colo. Sch. Med., Denver, CO 80262

We have demonstrated that trained circling rats have increased dopamine (DA) turnover in the caudate and nucleus accumbens (NA) contralateral to the circling direction. We now have studied rats running on circular treadmills of different diameters and at different turning intensities to see if DA in the caudate and NA is differentially affected by body posture and speed. Rats circled in place for 20 min and then were sacrificed. Caudate and NA were dissected and assayed for DA and DOPAC by HPLC with electrochemical detection. [C/I= contra/ipsi DOPAC concn.] N=6/group *p<.01 from controls.

	CAUDATE		N. ACCUMBENS	
	Posture (arc subtended by body)			
	120°	315°	120°	315°
SPEED(rpm)	C/I DOPAC concentration ratios			
0	0.96±.09	1.01±.03	1.00±.07	1.02±.02
2	1.20±.17	1.46±.04*	1.03±.07	1.06±.02
5	1.13±.09	1.56±.08*	1.29±.08*	1.30±.05*
10	1.14±.05*	1.52±.20*	1.44±.12*	1.60±.14*

DOPAC C/I ratios in caudate were more increased in animals turning in tight circles (body arc 315°) compared to animals circling in larger circles (body arc 120°). In NA, by contrast, DOPAC C/I concentration ratios were progressively increased by increasing speed of circling. Additional animals were placed on a straight treadmill at different speeds. The linear speeds were matched to the angular velocity of the animals which turned in circles. DA and DOPAC are represented as the mean concentration (nmoles/g) for both sides since no left-right differences were seen.

SPEED	CAUDATE		N. ACCUMBENS	
(cm/min)	DA	DOPAC	DA	DOPAC
0	53.0±0.8	5.8±0.4	24.5±1.6	6.7±0.2
145	56.8±2.7	6.8±0.3*	37.5±2.4*	8.1±0.3*
360	55.8±1.4	6.8±0.4*	47.5±1.8*	9.0±0.8*
720	68.1±1.9*	8.7±0.7*	57.6±5.6*	12.9±0.9*

These results show that DOPAC increases bilaterally both in caudate and NA during straight running. The magnitude of increase is greater in NA than caudate (192% control for NA vs. 150% control for caudate). We conclude that treadmill speed correlates with progressive increases in DA turnover in NA while increased body angle as well as speed correlates with increases in caudate DA.

124.5 DOPAMINE FUNCTIONS OF THE RODENT GLOBUS PALLIDUS: EFFECTS OF NEUROTENSIN AND NEUROLEPTICS. T.C. Napier and G.R. Breese. Biol. Sci. Res. Ctr., Univ. of North Carolina School of Medicine, Chapel Hill, NC 27514.

Systemically administered dopamine (DA) agonists alter neuronal activity in the globus pallidus (GP). However, since the GP serves as a major output for the caudate nucleus, these alterations may be reflective of changes produced in the caudate by DA-like drugs. The present study illustrates that the GP itself is sensitive to DA. The effects of neuroleptics and neurotensin (NT), a peptide known to affect DA functions, were also examined.

Standard microiontophoresis procedures were performed in male Sprague Dawley rats anesthetized with chloral hydrate. Systemic injections were via a tail vein cannula. Unilateral intracerebral microinjection was performed in rats previously implanted with injection guides over the GP. Drugs were infused in a 0.5 µl volume at 0.1 µl/min. Rotational behavior was quantified for 120 sec every 5 min for 45 min.

Approximately 90% of the pallidal cells recorded (n=53) exhibited a marked slowing of both spontaneous and glutamate evoked activity following iontophoretically applied DA (20 mM, 5-100 nA). Haloperidol, 0.25-1 mg/kg iv, did not consistently alter the DA response. Higher doses, 2-4 mg/kg iv, generally attenuated the DA-induced suppression by about 50%. Relatively lower doses of cis-flupenthixol (1-2 mg/kg iv) also antagonized DA responses by about 50%. The majority of the cells tested were not effected by iontophoresed NT (3.0 mM in 20 mM sodium acetate; 10-130 nA); 11/26 cells demonstrated a suppression of at least 30% and 4/26 were increased.

We observed ipsilated rotation following intrapallidal infusions of haloperidol, cis-flupenthixol and NT (2 µg/side) in amphetamine pretreated animals (1 mg/kg ip). Interestingly, 20 hrs after NT microinjection into the GP, amphetamine caused most animals to rotate contralaterally.

These results support anatomical studies which indicate a nigral-pallidal DAergic projection. Pallidal cells were very sensitive to iontophoretic DA and the lack of antagonism by low doses of iv haloperidol indicates that perhaps pallidal DA responses differ from those observed in the caudate nucleus. NT differed from neuroleptics in its actions in the GP. For example, NT was able to induce a long term change in the GP, an effect which we also saw following intranigral NT injections. These observations may indicate a new role for this peptide in the CNS. (Supported by F32-NS07247, HD-03110 and MH-36294)

124.6 THE EFFECTS OF DYNORPHIN A (1-8) IN THE SUBSTANTIA NIGRA. M. W. Friederich and J. M. Walker. Dept. of Psychology, Brown University, Providence, RI 02912.

High concentrations of the octapeptide dynorphin A (1-8) are found in the substantia nigra zona reticulata (SNR). In fact, 10-fold higher concentrations than dynorphin A have been demonstrated. It has been reported that dynorphin A affects motor behavior in rats after intranigral microinjections. The results of our experiments demonstrate that dynorphin A (1-8) has equivalent effects on the motor system when injected into the SNR. Specifically, unilateral dynorphin A (1-8) microinjections induced dose-dependent contraversive circling in rats. This effect of dynorphin A (1-8) is interesting because its potency so far has been reported to be only about 3% that of dynorphin A. The effect was naloxone reversible.

The results of preliminary experiments suggest that the effect of dynorphin A (1-8) may be mediated by kappa receptors. When equimolar doses of morphine (a mu receptor agonist) and U50,488 (a kappa receptor agonist) were compared on the induction of circling behavior, U50,488 appeared more potent than morphin.

Possible modes of action of dynorphin A (1-8) in the basal ganglia are via the dopaminergic and gabaergic systems. The blocking effect of selective destruction of dopamine cells in the substantia nigra zona compacta through ipsilateral 6-hydroxydopamine injections is under investigation, as well as the effects of systemic haloperidol administrations. The effect of the GABA antagonist bicuculline on the action of dynorphin A (1-8) will also be discussed.

124.7 EFFECTS OF SCH 23390-INDUCED BLOCKADE OF D-1 DOPAMINE (DA) RECEPTORS ON SINGLE UNIT ACTIVITY IN SUBSTANTIA NIGRA AND GLOBUS PALLIDUS. J.R.Walters, J.H.Carlson, D.A. Bergstrom and B.L.Waszczak. NINCDS, Bethesda, MD 20205 and George Washington Univ. Pharmacol. Dept., Washington D.C. 20037.

Several studies have suggested that SCH 23390 is a relatively selective D-1 DA receptor antagonist[1,2]. However, this drug has also been reported to reverse the behavioral effects of apomorphine[1], believed mediated through D-2 DA receptors. We have examined the effects of this drug on the activity of DA cells in the substantia nigra pars compacta (SNpc) and pars reticulata (SNpr) and in the globus pallidus (GP) to explore the consequences of D-1 receptor blockade on unit activity in these areas and to examine the ability of this drug to block or reverse the effects of apomorphine on these cells.

Extracellular single unit responses were recorded from spontaneously active cells of gallamine paralyzed, locally anesthetized and artificially respired rats. Firing rates of DA cells in the SNpc were generally unaffected by 1.0 mg/kg SCH 23390,i.v., but a subpopulation (3 out of 9) showed increased activity (>20% of baseline). The ED50 for apomorphine inhibition of DA cell firing was not significantly affected by SCH 23390 pretreatment (1.0 mg/kg, i.v., n=9); this inhibition was, in contrast, totally blocked by 0.025 mg/kg YM-09151-2, i.v. (n=5), a selective D-2 receptor antagonist[3]. In the GP, 2 cells showed slight decreases in rate (26%), while 7 showed no change (<20% of baseline) after 1.0 mg/kg SCH 23390 i.v. However, the stimulatory effect of 1.0 µmol/kg apomorphine (ave increase, 194% of baseline) on 9 cells was partially to fully reversed in 7 cases by subsequent administration of 1.0 mg/kg SCH 23390 (average reversal, 46%, sig. different from vehicle, p<.05, n=9). Complete reversal was consistently observed (n=7) with i.v. administration of 0.1 mg/kg YM-09151-2. In the SNpr, where effects of 1 µmol/kg apomorphine on unit activity are typically variable and haloperidol reversible, 6 of 11 cells showed increases or decreases in rate >20% of baseline. SCH 23390, 1.0 mg/kg i.v., partially or fully reversed 5 out of 6 of these changes (average reversal, 68%, n=6).

The results indicate that blockade of D-1 receptors has no consistent effect on the activity of DA cells or on apomorphine-induced inhibition of DA activity, a DA autoreceptor-mediated effect. However, SCH 23390 did partially reverse the effects of apomorphine on GP and SNpr cells, effects believed mediated through postsynaptic D-2 receptors. This action, although not robust, may be related to the observed blockade of apomorphine's behavioral effects[1] and reflect some interaction between striatal postsynaptic DA receptor subtypes or insufficient specificity of SCH 23390 at these sites.

[1]Iorio et al., J. Pharmacol. Exp. Ther., 226:462, 1983.
[2]Cross et al., Neuropharmacol. 22:1327, 1983.
[3]Grewe et al., Europ. J. Pharmacol. 81:149, 1982.

124.8 INVESTIGATION OF A ROLE FOR D-1 DOPAMINE (DA) RECEPTORS IN REGULATING TONIC ACTIVITY OF GLOBUS PALLIDUS NEURONS IN THE DA SUPERSENSITIVE RAT. J.H.Carlson, D.A. Bergstrom and J.R.Walters. NINCDS, Bethesda, Md 20205 and George Washington Univ. Pharmacology Dept., Washington, D.C. 20037.

The behavioral effects of DA agonists are currently believed mediated through D-2 receptor stimulation. Consistent with this is the finding that the selective D-1 receptor agonist, SK&F 38393, does not induce stereotypy or hyperlocomotion in normal rats. Unit recording studies in the rat globus pallidus (GP) have provided a neurophysiological correlate of these observations; agonists which stimulate D-2 receptors induce increases in GP activity; SK&F 38393 does not (Bergstrom et al., in press). In the present study, we have extended these observations by examining effects of apomorphine and SK&F 38393 on GP activity in rats made unilaterally supersensitive to DA by ipsilateral median forebrain bundle injections of 6-hydroxydopamine (6-OHDA). In contrast to its ineffectiveness in normal animals, SK&F 38393 induces an apomorphine-like rotation in these supersensitive rats (Setler et al., Europ. J. Pharmacol. 50:419, 1978).

Extracellular single unit responses of GP neurons were recorded in gallamine paralyzed, locally anesthetized and artificially respired rats. Five-6 weeks after 6-OHDA, apomorphine excited 55% of GP cells (ave increase, 181%), inhibited 17% (ave decrease, 79%) and left 28% unchanged (<20% baseline) (n=18). In normal rats, 30 μmol/kg SK&F 38393 i.v. increased 1(59%), decreased 2(29%) and had no effect on 7 cells. However, 2 weeks after 6-OHDA, firing rates of 50% of the cells were excited (ave increase, 44%), 20% were inhibited (ave decrease, 54%) and 30% were unaffected (<20% of baseline) (n=10). At 6 weeks post-lesion, the same dose increased the rates of 41% of the cells (ave increase, 94%), inhibited 32% (ave decrease, 57%) and 27% did not change (<20% baseline) (n=22). Administration of 0.1 to 1.0 mg/kg YM-09151-2, i.v., a selective D-2 antagonist, induced only a minor reversal of the significant changes induced by SK&F 38393 in the 6 week 6-OHDA rats; 1.0 mg/kg SCH 23390, i.v., a selective D-1 antagonist, was more effective, inducing a net reversal of 60% (n=12).

These results demonstrate that apomorphine and SK&F 38393 induce similiar changes in GP activity in DA supersensitive rats, actions qualitatively different from those induced in controls and paralleling their behavioral effects. The D-1 antagonist, SCH 23390, was comparatively more effective than the D-2 antagonist, YM-09151-2, in reversing effects of SK&F 38393 on GP activity, suggesting that a change in the functional consequences of D-1 receptor stimulation is involved in mediating the effects of SK&F 38393 observed here. However, recent studies (Walters et al., this vol.) have raised questions about the effects of SCH 23390 in the basal ganglia; other specific antagonists need to be examined before an action of SK&F 38393 on supersensitive D-2 receptors can be ruled out.

124.9 A RODENT MODEL OF PARKINSONISM: DOPAMINE DENERVATION OF THE MOUSE STRIATUM FOLLOWING ADMINISTRATION OF NMPTP Johnson, S.[1], Gerhardt, G.*[1], Rose, G.[1,2], Conboy, G.*[1], Jonsson, G.*[3], Olson, L.*[3] and Hoffer, B.[1] [1]Dept. of Pharmacology, UCHSC, [2]Medical Research, VAMC, Denver, CO and [3]Karolinska Institute, Stockholm, Sweden.

There have been several recent reports of a Parkinson's Disease-like syndrome in man which occurs following illicit injection of the Demerol analog N-methyl-4-phenyl-1,2,3,6-tetrahydrpyridine (NMPTP). In an effort to develop a rodent model for this problem, mice were injected with NMPTP (50 mg/kg, administered subcutaneously) and studies were carried out 3-6 months after injection using neuroanatomical, in vivo electrochemical, and electrophysiological methods to ascertain remaining dopaminergic function in the striatum.

Using Falck-Hillarp flourescence and tyrosine hydroxlyase immunocytochemistry, NMPTP-injected mice showed a marked loss of dopamine-containing fibers in the caudate nucleus compared to vehicle-injected controls. This denervation was much more pronounced in the dorsal (ca. 70% depletion) than in the ventral (ca. 30% depletion) striatum.

Dopamine (DA) release was induced by local pressure ejection of K^+ and measured using in vivo electrochemical detection. With this technique the striatum of NMPTP-treated mice was also found to be dopamine depleted (average release 5.0 um, versus 9.1 um in untreated mice). Again, greater depletion was observed in the dorsal than in the ventral striatum. In control mice, local ejection of the indirect DA agonist phencyclidine (PCP) elicited a dose-dependent slowing of striatal neuron discharge. The potency of PCP for causing depression of cell firing was reduced about 4-fold in the dorsal striatum of NMPTP-treated mice.

Taken together, the data suggest that NMPTP produces a significant, anatomically localized reduction in DA-containing afferents to the mouse striatum. Thus, this rodent may be useful as a model for studies of NMPTP-induced human neurotoxicity. (Supported by USPHS grants NS09199 & MH00289, grants from the Swedish MRC, and the VA Medical Research Service.)

124.10 CHARACTERIZATION OF THE BEHAVIORAL RESPONSE OF RATS TO MULTIPLE INJECTIONS OF N-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (NMPTP) M. Kent Shellenberger and Marsha E. Melnick. Ralph L. Smith Center for Mental Retardation Research, Univ. of Kansas, Kansas City, Kansas 66103.

NMPTP has been reported to produce a Parkinsons like syndrome in humans and monkeys which may be accompanied by loss of cells in the Substantia Nigra. It has not been clearly shown whether this compound has similar effects in rodents. We have undertaken studies utilizing a number of behavioral tests to determine if NMPTP produces behavioral effects in rats which might be ascribed to altered basal ganglia function. Rats used in these studies were male, retired breeders certified by the vendor (Sasco) to be at least 1 year old. These rats weighed between 420 and 530 gm. Groups of animals were evaluated during a series of 7 daily injections of 20 mg/kg NMPTP or solvent, 7-14 days after the series and during a second series of 7 daily injections at the same dose. Behaviors examined were walking patterns; exploratory behavior; and 24 hr. motor activity patterns recorded continuously during the time of treatment with these animals living in a figure eight maze. Injection of NMPTP produced a significant reduction in diurnal motor activity on each of the first 3 days of treatment and a reduction in nocturnal activity on days 3 and 4. On day 5 both diurnal and nocturnal activity returned to the normal range. Twelve days after the seventh injection, maze activity was again evaluated prior to any further injections. At this time the NMPTP treated animals were significantly hyperactive compared to the control group during the nocturnal phase of days 2-4 of the baseline period. The second series of injections did not alter diurnal activity; however, nocturnal activity was significantly reduced on all 7 nights. Evaluation of footprints for length and width of stride indicate that walking patterns were significantly altered by treatment with NMPTP. Acutely, the treated animals took shorter and wider steps and tended to walk on their heels more often than control. Walking patterns of the treated and control animals were similar on day 2, but on days 4 and 8 the treated animals showed significant increase in the width/length ratio. This effect persisted 10 days after the first series of injections.

The results of these experiments indicate that NMPTP produces residual behavioral and motoric effects in older rats. These alterations could be associated with basal ganglia damage. Supported by BRSG Funds 2 S07 RR05373SUB and USPHS Grant HD 02528.

124.11 IS MOTOR MOVEMENT ASSOCIATED WITH CHANGES IN DOPAMINE TURNOVER IN RAT NIGROSTRIATAL DOPAMINERGIC NEURONS? E.Melamed, G.Siegelman*,M.Globus* and M.Chipman.Dept. of Neurology, Hadassah University Hospital, Jerusalem,Israel.

Clinical and experimental observations suggest that the dopaminergic (DA) nigrostriatal neurons are important in the control and regulation of voluntary motor movement.While drug-induced acceleration or suppression of nigrostriatal DA transmission produce changes in motor behavior, there is very little information on the reverse situation i.e. effects of movement on DA synthesis and release by DA neurons.We therefore examined whether there is a relation between motor activity and DA turnover in rat corpus striatum.Male albino rats (Hebrew University strain)were decapitated after running on an electrically-activated threadmill at a speed of 24meters/min for periods of 2,4 or 5min.Controls were killed after staying for 5min in the threadmill chamber without running. Running for 2-5min did not induce any change in striatal DA and its metabolites DOPAC and HVA.Likewise,no changes occured in rats that were allowed to rest for 5min after running for 5min.Rats were injected with NSD-1015, a blocker of central dopa-decarboxylase (100mg/kg)and 30min later ran on the threadmill for 5min.Running did not cause any change in striatal accumulation of dopa.Rats were injected with probenecid (200mg/kg)and 2hr later ran for 5min.There were no changes in striatal accumulation of DOPAC and HVA among probenecid-injected runners and their controls. The nigrostriatal projection may be particularly important in the initiation of movement. However,striatal DA turnover did not change when threadmill was successively turned on and off for periods of 10sec for a total duration of 5min so that rats had many starts and stops in their running.To examine possible importance of running duration, male Fisher rats underwent 30min running trainingsessions on the threadmill for two consecutive days and were decapiated on the third after running for 30 or 60min at a speed of 24meters/min. There were modest,time-dependent increases in striatal DOPAC and HVA while DA levels remained unchanged. Study indicates that short-term threadmill running does not enhance striatal DA turnover.Mild elevations in DA metabolites after longer-term running may reflect movement-but also stress-or fatigue-induced alterations.Employed methods may not be sufficiently sensitive to detect subtle neurochemical changes.Alternatively,nigrostriatal DA turnover may not be affected by forced, non-goal oriented threadmill running.

124.12 CHRONIC HALOPERIDOL TREATMENT PRODUCES INCREASED NEURONAL SENSITIVITY TO IONTOPHORESED GABA WITHIN SUBSTANTIA NIGRA, PARS RETICULATA. J.M. FREY, M.K. TICKU, R.D. HUFFMAN, Dept. of Pharmacology, Div. of Neuropharmacology, Univ. of Texas Health Science Center, San Antonio, Texas 78284.

Several investigators have recently reported that chronically administered haloperidol (HAL) results in an increase in GABA receptor binding within the substantia nigra (SN) which may be due to a decrease in GABAergic outflow from the striatum. We have recently reported a significant increase in responsiveness to GABA applied microiontophoretically to single neurons in globus pallidus (GP) in rats that had been treated chronically with HAL (Frey et al., Neurosci. Abstr. 9:874, 1983). Since both the GP and pars reticulata (PR) of the SN receive a major GABAergic projection from the striatum, we decided to determine if the increased binding found within SN after chronic HAL treatment is associated with increased sensitivity of PR neurons to iontophoresed GABA. Rats were placed on a HAL diet for 30 days and withdrawn for 2 days, a time when GABA receptor binding within PR was significantly elevated by about 60% ($p<.01$). Control rats were fed plain chow for 32 days. The effects of GABA microiontophoretically applied to single PR neurons were compared at day 32 under chloral hydrate anesthesia utilizing 7-barrel microelectrodes and conventional extracellular recording techniques. Drug concentrations utilized were as follows: GABA (5 and 10nM in 0.2M NaCl, pH 3.8-4.0), glycine (10 and 50nM in 0.2M NaCl, pH 3.8-4.0), NaCl (3M for recording and 1M for current control). In order to quantitate the sensitivity of PR neurons to iontophoresed GABA, EC_{50} values were estimated from dose-response curves compiled from the results of the systematic application of various ejection currents of both GABA and glycine. Chronic haloperidol treatment produced a significant increase in the responsiveness of PR neurons to microiontophoretically applied GABA while there was no difference in glycine responsiveness. GABA EC_{50} values for the haloperidol-treated group were almost 2 times less than those of control animals (44.8±6.3nA versus 85.7±12.8nA, df50, $p<.01$). These results extend our observations relative to GP neurons to the PR neurons of the SN as well and provide physiological evidence to support the contention that the observed increases in {3H}-GABA binding within the basal ganglia after chronic HAL treatment reflect an increase in neuronal responsiveness to GABA.

124.13 STRIATAL 6OHDA ALTERS APOMORPHINE-INDUCED BEHAVIOR. S.L. Hartgraves*, P.H. Kelly, J.S. Randall and P.K. Randall. Physiology and Biophysics, and Andrus Gerontology Ctr., USC, L.A., CA 90089

Typical drug-induced stereotyped behavior in the rat includes sniffing, locomotion, rearing, head-weaving, licking and gnawing. An atypical response, self-mutilation, has previously been reported following systemic apomorphine administration in adult rats with bilateral substantia-nigra 6-OHDA lesion or in adult rats lesioned as neonates (Ungerstedt, Acta. Phys. Scand., 367:95,1971; Creese and Iversen, Br. Res. 55:369, 1973). Doses of apomorphine used to produce this atypical behavior ranged from 0.5 mg/kg to 5.0 mg/kg (the latter dose causing the animals to chew into their abdominal cavity).

The present study examined apomorphine-induced behavior in rats with bilateral 6-OHDA lesions (8ug/2ul) in the tail of the caudate nucleus (coordinates:anterior 0.8, lateral 3.5, from bregma and 6.0 ventral to the dura), producing specific striatal DA depletion, while leaving nucleus accumbens relatively intact. Sprague-Dawley rats weighing 275 to 300 gm at time of surgery were tested from 9-22 days post surgery with doses of apomorphine ranging from 0.0125 mg/kg to 0.2 mg/kg. Higher doses were not used, as the lower doses were found to elicit similar "stereotyped grooming/gnawing" behavior but at an intensity that did not produce injury. From 10 to 24 animals were tested at each dose, with the ED50 for this atypical behavior occurring at 0.034 mg/kg. In addition, the normal apomorphine response was not observed in rats with striatal DA depletions regardless of the presence of the aberrant behavior. The ED50 for the appearance of stereotyped licking/gnawing in intact rats was found to be 0.326 mg/kg. However, no intact animals showed any evidence of self-directed gnawing even at doses as high as 60 mg/kg. 6OHDA lesions of the nucleus accumbens also were ineffective in producing this behavior at doses of apomorphine up to 0.2 mg/kg, a dose increasing locomotor behavior substantially in these animals. The effectiveness of lesions in the caudate nucleus or the nucleus accumbens was evaluated by radioenzymatic assay.

The present results suggest that the atypical "stereotyped grooming/gnawing" caused by apomorphine in this study as well as prior studies may not be a "supersensitive" stereotypic response, but a behavior unique to striatal DA depletion.

124.14 MUSCIMOL IN THE NIGROTEGMENTAL TARGET AREA BLOCKS SELECTED COMPONENTS OF STEREOTYPY ELICITED BY AMPHETAMINE OR COCAINE. Susan E. Bachus & Karen Gale, Dept. Pharmacol., Georgetown Univ. Sch. Medicine & Dentistry, Washington, D.C., 20007.

Bilateral microinfusion of the GABA agonist muscimol into the nigrotegmental target area in the region of the pedunculopontine nucleus (PPN) abolishes stereotyped sniffing and gnawing induced by systemic apomorphine (Childs & Gale, Life Sci. 33:1007, 1983). Since other dopaminergic stimulants such as amphetamine and cocaine evoke a broader spectrum of stereotyped behaviors, we have examined effects of intrategmental muscimol on behaviors induced by these drugs.

Male Sprague-Dawley rats (310-375 g) were observed after bilateral infusion of 25 ng muscimol (or vehicle)/1 µl/5 min in the vicinity of PPN, followed by injection of either d-amphetamine sulphate (2.5 or 5.0 mg/kg s.c.) or cocaine (40 or 60 mg/kg s.c.). Muscimol in PPN alone did not alter spontaneous behavior. However, the PPN muscimol infusion eliminated sniffing, head movements and gnawing (which were major effects of amphetamine and cocaine in controls) in stimulant-treated rats. In contrast, locomotor activity persisted in the absence of the other stereotypies. In addition, maintenance of snout contact (without sniffing) was seen, and rats frequently assumed climbing postures marked by snout contact with a wall or corner. Interestingly, in the presence of muscimol in PPN, the locomotor activity, wall-climbing and snout contact induced by amphetamine and cocaine were more pronounced at higher doses. Since these behaviors are not normally observed with high doses of these drugs, it appears that they are unmasked in the absence of sniffing and gnawing. This dissociation of orofacial stereotypy from snout contact fixation re-emphasizes the need to separate components of stimulant-induced stereotypy in attempts to explore the underlying neural substrates (Szechtman et al., Eur. J. Pharm. 80:385, 1982; Teitelbaum et al., Handbook of Behavioral Neurobiology 6:23, 1983).

These observations, which extend those of Childs & Gale with apomorphine, further implicate the GABAergic nigrotegmental pathway in the mediation of orofacial dyskinesias of basal ganglia origin. In addition, our results demonstrate that cocaine- and amphetamine-induced locomotor activity is not dependent upon mediation through PPN.

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124.15 DOES ANALGESIA PLAY A ROLE IN MUSCIMOL-INDUCED SELF-INJURY? G.D. Frye and A.A. Baumeister*. Department of Medical Pharmacology and Toxicology, Texas A & M University, College of Medicine, College Station, TX 77843.

Bilateral microinjection of muscimol (30 ng/0.5 µl/site) into the medial substantia nigra zona reticulata of rats which were briefly anesthetized with ether evoked intense self-biting, frequent tissue damage and intense stereotyped sniffing, head bobbing, rearing and gnawing (Baumeister and Frye, Pharmacol. Biochem. Behav., in press, 1984). The role of muscimol-induced analgesia in this model of self-injurious behavior (SIB) was evaluated. Simultaneous bilateral intranigral injections of muscimol 30 ng/site blocked hindpaw licking by rats placed on a 55°C hotplate for 90 sec. This effect was observed between 30 and 120 min after injection. The hindpaw licking response returned by 240 min. Muscimol did not impair motor coordination since intense stereotypic behaviors were present at this time. The time course of hotplate analgesia correlated closely with the interval during which SIB was observed. Intraperitoneal administration of picrotoxin (5 mg/kg, ip), naloxone (1 & 10 mg/kg, ip) or p-chlorophenylalanine diethyl ester (500 mg/kg, ip) immediately after muscimol failed to block either SIB or hot-plate analgesia. Picrotoxin actually prolonged muscimol analgesia beyond 240 min. SIB could not be antagonized by local injection of lidocaine • HCl (2 mg) into injured appendages. These results indicate that intranigral muscimol evokes a centrally mediated analgesia that appears to be unrelated to GABAergic, serotonergic or naloxone sensitive nociceptive systems and that this analgesia may play an important role in the observed SIB. Supported in part by PHS AA06322, HD-07201.

124.16 ASYMMETRICAL ELICITATION OF HYPERACTIVITY BY UNILATERAL ELECTROLYTIC LESION OF THE NUCLEUS ACCUMBENS. K.L. Kubos*, T.H. Moran* and R.G. Robinson. (SPON: L. Fechter) Dept. Psychiatry and Behavioral Science, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Male Sprague Dawley rats, housed on a 12 hr. diurnal cycle were initially habituated to multiple measure activity chambers (OmniTech) overnight. Three days later they were again placed in the chambers and preoperative nocturnal activity levels were determined for all parameters. The following day rats were randomly assigned to one of 3 groups of 6 each: left or right lesion and sham operated. Under Chloropent anesthesia, exerimental groups received either a left or right unilateral electrolytic lesion of the nucleus accumbens (AP 9.2, L +/- 1.7, V 4.8) with respect to ear bar zero (Pellegrino, et al., 1979). Lesions were made by passing 2mA DC for 7 sec. through a blunt 26 ga. needle insulated with Epoxylite except for the tip. Sham operated animals received a craniotomy alone. Rats were then placed individually in activity chambers at 1 wk. intervals for 4 postoperative wks and their spontaneous nocturnal activity recorded.

Measurement of postoperative nocturnal activity revealed that lesion groups were significantly hyperkinetic compared with controls on the following measures: total distance travelled, movement time, mean velocity, # of movements and mean distance. In all horizontal measures the order of activity was right > left > sham.

A body of evidence suggests an asymmetrical potency of right over left left hemispheric cortical lesions in the production of hyperkinesis in rats (Robinson, R.G. and Coyle, J.T., Brain Res. 188: 63-78, 1980). Severence of right cortico-subcortical communicating fibers produces hyperkinesis while similar undercuts of the left frontal cortex do not (Kubos, K.L. and Robinson, R.G. Exp. Neurol. 83: 646-653, 1984). The present findings suggest a corresponding mesolimbic asymmetry for the elicitation of hyperactivity following subcortical lesions.

124.17 UNIT ACTIVITY VARIATION IN MONKEY VENTRAL TEGMENTAL AREA DURING OPERANT FEEDING

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To elucidate functional roles of the ventral tegmental area (VTA) in the initiation and execution of motivated feeding behavior, variation of unit activity in the VTA was investigated during bar-press feeding. The feeding behavior was divided into 3 stages : 1) discrimination (food or non-food) (1st stage), 2) bar-pressing to obtain food (2nd stage), and 3) food acquisition and ingestion (3rd stage). In the 1st stage when food or non-food was presented only a few neurons responded, but in the 2nd and 3rd stages almost half of the neurons responded in relation to feeding motor acts, such as arm extension, flexion, bar-pressing, chewing, etc. Almost the same response appeared when the monkey extended the arm contra- or ipsi-lateral to recording hemisphere, but no significant response was observed when the arm was extended passively. Most prominent firing pattern was increase upon arm extension or during bar pressing to procure food, and decrease after food acquisition and ingestion. Patterns and magnitudes of procurement responses differed depending on the nature of the food. For normal diet, firing increased only in the first third of the 2nd stage and thereafter returned to control level even if the monkey continued bar-pressing, but for preferred food firing increased throughout the 2nd stage. The magnitude of ingestion responses was less dependent on the nature of food in the 3rd stage. Firing was also modulated by the motivational state or external stimuli; it increased during vocalization to ask for food or when the experimenter made sounds associated with food manipulation, and decreased when the experimenter touched a finger, arm, leg, or fur of the animal.

Data suggest that activity in the VTA is intimately related to motor initiation and motion to procure food (motor drive), and to internal or external inhibition (drive reduction or arousal) of initiation of motor acts.

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PHARMACOLOGY OF SYNAPTIC TRANSMISSION II

125.1 NEUROTENSIN AND FLUNARIZINE INTERACTIONS ON NEUROTRANSMITTER RELEASE AND CALCIUM IONS MOVEMENTS IN RAT STRIATAL SLICES - F. Battaini, S. Govoni, M. Memo[a], P.F. Spano[a] and M. Trabucchi[b]. Institute of Pharmacology and Pharmacognosy, University of Milan, Milano 20129, [a]Dept. of Pharmacology, University of Brescia and [b]Chair of Toxicology, II University of Rome Italy.

The interaction between neurotensin and dopamine at central level is well documented and comes mainly from observations showing i, a direct effect of this peptide in increasing dopamine turnover ii, the presence of neurotensin immunoreactivity and receptors in dopaminergic areas iii, behavioural and biochemical correlation with neuroleptic treatment. Recently neurotensin has been shown to promote the release of dopamine in striatum in a calcium dependent manner. On this line we studied the effect of a calcium entry blocker, flunarizine, and neurotensin on endogenous dopamine release from rat striatal slices under basal and depolarizing conditions. The basal release of dopamine was stimulated more than 10 fold by KCl (30 mM). The in vitro addition of 10 μM flunarizine, a concentration that is reached in vivo in brain after oral administration of therapeutic doses of this drug, inhibited the K^+-stimulated but not the basal release of dopamine; neurotensin, while not modifying either the basal or the depolarized release of dopamine, abolished the inhibitory action of flunarizine at equimolar concentrations. In our experimental conditions K^+-stimulated dopamine release is almost completely dependent on the presence of calcium in the medium. The participation of this ion to the observed changes was studied using ^{45}Ca. Flunarizine inhibited calcium uptake and neurotensin, besides not modifying calcium movements by itself, was able to antagonize the inhibitory action of flunarizine. In addition, in vitro data indicate that neurotensin may interact with ^3H-Nitrendipine binding to rat brain membranes.

125.2 POSSIBLE NMDA RECEPTOR MEDIATION OF SYNAPTIC TRANSMISSION IN THE HIPPOCAMPAL CA1 REGION. J. J. Hablitz and I. A. Langmoen Dept. of Neurol., Baylor Col. Med., Houston, Tx. and Dept. of Neurosurgery, Ullevål Hospital, Oslo, Norway.

Glutamate or a glutamate-like acidic amino acid has been suggested as the excitatory neurotransmitter in the CA1 area of the hippocampus. It has also been suggested that there are several pharmacologically distinct excitatory amino acid receptors. We have examined the effect of selected receptor antagonists on intracellularly recorded EPSPs in CA1 neurons in an attempt to identify the receptor type mediating excitatory transmission in this system.

Intracellular recordings were obtained from neurons in the CA1 region of rat hippocampal slices maintained in vitro. In order to study EPSPs without contamination from IPSPs, slices were perfused with a saline containing 50-100μM picrotoxin, a potent antagonist of GABA-mediated inhibition. The incidence of epileptiform activity was reduced by increasing the concentration of calcium and magnesium to 4mM. The antagonists DL-alpha-aminoadipate (DAA), DL-2-amino-4-phosphonobutyrate (APB), DL-2-amino-5-phosphonovalerate (APV) and gamma-D-glutamylglycine (DGG) were then bath applied at 100 or 500 μM.

The most potent EPSP antagonist was APV. When applied at 100μM, it reliably reduced subthreshold EPSPs. Responses to higher stimulation strengths which evoked action potentials were also reduced but to a lesser extent. DAA and APB, at 100 μM were less effective but also reduced EPSPs in most cells. DGG reduced orthodromically evoked responses only at 500μM.

To test the specificity of APV and APB, their effect on responses to iontophoretically applied N-methyl-D-aspartate (NMDA) and quisqualate was examined. In slices bathed in TTX and manganese, APV was found to be a selective NMDA antagonist while APB reduced both quisqualate and NMDA responses. Furthermore, APV appeared to act as a competitive antagonist since responses to small but not large doses of NMDA were reduced by this agent.

These results indicate that, under the conditions used, APV is a selective NMDA antagonist in the CA1 area and that an NMDA-type receptor may be involved in synaptic excitation. Previous reports of a lack of effect of APV may have resulted from a failure to examine subthreshold responses where APV's action is apparently greatest.

(Supported by NS-11535 and NS-18145)

125.3 EFFECTS OF PENICILLIN AND PENTOBARBITAL ON INHIBITORY MECHANISMS IN NEOCORTEX. D. S. Weiss* and J. J. Hablitz (SPON: P. Kellaway). Dept. of Neurol., Baylor Col. of Med. Houston, TX 77030.

Alterations in GABAergic function have been implicated in both the etiology and treatment of convulsive disorders. In this study we characterized the response of neocortical neurons to iontophoretically applied GABA and examined how these responses, as well as IPSPs, were affected by convulsant (penicillin) and anticonvulsant (pentobarbital) drugs. Intracellular recordings were obtained from slices of rat neocortex maintained in vitro. Dye-injection studies indicated that our recordings were primarily from pyramidal neurons.

Orthodromically evoked responses were always depolarizing at the cell's resting potential. Depolarizing the cell by 10-20 mV reversed the response, suggesting that it predominantly consisted of an IPSP. This depolarization often unmasked a small, short-latency EPSP. The apparent reversal potential for the IPSP was -55 mV in a population of cells with a mean resting potential of -76 mV (n = 6). Responses to GABA were also depolarizing at rest. In some neurons, the depolarizing GABA response appeared as a biphasic hyperpolarizing/depolarizing response if the cell was depolarized slightly (5-10 mV).

Bath application of penicillin (1.7-3.4 mM) decreased the amplitude of IPSPs and also reduced the response to GABA application without affecting the dose required for a half-maximal response. These effects were associated with the development of spontaneous and evoked epileptiform activity. Spontaneous bursts in neocortex showed no periodicity and usually preceded an episode of apparent spreading depression. This was characterized by a depolarization to approximately 0 mV and a >90% decrease in input resistance. This response recovered over a 1- to 2-min period. Pentobarbital (100-200 μM) prolonged the time course and increased the amplitude of IPSPs while producing a leftward shift in the GABA charge-response relation. Pentobarbital did not affect either the maximum GABA response or its time course.

These studies indicate that a good correspondence exists between GABA responses and IPSPs in neocortical pyramidal neurons, suggesting that GABA is the transmitter mediating neocortical IPSPs in vitro. Convulsant and anticonvulsant drugs have opposing effects on cortical inhibition. The effects of each type of drug on GABA responses were similar to those on IPSPs. (Supported by the Epilepsy Foundation of America and NIH grant NS11535).

125.4 STIMULATORY EFFECT OF THE ATYPICAL NEUROLEPTIC SULPIRIDE ON REGIONAL BRAIN METABOLISM IN THE RAT. G. Pizzolato*, T.T. Soncrant*, D. Larson*, S.I. Rapoport. Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health, 10/6C103, Bethesda, MD 20205.

Sulpiride (SULP) is a neuroleptic drug which, despite its antipsychotic activity, possesses only some of the properties of more typical neuroleptics, such as haloperidol (HAL). SULP has minimal cataleptic activity, and has no inhibitory effect on apomorphine-induced stereotypy.

Neuroleptics act by antagonism at central dopamine (DA) receptors, of which there are different classes. SULP is a selective antagonist of DA receptors which are not linked to adenylate cyclase (D2 receptors).

We used the quantitative autoradiographic [14C]-deoxyglucose (DG) technique of Sokoloff et al. (J. Neurochem. 28:897, 1977) to determine the time-course and the regional distribution of alterations in local cerebral glucose utilization (LCGU) after SULP administration to awake Fischer-344 rats. Animals received SULP 100 mg/kg or vehicle i.p. at 1, 2 or 3 h before DG. LCGU was determined in 55 regions.

SULP increased LCGU in 11% of regions at 1 h, and in 24% after 2-3 h. Increases occurred in the nigrostriatal (caudate-putamen and substantia nigra compacta) and mesolimbic (olfactory tubercle and accumbens) DA systems, but not in the ventral tegmental area or mesocortical system (anterior cingulate and frontal cortex). Large and early increases were found in DA regions associated with endocrine functions (suprachiasmatic and paraventricular hypothalamic nuclei, and median eminence). LCGU also rose in the lateral habenula and in the ventral lateral geniculate.

The time-course of the effect of SULP on LCGU does not correspond to the time-course of the brain concentration of SULP, which is maximal at 0.5-1 h after i.p. administration (Mizuchi et al., Psychopharmacol. 81:195, 1983). Instead, the metabolic effect parallels the time-course of changes in DA metabolism induced by SULP (Hofman et al., J. Neurochem. 32:195, 1979), which indicates that the effect of SULP on LCGU is mediated by its action at specific receptor sites.

The effects of SULP on LCGU are different from those of HAL, a mixed D1 and D2 receptor antagonist (Pizzolato et al., J. Neurochem., 1984). HAL decreases LCGU in most DA regions, and also in many non-DA regions, whereas SULP has a rather selective action on DA regions in the brain. Elevations in LCGU after SULP may be related to selective antagonism at D2 receptors, and suggest that DA acts at these receptors mainly to inhibit neuronal activity.

125.5 EFFECT OF GALLAMINE AND PIRENZEPINE ON RESPONSES OF RABBIT SUPERIOR CERVICAL GANGLION TO CATECHOLAMINES AND MUSCARINIC AGONISTS. C.A. Yarosh, John H. Ashe and L.M. Kooyman*. Dept. of Psychology, Univ. of Calif., Riverside, CA 92521.

Studies using pharmacological and biochemical techniques have demonstrated multiple muscarinic binding sites in the central and peripheral nervous system(for review see: Trends Pharmacol. Sci., Suppl. 1, 1984). The physiological significance of multiple muscarinic binding sites has recently been examined in the in vitro rabbit superior cervical ganglion where gallamine and pirenzepine selectively antagonize the muscarinically mediated slow-IPSP and slow-EPSP respectively (Ashe & Yarosh, Neuropharm., in press). Since production of the s-IPSP may require the release of dopamine from intraganglionic SIF cells (Ashe & Libet, Brain Res., 242, 1982) it was of interest to examine the effect of gallamine superfusion on ganglionic hyperpolarization elicited by dopamine (DA) and norepinephrine (NE). Also, the ability of gallamine and pirenzepine to selectively antagonize hyperpolarization (HP) and depolarization (DP) produced by the muscarinic agonists methacholine (MCh) and bethanechol (BCh) was studied. Changes in membrane potential were recorded at room temperature (23°C) using the sucrose-gap technique. Small volumes of drugs were rapidly injected into the perfusion current and diluted in the perfusion line and chamber volume (540ul).

Gallamine (28uM) superfusion had no effect on the baseline membrane potential and its potency in blocking the s-IPSP at 23°C was similar to that previously observed at 35°C. The s-IPSP was selectively suppressed following 45min superfusion of ganglia with gallamine (28uM) without suppression of the HP elicited by DA (100ul of 2mM) or NE (100ul of 0.2mM).

In contrast, HP elicited by the muscarinic agonists MCh (100-300ul of 10mM) and BCh (100-200ul of 10mM) was reversibly blocked following superfusion for 45min with gallamine (28uM). Gallamine had no suppressive effect on the agonist induced muscarinic DP; usually facilitation of the amplitude of the DP was observed.

Superfusion with pirenzepine (0.1-0.2uM) suppressed the DP elicited by MCh and BCh with little effect on the amplitude of ganglionic HP, although the duration of the HP was often increased relative to control responses. (Supported by NSF PRM-8200575 and NIH BRSG-RR07010-17).

125.6 ALUMINUM INHIBITS Ca^{++}/Mg^{++} ATPase: CONSEQUENCES FOR ALZHEIMER'S DISEASE. R. Garza,*, C. Equivel* and D.H. Ross. (SPON: W. Stavinoha). Division of Molecular Pharmacology, The University of Texas Health Science Center, San Antonio, TX 78284.

Aluminum (Al^{+3}) neurotoxicity has recently been implicated as a causative factor in the development of Alzheimer's Disease (AD). The sequence of this disease may be replicated by administration of μg quantities of Al^{+3} into the hippocampal ventricle of cats. The underlying factors of Al^{+3} toxicity are unknown; however, it is chemically very similar to calcium (Ca^{+2}) and is a potent Ca^{+2} antagonist. Al^{+3} has been shown to bind to calmodulin to reduce the % helix of the protein and to reduce phosphodiesterase activity. It was our interest to test the effects of Al^{+3} on calmodulin-dependent Ca^{++}/Mg^{++} ATPase in view of this enzyme's role in regulation of intracellular calcium levels in brain. Synaptic membranes prepared from whole rat brain were assayed for Ca^{++}/Mg^{++} ATPase by measuring phosphate release. Al^{+3} was tested over a range of 10 - 150 μM. Al^{+3} produced a concentration-dependent decrease in enzyme activity over 20 - 150 μM. Ca^{++}-dependent Ca^{++}/Mg^{++} ATPase activity was inhibited over the same range. Mg^{++} ATPase (Mg^{++} = 20 - 250 μM) was unaffected by Al^{+3} concentrations. Mg^{++} ATPase activity at ATP = 20 - 250 μM was also unaffected. ATP-dependent Ca^{++} uptake was inhibited at 10 - 75 μM Al^{+3}. Inhibition of Ca^{++}/Mg^{++} ATPase and ATP-dependent Ca^{++} uptake by Al^{+3} may contribute to elevated cytosolic Ca^{++} levels in the nerve cell, eventually leading to cell death. The destruction of cells in the hippocampal regions of brain by this mechanism may underlie anatomical and biochemical changes associated with Alzheimer's Disease.

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125.7 HIPPOCAMPAL NEURONS IN CULTURE: ELECTROPHYSIOLOGICAL EXAMINATION OF SPONTANEOUS AND TRANSMITTER EVOKED ACTIVITY. A.T. Malouf and D.L. Gruol. Division of Preclinical Neuroscience and Endocrinology, Scripps Clinic and Research Foundation, La Jolla, CA 92037

We have recently begun electrophysiological analysis of the cellular and membrane physiology of hippocampal neurons (HNs) using an in vitro model system of cultured rat neurons. In our first series of experiments we have used morphological criteria and extracellular unit recording to identify and characterize the neuronal types in the cultures and to assess their sensitivity to putative excitatory amino acids and neuropeptide transmitters. The cultures, prepared from 20 day rat embryos, retain much of the histotypical organization of the hippocampus while affording an unobstructed view of the cell body and dendrites. Extracellular unit recordings were made with saline filled patch electrodes from the somal region of HNs in 1 to 6 week cultures. Transmitters were dissolved in recording media at a concentration of 50 μM and applied by micropressure from large-tipped (10 to 30 μ) glass pipettes. After one week in culture, the neurons are still relatively immature both morphologically and physiologically. At this age, the majority of HNs tested did not display spontaneous activity and were insensitive or only weakly responsive to the putative transmitters. By 2 weeks, the cultures contain neurons which morphologically resemble the major cell types from this cortical area. At this age the majority of HNs displayed spontaneous activity. About 50% of the spontaneously active HNs exhibited bursting patterns, some of which resembled patterns typical of CA3 pyramidal neurons. HNs also developed a sensitivity to exogenously applied glutamate (GLU). The GLU response consists of a fast onset, fast offset burst of 12 to 100 single spikes. The excitatory amino acid N-Methyl-D-aspartate (NMDA), unlike GLU, did not elicit spike activity in any of the HNs tested. The dipeptide N-acetylaspartylglutamate (NAAG), which has been shown by Zaczek et al (PNAS 80, 1983) to displace ^{3}H-GLU from its high affinity sites in brain homogenates, was also tested. Preliminary results indicate that this compound has neuroactive properties in our system. These data suggest that HNs in culture develop complex morphological and physiological characteristics reflective of those observed in vivo. Correlation of these two factors should provide valuable information about the type of information HNs process and the physiological mechanisms responsible. (supported by NIAAA 06420 and 07456)

125.8 ANTAGONISM OF ORGANOPHOSPHATE-INDUCED DEPRESSION OF REFLEX ACTIVITY IN THE NEONATAL RAT SPINAL CORD. Qin Z. Yang and Jordan E. Warnick. Dept. of Pharmacol. & Exp. Ther., Univ. of Maryland School of Medicine, Baltimore, MD 21201.

We recently reported that sarin (5-20 nM) facilitated reflex activity in the isolated neonatal spinal cord and reduced homosynaptic depression; at 50 nM and above it continued to reduce homosynaptic depression but depressed reflex activity (Yang and Warnick, Fed. Proc. 43:929, 1984). Atropine (ATR), but not (+)-tubocurarine, reversed sarin-induced depression of reflex activity but prior inhibition of AChE with DFP did not.

We now report on the effects of DFP and soman, and on the antagonism of DFP-, sarin- and soman-induced depression of reflex activity. Spinal cords were removed from 7-9-day old rats, hemisected, placed in a chamber and superfused with physiological solution at 25 C. Reflex activity was evoked by stimulating L_3 dorsal root and recording from L_3 ventral root with suction electrodes. DFP, soman and sarin caused a dose-dependent depression of reflex activity. Neither DFP nor soman caused facilitation. Although having no effects by themselves, ATR and pirenzapine, respectively, completely and partially antagonized the depression caused by all three agents. At 200 nM, sarin produced maximal depression of the reflex to 25% of control. At 2, 20 and 200 nM ATR (+ 200 nM sarin), the reflex was 30%, 70% and 100% of control. At 500 nM ATR (+ sarin 200 nM), the reflex increased to 120% of control. Maximal reversal of depression with pirenzapine occurred at 100 nM to 75% of control; when ATR (500 nM) was added, the reflex increased to 150% of control. At 5, 10, 20 and 50 nM soman, reflex activity was also depressed to 80%, 70%, 50% and 16% of control. Higher concentrations did not cause greater depression but with continued exposure to soman there was some slow reversal of depression. ATR (500 nM) reversed soman induced depression completely. DFP caused 50% depression at 100 μM and was also reversed with ATR. DFP pretreatment had no effect on the depression caused by sarin. DFP and soman depress reflex activity, but sarin both facilitates and depresses. The depression appears to be receptor specific since antinicotinic agents are ineffective. The persistence of their action after inhibition of AChE as well as the antagonism of depression by ATR and pirenzapine suggests that: i) the effects of these agents are unrelated to AChE inhibition; and ii) muscarinic cholinoceptors are involved in their depressant actions. (Supported by U.S. Army Medical Research and Development Command contract DAMD17-81-C1279.)

125.9 TABUN FACILITATES AND DEPRESSES SPINAL REFLEXES IN CAT AND NEONATAL RAT SPINAL CORDS. Karen L. Swanson and Jordan E. Warnick. Dept. of Pharmacol. & Exp. Ther., Univ. of Maryland School of Medicine, Baltimore, MD 21201.

We have studied the actions of tabun (ethyl N-dimethylphosphoramidocyanidate), a potent inhibitor of acetylcholinesterase (AChE), on spinal reflex activity in cats in vivo and in the hemisected spinal cord of neonatal rats in vitro. The cats were anesthetized with α-chloralose (70 mg/kg, i.v.) and a dorsal laminectomy was performed at L_4 to L_7. Prior to recording reflex activity, the anesthetic state of the animal was assured; it was paralyzed with pancuronium and artificially respired. Blood pressure at the femoral artery and end-tidal CO_2 were continuously monitored.

In the cat, reflex activity was studied by stimulating the L_7 dorsal root and recording from the L_7 ventral root. Low doses of tabun (5-10 μg/kg, i.v.) facilitated the monosynaptic reflex. After further administration of tabun (cumulative dose of 20 μg/kg) the monosynaptic reflex was depressed but could be reversed by atropine (1 mg/kg).

In the isolated neonatal rat spinal cord preparation, reflex activity was elicited from spinal cords from 8- to 9-day old rats by stimulating the L_5 dorsal root and recording from the L_5 ventral root with suction electrodes. Tabun facilitated the reflex to a small extent at low concentrations (10-100 nM) but depressed reflex activity at higher concentrations (500 nM). Both facilitation and depression of the reflex were accompanied by an increase in homosynaptic depression at low frequencies of stimulation (0.2 to 1.0 Hz). Atropine (500 nM), which had no effect by itself at concentrations up to 10 μM, completely reversed the depression of reflex activity and the increase in homosynaptic depression. Tabun (500 nM) produced maximal depression of reflex activity to 25% of control; with the subsequent addition of 3, 10 and 30 nM atropine to the solution containing tabun (500 nM), the reflex attained 55%, 65% and 90% of control, respectively. Since the depressant effects of tabun appear to be related to muscarinic receptors we examined the effects of oxotremorine and of carbamylcholine on reflex activity. Oxotremorine (1-10 μM) and carbamylcholine (>10 μM) both caused a depression of reflex activity which was reversed by atropine (500 nM). These data suggest that the depression of reflex activity by tabun is mediated through muscarinic receptors within the spinal cord. (Supported by U.S. Army Medical Research and Development Command contract DAMD17-81-C1279.)

125.10 ENFLURANE PRODUCES EXCITATORY AND DEPRESSANT EFFECTS ON HIPPOCAMPAL CA 1 PYRAMIDAL NEURONS IN VITRO. M.B. MacIver, D.P. Harris*, and S.H. Roth. Departments of Pharmacology & Therapeutics and Anaesthesia, Faculty of Medicine, University of Calgary, Alberta, CANADA.

General anaesthetics are known to produce both excitatory and depressant actions on neuronal excitability in vivo, including seizure-like activity as measured in the cortical EEG. Enflurane has been particularly noted for excitatory effects, in vivo; however, little is known about its actions at the cellular level. In order to elucidate the cellular mechanism(s) underlying cortical excitation, the present study investigated the effects of enflurane on synaptic transmission in the in vitro hippocampal slice preparation.

Rat hippocampal slices (400 μ) were prepared using standard methods and maintained in a McIlwain tissue chamber (35°C, 1.0 ml/min). Bipolar metal stimulating electrodes were placed on stratum radiatum fibers to activate synaptic inputs to CA 1. Recording electrodes (2 M NaCl, 2 to 10 Mohm) were located in somal or dendritic layers of CA 1 to record evoked field potentials. Paired stimulus pulses of 0.01-0.05 ms duration, 15 to 70 μA, were delivered at 0.20 Hz. Interpulse interval delays were varied between 5 and 120 ms, in 5 ms increments, to examine the time course of short-term potentiation as a measure of synaptic inhibition.

Enflurane produced a concentration dependent continuum of effects which included both synaptic facilitation and depression. At low concentrations (approx. 5 to 10 μM) threshold for discharge of the CA 1 neurons appeared to be decreased, while synaptic inhibition increased. Input-output curves (EPSP vs. POP spike) were shifted to the left and attenuated; however, no change in slope was observed. Background unit firing activities were also increased at low concentrations. At higher concentrations (approx. 10 to 50 μM) EPSP amplitudes were depressed, response latencies increased, and unit firing decreased. The results suggest that both postsynaptic excitation and presynaptic depression may contribute to alteration of synaptic function. Multiple spiking was occasionally observed in the presence of enflurane. A combination of excitatory and depressant actions could explain seizure-like discharge in the cortical EEG.

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125.11 γ-AMINOBUTYRIC ACID RECEPTORS IN MOUSE BRAIN: COMPARISON OF BINDING AND CHLORIDE CHANNEL ACTIVATION. J. Yang-Ransom, J.B. Fischer, and R.W. Olsen, Department of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024.

[^{3}H]Muscimol binding to γ-aminobutyric acid (GABA) receptors in Swiss mouse brain showed multiple components in equilibrium and kinetic curves with a low affinity subpopulation or conformational state implicated in functional activation of chloride channels. Crude membrane homogenates of whole brain either were assayed unfrozen(M) or frozen(Mf). Physiological saline or 10 mM KPO4, pH 7.5 , 0.1 M KCl, gave similar results at 0° C. Saturation binding curves showed 2 components. In M, high affinity(H), Kd=2-20 nM, Bmax=0.8-1.4 pmol/mg protein, and low affinity(L), Kd=200-350 nM, Bmax=2-4. In Mf, Kd=1-8 nM and 15-30 nM, Bmax=0.1-0.8 and 1-3 pmol/mg. Bound [^{3}H]muscimol dissociated with multiple rates: in M, 50% of the population with $t_{½}$=20 min, 50%:17 sec; in Mf, 45%:10 min, 40%:37 sec and 15%:under 2 sec. Superlow affinity(SL) superfast off rate sites were detectable only in Mf (possibly due to even lower affinity and faster dissociation in M) and can be assayed with the antagonist [^{3}H]bicuculline methochloride. In the presence of 1 mM pentobarbital(PB), some L sites and possibly SL were converted to H ($t_{½}$=20 min), and $t_{½}$ for L increased from 17 to 60 sec. Dose-response curves for GABA receptor agonist-activated $^{36}Cl^-$efflux (Wong et al., Soc. Neurosci. Abstr. 8, 796, 1982) from cerebral slices were compared with binding data. GABA and muscimol produced a dose-dependent bicuculline-sensitive increase in chloride permeability, with EC_{50} values of about 200 nM, corresponding approximately with the affinities for L. PB by itself increased $^{36}Cl^-$flux(EC50=0.3 mM) and enhanced the response to submicromolar muscimol suggesting that L or even H GABA receptor sites contribute to chloride channel activation despite their slow off-rates. Nevertheless, H sites which dominate binding data apparently normally do not activate channels. It is possible that SL sites contribute much of the function but these data suggest that L sites(K_D=300 nM for muscimol) can activate channels, consistent with similar potencies for allosteric interactions with benzodiazepine and cage convulsant/barbiturate modulatory sites on the receptor complex.

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125.12 THE EFFECTS OF BUSPIRONE, BMY-13805AND BMY-13653 ON SPINAL REFLEXES IN THE ALPHA-CHLORALOSE ANESTHETIZED CAT. G. Keith Matheson. Department of Anatomy, Indiana University, School of Medicine, Evansville, Indiana, 47714.

Buspirone (Buspar™) is a nonbenzodiazepine antianxiety agent. BMY-13805 is a structural analog of buspirone and BMY-13653 (1-(1-pyrimidinyl) piperazine) is the major metabolite of both buspirone and BMY-13805. The effects of these compounds were tested on evoked potentials in ventral roots of the lumbar region elicited by stimulating dorsal roots. In addition to the monosynaptic reflex (MSR), the condition/test (C/T) paradigm was used to study their effects on the EPSP, IPSP and pre-synaptic inhibitory phases of spinal reflexes. In the intact cat buspirone (1 mg/kg. i.v.) caused a transitory decrease in the MSR. Three hours after administration, buspirone also produced a marked reduction in the magnitude and duration of pre-synaptic inhibition, while not significantly affecting either excitatory or inhibitory post-synaptic potentials. When the spinal cord was transected at the upper lumbar levels a different picture developed, neither buspirone or BMY-13805 (1 mg/kg. i.v.) produced the transitory change in the MSR seen in the intact preparation. In addition, there is no change in the pre-synaptic mechanism. This suggests that buspirone's effects on the MSR and pre-synaptic inhibitory mechanism are mediated via descending pathways from higher brain centers in the intact preparation. Additionally, there is a significant increase in the amplitude of potentials evoked during the excitatory phase of the C/T stimulatory paradigm following buspirone. BMY-13805 elicited a slight decrease in the early stages of pre-synaptic inhibition in the spinal cat, otherwise there were no significant changes. BMY-13653 did not have any significant effect on the excitatory, inhibitory or pre-synaptic inhibitory phases of the C/T paradigm in the spinal preparation.

125.13 LONG-TERM POTENTIATION (LTP) INDUCED BY IBMX IN THE CA1 REGION OF RAT HIPPOCAMPUS. I. Mody and J.J. Miller, Dept. of Physiology, University of B.C., Vancouver, B.C. V6T 1W5.

Several studies have shown that compounds such as neuroleptics and EGTA, known to interfere with Ca^{2+}-mediated events, block LTP in the hippocampal formation. Other reports have stressed the importance of a Ca^{2+}-requirement in this phenomenon and have demonstrated that calcium itself can produce LTP. The present study describes the action of 3-isobutyl-1- methylxanthine (IBMX), known for its property to release intracellular Ca^{2+}, on the stratum radiatum (SR) evoked field potentials of the CA1 region.

Experiments were carried out using the hippocampal slice preparation. Extracellular recording electrodes were placed in the SR and stratum pyramidale of CA1 to monitor Schaffer collateral/commissural evoked population responses. In some slices intracellular recordings were obtained to assess any changes in RMP and R_N of CA1 pyramidal cells.

A 10 min exposure to IBMX(100 μM) resulted in a consistent non-reversible (up to 2-3 hrs) enhancement of EPSPs and population spikes(range: 300-900%). Presynaptic excitability or recruitment, as reflected by the amplitude of the fiber-volley (FV), was unaltered. IBMX consistently shifted the FV v EPSP dV/dt and FV v EPSP amplitude curves to the left and in some cases also changed the slope of the relationship. The EPSP dV/dt v pop.spike amplitude curves were usually shifted to the right or were unaffected. These changes were not due to alterations of RMP or R_N of CA1 pyramidal cells, however, a reduced firing threshold was observed. In addition, IBMX was able to exert its effect when synaptic transmission was significantly depressed in the presence of Co^{2+} (1.5mM).

Methylxanthine derivatives also show potent phosphodiesterase (PDE) inhibitory activity and act as adenosine antagonists. Theophylline (100 μM) had a comparable effect to that of IBMX, although of lesser magnitude and the changes were fully reversible after a 10-15 min washout period. Another PDE-inhibitor, papaverine (100 μM) had an opposite effect to that of IBMX.

On the basis of these data, the LTP-inducing effect of IBMX does not appear to involve adenosine antagonism and/or PDE-inhibition, but rather the release of intraneuronal Ca^{2+} from calcium-storage sites.

125.14 **NMDA Receptors in the Rat Brain. II. Physiological Analysis and Involvement in Potentiation.** E.W. Harris, A.H. Ganong, D.T. Monaghan and C.W. Cotman, Dept. Psychobiology, University of California, Irvine CA 92717.

Excitatory amino acid receptors appear to mediate neurotransmission at many synapses in the mammalian CNS. The best characterized of these receptors are preferentially activated by N-methyl-D-aspartate (NMDA). The role of NMDA receptors in synaptic transmission remains unclear, and specific antagonists of NMDA are not potent blockers of any identified monosynaptic pathway. We have studied NMDA responses and synaptic transmission in stratum radiatum of rat hippocampus, where NMDA sites are relatively abundant, using a series of ω-phosphono acidic amino acid analogs that have a characteristic pattern of antagonism of NMDA sites.

Bath application of 10uM -AP5 (2-amino-5-phosphonopentanoate) or ±AP7 (2-amino-7-phosphonoheptanoate) reduced focal depolarizations produced by ionophoretic application of NMDA by 68%(± 2) and 44%(± 2) respectively. Solutions of 100uM ±AP4 (2-amino-4-phosphonobutyrate) and ±AP6 (2-amino-6-phosphonohexanoate) reduced NMDA responses less than 20%. None of the ω-phosphonates were potent antagonists of ionophoretically-applied kainate or quisqualate.

A similar spectrum of activity was seen for blocking long-term potentiation (LTP) of synaptic responses in stratum radiatum (Schaffer collateral pathway). Addition of 50uM -AP5 or ±AP7 had no effect on control synaptic potentials, but completely blocked the induction of LTP by high frequency stimulation (100Hz x 1s, three times). LTP could be induced once either drug was washed out, however. AP5 and AP7 did not reduce a response potentiated by previous high frequency stimulation. Neither +AP5, ±AP4 nor +AP6 had any effect on synaptic potentials or the development of LTP.

Extracellular and intracellular recordings in CA1 revealed that application of NMDA agonists induced burst firing of sodium action potentials, TTX-resistant recurrent spikes and apparent increases in input resistance. These effects were elicited by ibotenate, ADCP and quinolinate, but never by kainate, quisqualate, or AMPA.

Hippocampal NMDA receptors are pharmacologically similar to those previously described in spinal cord. These sites are also enriched in synaptic membranes isolated from whole rat brain. Although the synaptic potential probably does not result from activation of NMDA receptors, they are important for the induction of LTP, possibly via a voltage-dependent calcium conductance. (Supported by DAMD 17-83-C-3189)

125.15 **NMDA Receptors in the Rat Brain. I. Subcellular and Anatomical Distribution.** D. Yao*, D.T. Monaghan, A.H. Ganong, E.W. Harris and C.W. Cotman. Dept. Psychobio., Univ. Cal., Irvine CA, 92717.

Considerable evidence has accumulated indicating that L-glutamate is a major excitatory neurotransmitter. Three receptors which mediate glutamate's excitatory action have been identified by their selective interaction with N-methyl-D-aspartate (NMDA), kainate, or quisqualate. Of these, the NMDA class has the best defined agonist/antagonist pharmacology, however, relatively little is known regarding its distribution and ligand-binding properties. In this study we have used radioligand binding techniques to determine the subcellular and anatomical distribution of NMDA-displacable ^{3}H-L-glutamate binding sites.

Ligand binding to purified rat brain membranes was determined by a microfuge assay using 10 nM ^{3}H-L-glutamate in 50 mM Tris-acetate. In synaptic plasma membranes (SPMs), approximately 50% of the binding exhibits the distinctive NMDA receptor pharmacology: -AP5 (2-amino-5-phosphonopentanoate) and +AP7 (2-amino-7-phosphonoheptanoate) are potent and selective displacers of the NMDA-sensitive site, whereas +AP4 and +AP6 (the 4 and 6 carbon derivatives) are both substantially less potent, as is +AP5.

Displacement of bound glutamate by 100 µM NMDA was determined in various membrane fractions. Relative to original brain homogenate, NMDA sites were distributed — P_1:0.42, P_2:-0.74, mito.: 0.39, SPMs : 3.29, and synaptic junctions 13.63. In the original homogenate NMDA sites account for 15% of the specific binding, but in synaptic junctions they represent 65%.

The anatomical distribution of NMDA sites was determined by quantitative autoradiography as previously described (Nature, 1983, 306:176). The highest concentrations are found in the outer layers of cerebral cortex, pyriform cortex, ant. olfactory nuclei, n. accumbens, and s. radiatum and s. oriens of hippocampus. High levels are found within the caudate/putamen, middle and deep cerebral cortical layers, n. reuniens, lateral septum and external plexiform layer of the olfactory bulb. Moderate levels are found in thalamus, granule cell layer of the cerebellum, inferior olive, medial vestibular nucleus, n. solitary tract, cuneate nucleus, dorsal cochlear nuc., dorsal horn of spinal cord, and medial septum. Low levels are found in the globus pallidus, habenula, hypothalamus, midbrain, and molecular layer of the cerebellum.

NMDA-sensitive ^{3}H-L-glutamate binding sites are greatly enriched in the synapse. Since NMDA receptor activity is necessary for long term potentiation (LTP) in the s. radiatum of hippocampus, the distribution of binding sites described here, may indicate regions which exhibit LTP which can be blocked by AP5.

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125.16 EVALUATION OF THE ABILITY OF SEROTONERGIC DRUGS TO MODULATE THE RELEASE OF ^{3}H-5HT FROM RAT SPINAL CORD SYNAPTOSOMES. P.J. Monroe and D.J. Smith Depts. of Anesthesiology and Pharmacology, West Virginia University Medical Center, Morgantown, WV 26506

The existence of a presynaptic receptor capable of modulating the release of serotonin (5HT) from a rat spinal cord synaptosomal preparation has previously been demonstrated (Monroe and Smith, Soc. Neurosci Abstr. 9, 1983). In the present study, the functional characteristics of the autoreceptor were determined through an evaluation of the ability of serotonergic drugs to alter release. These drugs were examined for their ability to directly initiate release (measured as an increase in basal ^{3}H-efflux), modulate K^+ stimulated ^{3}H-efflux, or antagonize the interaction of exogenous 5HT with the autoreceptor.

Synaptosomes were prepared from spinal cord tissue and were suspended in a Tris-buffered Kreb's medium. After 2-10 min. incubations (37°C) in the absence then in the presence of 100 nM ^{3}H-5HT, an aliquot (0.4ml) of tissue suspension (50 mg/ml) was loaded onto a bed of Sephadex G-15 and superfusion was begun. The standard procedure was to wash the tissue for 50 min., then to change to a medium containing drugs 20 min. prior to exposure of the tissue to medium containing high K^+ (15 mM). Tissue was lyzed at the termination of the experiment with 1N HCl. ^{3}H-5HT release was expressed as a percentage of total ^{3}H available. Fluoxetine (1µM) was present during all superfusions.

Methiothepin (MET) and quipazine (QP) were found to have no effect on basal, nor on K^+ stimulated ^{3}H-5HT efflux. However, both drugs significantly ($p<.05$) antagonized the depressant effect of 30nM 5HT on K^+ stimulated release.

Ketanserin (in concentrations $\leq$ 30nM) was found to have no effect on K^+ stimulated ^{3}H-5HT release, nor on the ability of 5HT to modulate the release. Higher concentrations of drug evoked increases in basal ^{3}H-5HT and ^{3}H-5HIAA efflux, indicative of a non-specific action. Spiperone produced similar increases in basal ^{3}H-efflux. The inability of ketanserin to alter 5HT-mediated regulation of release suggests that the autoreceptor function is not associated with $5HT_2$ binding sites. The idenity of the $5HT_1$ subtype associated with the regulation of release is currently under investigation using spiperone and LSD. Preliminary data suggest that LSD possesses agonist properties at the autoreceptor.

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125.17 DEVELOPMENT OF INHIBITORY MECHANISMS IN DISSOCIATED CULTURES OF RAT HIPPOCAMPUS. Deborah M. Barnes and Marc A. Dichter, Dept. of Neuroscience, The Children's Hospital, Boston, MA 02115.

Hippocampal tissue was obtained from E20-22 d rats, dissociated mechanically, and plated (6.5×10^5 cells) onto collagen and polylysine-coated coverslips in 35mm plastic dishes. Media supplemented with 5% horse and 3.5% rat serum was changed 3x per week. At 1,2, and 3 weeks in culture cultures were examined by ^{3}H-GABA uptake autoradiography, for the presence of pairs of neurons coupled by chemical synapses, and for physiological response to applied GABA. Other experiments involving spike afterhyperpolarizations (AHP) were performed on neurons after 3-5 weeks in vitro.

Hippocampal neurons are positive for ^{3}H-GABA uptake as early as 1 week in vitro and continue to be positive at 2 and 3 weeks. Similarly, neurons at 1,2, and 3 weeks show membrane hyperpolarization and a conductance increase with GABA, and the sensitivity to GABA does not vary significantly with age. Both excitatory and inhibitory chemical synapses occur as early as 1 week, with an increase in the percentage of inhibitory synapses in older cultures. Inhibitory synaptic potentials, either spontaneously occurring or driven, are bicuculline-(10 uM) sensitive and appear to be due to an increased Cl^- conductance.

Action potentials in the hippocampal neurons in culture are followed by complex hyperpolarizing events, consisting of a 'fast' K^+-dependent AHP and a longer latency and duration hyperpolarization which is bicuculline-sensitive and Cl^--dependent. The latter is likely to be a recurrent IPSP. Sometimes the recurrent IPSP occurs without a detectable isopotential segment after the spike and appears as a long Cl^--dependent AHP.

Supported by NIH grants NS06869, NS15362, and the CHMC Mental Retardation Core Grant HD06276.

125.18 SIMULTANEOUS MONITORING OF ION-SELECTIVE AND VOLTAMMETRIC ELECTRODES IN MAMMALIAN BRAIN. B. Moghaddam*, G. Nagy* and R.N. Adams. Department of Chemistry, University of Kansas, Lawrence, KS 66045.

Ion-selective microelectrodes (ISMs) are now used routinely to measure ion fluxes in brain extracellular fluid. Miniature voltammetric electrodes which detect changes in biogenic amine neurotransmitter levels are also available. We have combined these measuring techniques to simultaneously monitor both neurotransmitter and ionic fluxes elicited by various drug stimulations to rat brain.

A multi-electrode assembly consisting of a K^+-sensitive ISM and a Nafion-coated voltammetric electrode are cemented together with a pressure-ejection micropipette. Various drug ejections are made next to the detector electrodes and one can follow changes in both $[K^+]_o$ and biogenic amine release.

Compounds such as veratridine and glutamic acid which cause neuronal depolarization give rise to increases in both ISM and voltammetric signals as expected. The magnitudes of the $[K^+]_o$ increases with typical depolarization drugs are ca. 2-5 mM--values which are expected in view of previous ISM studies. On the other hand, drugs which are believed to operate via carrier-mediated release of biogenic amines and which do not involve neuronal depolarization, show little or no ISM signal when the voltammetric signal increases.

The time courses and magnitudes of the ISM and voltammetric signals have very different profiles. Furthermore, by various pharmacological and neuronal pathway manipulations, one can begin to "tease out" some of the pre- and postsynaptic chemical fluxes. The results clearly show that this combined measuring technique has a breadth greater than the sum of its component parts and provides a powerful tool for studying the brain cell microenvironment.

125.19 PRESYNAPTIC AND POSTSYNAPTIC EFFECTS OF CHROMIUM (Cr^{3+}) AT THE FROG NEUROMUSCULAR JUNCTION. G.P. Cooper and J.B. Suszkiw, Depts. of Environ. Health & Physiol., Univ. of Cincinnati, Col. of Med., Cincinnati, OH 45267-0056.

Except for La^{3+} and several other members of the lanthanide series, there is little information concerning the effects of elements having a valence state of $^{3+}$ on synaptic transmission. La^{3+} and many divalent heavy metals prevent the influx of Ca^{2+} into nerve terminals and thereby block the release of neurotransmitters normally evoked by nerve action potentials. Many of these same metals increase the "spontaneous" release from non-depolarized nerve terminals. In the experiments described here we examined both the pre- and post-synaptic actions of Cr^{3+} on neuromuscular transmission. Conventional microelectrode and electrophysiological techniques were used in experiments on the isolated sciatic nerve-sartorius muscle preparation of the frog Rana pipiens. Preparations were mounted in a suitable chamber which permitted the temperature to be maintained at 16°C and which allowed easy introduction and removal of physiological solutions. Ringer solutions contained 111 mM NaCl, 2.5 mM KCl, 4 mM tris-maleate, adjusted to a pH of 7.0-7.2, and 1 µg/ml neostigmine bromide. Chromium was added as $CrCl_3$. Average endplate potential (EPP) amplitude was obtained by electronically averaging individual responses. The postsynaptic effects of Cr^{3+} were determined by iontophoretically applying acetylcholine (ACh) to the endplate from an extracellular microelectrode filled with 1 M ACh. The effects of Cr^{3+} on ^{45}Ca uptake by rat brain synaptosomes was determined during 5 sec depolarizations using a high K^+ (52.5 mM) HEPES-buffered modified Krebs-Ringers solution. Neuromuscular preparations exposed to 10-50 µM Cr^{3+} exhibited irreversible reduction evoked transmitter release. No significant change in miniature endplate potential (MEPP) amplitude or frequency were noted. However, in contrast with many other heavy metals Cr^{3+} did not reduce the uptake of ^{45}Ca into rat brain synaptosomes. Exposure to 2 mM Co^{2+}, which normally has little or no effect on MEPP frequency, produced large increases in frequency following exposure to 100-200 µM Cr^{3+}. Exposure to 100-200 µM Cr^{3+} depressed postsynaptic response to ACh as evidenced by a reduction in MEPP amplitude and in the response to iontophoretically applied ACh. These results suggest that Cr^{3+} might act by altering membrane Na^+ conductance and/or by membrane depolarization. (Supported by NIH grants ES-00159 and NS-17968.)

125.20 **CORRELATION OF BENZODIAZEPINE HYPNOTIC POTENCY WITH INHIBITION OF VOLTAGE-DEPENDENT CALCIUM UPTAKE INTO MOUSE WHOLE BRAIN SYNAPTOSOMES.** **L. Judson Chandler*, S.W. Leslie, A.Y. Chweh and E.A. Swinyard* (SPON: R.V. Smith), Division of Pharmacology, College of Pharmacy, The University of Texas at Austin, Austin, TX 78712, and Department of Biochemical Pharmacology and Toxicology, College of Pharmacy, University of Utah, Salt Lake City, Utah 84112.**

Previous work has shown that chlordiazepoxide inhibits voltage-dependent $^{45}Ca^{++}$ uptake into synaptosomes and that chronic administration of this drug results in a reduction in its inhibitory potency (Leslie et al. Biochem. Pharmacol. 29:2439, 1980). Other recent studies have shown that a variety of benzodiazepines block synaptosomal $^{45}Ca^{++}$ uptake (Taft and DeLorenzo, Soc. Neurosci. Abs. 9:1040, 1983; Shreeve and Ross, Soc. Neurosci. Abs. 9:1041, 1983). In the present study, the hypnotic potency of a series of benzodiazepines was examined and compared with their potency in blocking $^{45}Ca^{++}$ uptake into mouse whole brain synaptosomes. Benzodiazepines which produced hypnosis, as measured by loss of righting reflex, all inhibited fast-phase, voltage-dependent $^{45}Ca^{++}$ entry. In addition, there was a direct correlation between the hypnotic potency of these drugs and their ability to inhibit synaptosomal $^{45}Ca^{++}$ uptake. These results support previous findings with other sedative/hypnotic drugs and suggest that inhibition of presynaptic calcium uptake may be linked with the sedative/hypnotic actions of benzodiazepine drugs. (Supported in part by NIAAA grant AA05809 and RSDA AA00044 to S.W.L.)

NEUROANATOMICAL METHODS

126.1 RETROGRADE TRANSPORT OF CHOLINE IN CEREBELLOTHALAMIC NEURONS IN THE CAT. G.B. Stanton and A. Orr*. Dept. of Anatomy, Howard Univ. College of Medicine, Washington, D.C. 20059.

Studies in carvinvores have shown that acetylcholinesterase staining or choline acetyltransferase (ChAT) levels in the red nucleus and ventral lateral nucleus (VL) of the thalamus are dependent upon intact cerebellar projections to the midbrain and thalamus. There is also evidence that cerebellothalamic activity is enhanced or suppressed in response to acetylcholine potentiators or inhibitors. These results suggest the possibility that ascending cerebellar projections may be cholinergic. Recent reports that uptake and retrograde axonal transport of tritiated choline (^{3}HCh) is specific for cholinergic neurons prompted us to test the hypothesis that cerebellothalamic neurons would also label by retrograde transport of ^{3}HCh.

Stereotaxically placed injections of ^{3}HCh were made into VL in three cats. After a postoperative period of 20hrs, the animals were deeply anesthetized and their cerebelli were removed, quick-frozen, and sectioned in a cryostat. Cerebellar sections were mounted directly from the cryostat knife onto glass slides and dried on a warming plate. Following cerebellectomy, the animals were perfused intracardially with saline and buffered formalin. Brain blocks containing the injection sites were frozen and sectioned on a sliding microtome. Glass-mounted thalamic and cerebellar sections were processed for routine autoradiography.

Retrograde labeling was present in the cerebellar nuclei perikarya after autoradiographic exposure times as short as 17 days. Labeled cell bodies ranged in size and labeling density and were topographically distributed within the lateral deep cerebellar nuclei with respect to the injection sites. This topography was similar to other published maps of cerebellothalamic projections. A combined injection of ^{3}HCh and tritiated amino acids into dorsal VL labeled cells in the ventral dentate and interpositus posterior nuclei and axon terminals in layer I of the middle suprasylvian gyrus (areas 5,7). These results suggest that cerebellothalamic neurons are cholinergic. However, absence of ChAT antibody labeling of the cerebellar nuclei (Mesulam et al.,'83) suggests an alternative hypothesis that uptake and transport of ^{3}HCh is not restricted solely to cholinergic neurons. Supported by NIH grant EY03763 to GBS.

126.2 AN ELECTRON MICROSCOPIC METHOD TO DEMONSTRATE COLLATERAL PROJECTIONS IN THE CENTRAL NERVOUS SYSTEM USING GOLD-WHEAT GERM AGGLUTININ-HORSERADISH PEROXIDASE. C.L. Lee and D. Menétrey*. Univ. of North Carolina Sch. of Med., Chapel Hill, NC 27514.

Among the different substances that have been used recently to demonstrate the diverging axon collaterals of a single neuron, few have proven suitable for electron microscopic (EM) analysis. Other, more effective, markers are electron dense substances, e.g., gold, ferritin and silica. Colloidal gold conjugated to wheat germ agglutinin-horseradish peroxidase (gold-WGA-HRP complex), was, therefore, used as a marker to demonstrate divergent axon collateral projections, since the gold can be made in a variety of round particles of nonoverlapping sizes. This method was tested on the efferent projections of the rat's lateral mammillary nucleus.

Following pressure injections of complexes containing different sizes of colloidal gold (6-12, 12-16, and 20-30 nm) into the pontine tegmentum and the ipsilateral and contralateral anterior thalamus, retrogradely labeled cells were demonstrated in a two step procedure. Initially HRP-labeled cells (tetramethylbenzidine procedure) were observed under a light microscope and drawn with a camera lucida. Sections with labeled cells were then osmicated, dehydrated and embedded in epon-araldite for EM analysis. Examination of the ultrathin sections revealed cells that were labeled with populations of gold particles of one, two or even three different sizes. These gold particles were invariably localized in membrane-bound, spherical-ovoid organelles that resemble lysosomes. Gold particles of different sizes were found in the same organelle, suggesting a fusion of the retrogradely transported organelles. This is consistent with the fate of phagosomes. Furthermore, since raphe magnus neurons can be double labeled with gold (following spinal injection of gold-WGA-HRP) and peroxidase-antiperoxidase-diaminobenzidine (PAP-DAB) reaction product for serotonin, it may be that this method can be used in conjunction with immunocytochemistry to correlate neurochemistry with neuroanatomy.

As we had no evidence that gold particles were transported in the anterograde and/or transganglionic direction, this technique may be specific for retrograde transport and, therefore, can be applied where there may be overlapping anterograde projections.

Funded by NINCDS grants NS10321 and NS16433.

126.3 CONCANAVALIN A-HORSERADISH PEROXIDASE (CON A-HRP) CONJUGATE IS A USEFUL NEUROANATOMICAL TRACER. R.G.Wiley, R.Baker and H.Baker, Vanderbilt U.Med Schl & Nashville VAMC, Nashville, TN, 37203, and NYU Med Schl and Cornell U.Med Coll., NYC.

Wheat germ agglutinin (WGA) and cholera toxin (CT) coupled to horseradish peroxidase (HRP) are more efficient neuroanatomical tracers than free HRP producing larger numbers of more intensely labelled neurons from smaller injection sites. These conjugates work better on some neural systems than others and produce a mixture of retrograde, anterograde, transganglionic and transsynaptic labelling. Because Concanavalin A (Con A) has been reported to bind to nerve terminals and axonal transport of Con A has been demonstrated by immunohistochemical techniques, we sought to determine if the newly available Con A-HRP (Sigma Chemical) was a useful tracer in comparison to WGA-HRP and CT-HRP. Initially, each tracer was microinjected into rat caudate nucleus or cervical vagus nerve. After 24-48 hrs survival, animals were perfused with aldehyde fixative followed by sucrose. Brains and ganglia were promptly removed, sectioned in a cryostat, mounted on gel-coated slides, reacted with tetramethylbenzidine and H_2O_2 to demonstrate HRP and then examined with darkfield optics. Compared to CT-HRP and WGA-HRP, Con A-HRP produced somewhat fewer and less intensely labelled cell bodies in thalamus and substantia nigra after caudate injection and in dorsal motor nucleus of the vagus and nucleus ambiguus after vagal injection. Also, Con A-HRP consistently produced significantly less anterograde labelling in the substantia nigra after caudate injection and in nucleus tractus solitarius after vagal injection which corresponded to weak labelling of nodose ganglion sensory neurons in vagal experiments. Similar retrograde labelling results were also obtained after application of Con A-HRP to rat olfactory mucosa and rat and cat extraocular muscles. No transsynaptic label was detected after intraocular or olfactory mucosa application in rats or cats; under identical experimental conditions, these systems demonstrate transsynaptic labelling with WGA-HRP. In summary, Con A-HRP is a useful tracer with a predilection for retrograde transport, particularly by motor neurons, and a paucity of anterograde, transganglionic and transsynaptic transport in the systems tested. This selectivity of transport presumably reflects a differential distribution of Con A surface membrane receptors between cell bodies and nerve terminals and among various types of neurons. (This work supported by VA Merit Review Award, Vanderbilt U.Research Council and NIH grant NS 13742.)

126.4 NEURONAL TRANSPORT OF WHEAT GERM AGGLUTININ (WGA)-HRP IN VIVO: ENDOCYTIC AND EXOCYTIC PATHWAYS. B.J. Balin* and R.D. Broadwell. Dept. Path., Univ. MD Med. Sch., Balt., MD

Our study demonstrates that in neurons internalized cell surface membrane tagged with WGA-HRP is recycled through the Golgi complex and transported to axon terminals, not by the endoplasmic reticulum but by vacuoles derived largely from the transmost Golgi saccule. WGA-HRP binds to surface membrane oligosaccharides and is taken into cells by receptor-mediated endocytosis. The brains of control and chronically salt-stressed mice received 10-50 ul of 1% WGA-HRP delivered into the right lateral ventricle; post-injection survival times were 0 mins. to 24 hrs. The binding of the lectin conjugate to the ependymal cell surface prevented an appreciable diffusion of WGA-HRP into the brain parenchyma. Nevertheless, neuronal cell bodies and processes in periventricular areas were exposed extracellularly to the protein. Neurons labeled with WGA-HRP by retrograde transport or cell body/dendritic uptake included those of the hippocampus, midbrain raphe, hypothalamic paraventricular nuclei, accessory neurosecretory cells, and abducens nuclei. In salt-stressed mice only, WGA-HRP spread extracellularly through the neocortex, striatum, and dorsal thalamus. Numerous cell groups in the forebrain and rostral midbrain of these mice were labeled with WGA-HRP. All labeled perikarya observed ultrastructurally contained WGA-HRP reactive endocytic vesicles, vacuoles and tubules clustered around similarly reactive lysosomal dense bodies. The transmost Golgi saccule was labeled as were vesicles and vacuoles in the immediate vicinity. Neurosecretory granules forming from this Golgi saccule in paraventricular somata also contained WGA-HRP reaction product. Axons and terminals not exposed extracellularly to WGA-HRP exhibited concentrations of reactive neurosecretory granules (posterior pituitary), tubules and vacuoles 100 nm wide or larger (posterior pituitary, lateral hypothalamus, brainstem); the axonal reticulum and synaptic/endocytic vesicles were never labeled. The endoplasmic reticulum in entire neurons and fiber bundles exposed extracellularly to WGA-HRP was never labeled. Our results suggest exportable materials processed within the Golgi complex are packaged predominantly as vacuoles that can be transported throughout the neuron independent of the endoplasmic reticulum. This mode of transport is similar to that which occurs in non-neural cells. Perikaryal secondary lysosomes may contribute to the anterograde axonal transport of WGA-HRP labeled tubules and vacuoles. Supported by NIH grant NS13030.

126.5 SUCCINYL CONCANAVALIN-A AS A NEUROANATOMICAL TRACER. D.M. Nance. Dept. of Anatomy, Faculty of Medicine, Dalhousie University, Halifax, N.S., B3H 4H7, Canada.

The lectin concanavalin-A (Con-A) is transported by neurons in both retrograde and anterograde directions but the poor solubility of Con-A relative to wheat germ agglutin (WGA) may limit its usefulness. Since, there are a variety of different forms of Con-A (HRP and biotin conjugates, and succinylated Con-A) and antibodies to Con-A that are commercially available, these forms of Con-A were tested as tracers with reference to their compatibility with immunocytochemical (ICC) localization of neural transmitters. The lectins were injected as 1.0-5.0% solutions into the lateral septum, lateral hypothalamus or striatum of rats and the degree of axonal transport was assessed by several procedures. Rats were perfused with fixatives compatible with ICC of neurotransmitters. Con-A-HRP was not compatible with the ICC fixatives and there was minimal evidence of axonal transport although the injection sites could be visualized. Biotinated Con-A (Bio-Con-A), as well as Bio-WGA, with avadin-biotin-HRP complex (ABC) reagents were visualized only at the injection sites and in a few labeled cell bodies located close to these sites. Thus, Bio-Con-A and Bio-WGA may be transported but the biotin may be in too low of a concentration or not accessible to the avadin. In support of this, Bio-Con-A was visualized in both retrograde and anterograde directions when developed with ICC (rabbit anti-Con-A, ABC reagents). Succinyl Con-A (Suc-Con-A) proved to be an ideal and sensitive retrograde and anterograde tracer and compatible with other procedures. Suc-Con-A is highly soluble and detected by ICC using a commercial antibody (U.S. Biochem.). Typically the Suc-Con-A was visualized by agitating sections overnight at room temperature in anti-Con-A (1/7500 + 1% normal goat serum, 1% triton X-100). Using the ABC procedure with DAB, the sections could then be mounted or further processed for ICC of neurotransmitters (PAP procedure with 3-amino-9-ethylcarbazole). In addition Bio-anti-Con-A detected transported Suc-Con-A (saving 1 step in the ICC procedure) but it lacked the sensitivity of the regular ABC technique. Finally, Bio-goat anti-rabbit antibody followed by avadin conjugated fluorescein produced excellent results. Thus, Suc-Con-A is a very sensitive and versatile anatomical tracer. (Supported by MRC of Canada).

126.6 LIGHT AND ELECTRON MICROSCOPIC IDENTIFICATION OF NEURONAL SOMATA, DENDRITES, AXONS AND AXON TERMINALS WITH PHASEOLUS VULGARIS LEUCOAGGLUTININ (PHA-L). H.J.Groenewegen and F.G. Wouterlood*, Department of Anatomy, Vrije Universiteit, Amsterdam, The Netherlands.

The recently developed technique using the anterograde transport of the lectin Phaseolus vulgaris leucoagglutinin (PHA-L) (Gerfen and Sawchenko, 1984) has several advantages over the autoradiographic tracing technique. The injection sites of PHA-L are small and can accurately be defined, and there is virtually no uptake by passing fibers or retrograde transport. Neurons at the injection site are completely labeled including their somata, dendrites and dendritic appendages. Also the axons, their varicosities and terminal specializations appear to be excellently marked. Thus far PHA-L labeled material has only been studied at the lightmicroscopical level, which does not offer the high resolution required to identify synaptic contacts. Therefore in the present study the original method of Gerfen and Sawchenko (1984) has been adapted such that material containing PHA-L stained neurons can be subjected first to light microscopy and subsequently to electron microscopy. PHA-L was injected iontophoretically in various brain areas of deeply anesthetized rats. Following survival times of 2-6 days the animals were perfused with a buffered mixture of paraformaldehyde and glutaraldehyde. The brains were sectioned on a vibratome and the sections were reacted according to the unlabeled antibody technique. To the primary antisera 0.05-0.075% Triton was added. To visualize the labeled structures, the peroxidase-antiperoxidase (PAP) method was applied. In the electron microscope the DAB reaction product is visible as an electron dense precipitate with a cytoplasmic localization. Axon terminals on PHA-L labeled neurons can be distinguished by their contents of synaptic vesicles and their membrane specializations. In PHA-L labeled axons the reaction product seems to be precipitated around microtubules. In order to determine whether axonal varicosities observed in the light microscope represent axon terminals, we serially thin sectioned and reconstructed several axons carrying these axonal varicosities. All the axonal swellings and varicosities studied sofar contain mitochondria and appear to be involved in single or multiple synaptic contacts with dendritic spines and shafts, or neuronal somata. These results indicate that: 1. PHA-L labeled material identified in the optical microscope can be studied at the ultrastructural level, 2. PHA-L may be used for the electron microscopical study of neuronal circuits.

126.7 VITAL STAINING OF PRESYNAPTC NERVE TERMINALS WITH FLUORESCENT DYES. L. M. Okun* and D. Yoshikami, Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112.

It was recently reported that certain positively charged, membrane-permeant fluorescent dyes can be used as vital stains for mitochondria in cultured cells (Johnson et al. J. Cell Biol. 88: 526, 1981). We have found that some dyes of this type can also serve as vital, fluorescent stains for presynaptic motor nerve terminals, which are typically rich in mitochondria. This provides a new and simple approach for the visualization of living nerve terminals.

Motor-nerve terminals in live, skeletal muscle preparations from frog and mouse could be seen with great clarity by epifluorescence microscopy after a few minutes of exposure to the dye 3,3'-diethyloxadicarbocyanine iodide ($DiOC_2(5)$, 0.5μM). The definition of terminal structure afforded by this approach exceeded that obtained with differential interference contrast optics, and substructure, presumably mitochondria, could also sometimes be seen within the terminals. The morphology of presynaptic terminals revealed by fluorescence in stained, live endplates correlated precisely with that seen after subsequent zinc iodide-osmium staining of the same endplates.

When tested on frog muscle, staining by $DiOC_2(5)$ and subsequent low-level fluorescence-exciting illumination had no detectable effects on nerve-evoked muscle contractions, endplate potentials, or spontaneous miniature endplate potentials (mepps). However, strong illumination of stained terminals could produce increases of mepp frequency (possibly because of photo-induced Ca^{++} release from stained mitochondria in terminals). Other effects produced by strong illumination of stained preparations included selective enhancement of nerve-terminal fluorescence and induction of fluorescence fluctuations ("twinkling"; periodicities ~1 sec.) in subcellular muscle fiber elements, presumably mitochondria.

Staining with $DiOC_2(5)$ also allowed epifluorescence visualization of motor nerve terminals in live (anesthetized) frog, and subcutaneous injections of the dye in amounts 20-fold greater than those used for staining had no evident toxic effects. This suggests that the approach might be used for chronic studies in vivo; e.g. of development and turnover at individual junctions.

The dye Rhodamine 123 provided good visualization of nerve terminals at neuromuscular junctions of larval Drosophila as well as at those of frog and mouse, with a less detailed staining of background muscle fibers than seen with $DiOC_2(5)$.

Preliminary observations indicate that synaptic terminals in autonomic ganglia of frog can also be seen with this approach, so its usefulness in studies of the nervous system may extend beyond the neuromuscular junction.

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126.8 IDENTIFICATION OF DISSOCIATED RAT CNS NEURONS USING A RETROGRADE FLUORESCENT LABEL. S.L. Powell*, M. Goldberg*, A.R. Kriegstein and D. Prince (SPON: M.T. Lee). Dept. Neurol., Stanford Univ. Sch. of Med., Stanford, CA 94305.

New techniques for dissociation of cortical slices allow acute study of isolated mammalian neurons in vitro (Numann et al, Neurosci. Abst. 8:413, 1982). Such preparations contain a mixture of cell types which may have varying electrophysiological properties. We therefore developed a technique to identify subclasses of isolated neurons which have been dissociated from different brain areas.

Neurons were retrogradely labeled by making focal injections of the fluorescent dye bisbenzamide (Bb, 2%) into the following CNS areas in different animals: 1) thoracic spinal cord containing axons of cortical pyramidal cells; 2) substantia nigra (SN) containing axons from neostriatal neurons; and 3) rostral caudate-putamen containing axons from SN cells. Adult rats (200-300 g) were used for experiments requiring stereotaxis; rats age 1 day-adult were used for spinal cord injections. After 24 hours animals were killed and brains rapidly removed and cut into 600 μm slices with a tissue chopper. Sensorimotor cortex, anterior neostriatum, or SN was dissected free and subjected to enzymatic and mechanical dissociation according to the technique of Wong and colleagues (Neurosci. Lett., in press). Adjacent slices demonstrated bright, specific neuronal labeling throughout each target area. Glia were labeled as well if survival time exceeded 48 hrs, if Bb was inadvertently injected into the subarachnoid space, or if slices were allowed to stand at room temperature.

Dissociations of each target structure yielded some labeled neurons with bright fluorescent nuclei. Dissociated labeled cells were phase bright and excluded vital dyes, suggesting they were viable neurons.

Dye-labeled cortical neurons, pyramidal in shape, were recovered as early as day P1. Dissociations from neonatal cortex yielded large numbers of cells with long processes. Bipolar as well as pyramidal configurations were common in the first postnatal days.

The combination of retrograde fluorescent labeling and acute dissociation techniques will allow detailed physiological studies of identified isolated neurons from selected brain areas. Supported by NIH grant NS 12151 from the NINCDS (DAP) and a Klingenstein Fellowship in Neuroscience (ARK).

126.9 LIGHT MICROSCOPE QUANTIFICATION OF IMMUNO- AND HISTOCHEMICAL STAINING BY DIGITAL IMAGE ANALYSIS. J. Rogers[1] and F.S. Fay[2], Departments of Neurology[1] and Physiology[2], U. Mass. Medical School, Worcester, MA 01605.

We have developed a computer assisted digital imaging approach that permits quantification of immunochemical or histochemical staining at the light microscope level. Measurements of reactivity or cross-sectional area of stained elements are obtained within directly visualized, discretely localized microscope fields up to 200,000 um^2.

Briefly, sections are imaged by conventional microscopy. Selected fields are then recorded using an ultrasensitive video camera, the output of which is digitized and stored. Software digitally corrects the image for background, and enhances the image for high resolution display. Pixel by pixel analyses of optical densities (or fluorescence intensities) yield estimates of background staining, reactivity and cross-sectional areas of neurites, and reactivity and cross-sectional areas of perikarya within the field.

Experiments to evaluate the system's performance have been conducted using a neurotransmitter (somatostatin) specific antiserum, an organelle (neurofilament) specific antiserum, a conventional (neutral red) histochemical stain, and a common (Bielschowsky) neuropathology stain. For immunocytochemistry, both PAP and fluorescence techniques proved amenable to assay.

The results show that: 1) readings on the same field or adjacent fields of the same section are highly replicable; 2) readings on similarly localized fields in adjacent sections are highly replicable; 3) readings on similarly localized fields in sections from different experimental groups (e.g., Alzheimer's Disease vs. controls) are sufficiently consistent to detect significant differences between groups; and 4) system measurements of cross-sectional areas of stained elements correlate highly with hand measurements of the same regions. Experiments are now in progress to develop similar correlations between system estimates of reactivity and RIA estimates.

Supported by NIH 14523 (F. Fay) and the Alzheimer's Disease and Related Disorders Assoc. (J. Rogers).

126.10 IN VITRO APPROACH FOR THE RADIOAUTOGRAPHIC VISUALIZATION AND QUANTIFICATION OF REGIONAL DOPAMINE, NORADRENALINE OR SEROTONIN INNERVATIONS IN RAT CNS. Guy Doucet and Laurent Descarries. Centre de recherche en sciences neurologiques Université de Montréal, Montréal, Québec, Canada H3C 3J7.

In line with earlier work by Nguyen-Legros et al ('81) and Azmitia & Marovitz ('80), we searched for conditions providing integral yet specific labeling of the 3 main types of monoamine (MA) axonal varicosities in rat CNS. Vibratome slices (200 μm) of whole hemisphere from brains freshly perfused with cold, oxygenated MEM were incubated at 36°C for 15-20 min in MEM containing pargyline (10^{-4}M) and 10^{-7} to 10^{-5}M [^{3}H]dopamine (DA) or noradrenaline (NA), or $5 \cdot 10^{-8}$ to $5 \cdot 10^{-7}$M [^{3}H]serotonin (5-HT), with or without specific uptake inhibitors added. The slices were then fixed in 3.5% glutaraldehyde, postfixed with osmic acid, flat-embedded in Epon, sectioned semi-thin (4 μm) on a Polycut and radioautographed by dipping (1-30 days of exposure). After incubations in [^{3}H]DA or [^{3}H]NA without uptake inhibitors, labeled varicosities were seen throughout the cerebral cortex and septum. Their number was usually greater after 10^{-6} than 10^{-7}M incubations but reached a plateau, at these or higher molarities, following prolonged exposure times. In the presence of desipramine (DMI, $5 \cdot 10^{-5}$ or $5 \cdot 10^{-6}$M), labeled varicosities were found only in areas already known to receive a DA innervation, where their distribution patterns were also typical. In the presence of benztropine (BZ), this labeling of DA endings was clearly suppressed. With $5 \cdot 10^{-5}$M BZ and 10^{-6}M [^{3}H]DA or [^{3}H]NA, remaining silver grain clusters appeared reduced in size and number, while at 10^{-5}M BZ, their distribution suggested integral labeling of NA terminals. In the presence of both DMI and BZ, all labeling of varicosities was abolished even after the highest molarities of tracer. In the striatum, incubations in 10^{-6}M [^{3}H]DA or [^{3}H]NA induced a labeling of varicosities confined to the border of this anatomical region. Fivefold higher concentrations of tracer were required to overcome this gradient. In the presence of $5 \cdot 10^{-5}$M BZ, no gradient was visible, but the labeling was solely diffuse. Incubations with $5 \cdot 10^{-7}$M [^{3}H]5-HT together with $5 \cdot 10^{-6}$M cold NA resulted in optimal detection of 5-HT terminals in all regions examined. This labeling was abolished by $5 \cdot 10^{-6}$M citalopram or $2.5 \cdot 10^{-5}$M fluoxetine. Thus, under some of these conditions, counts of the varicosities combined with electron microscopic measurement of their diameter should yield precise quantitative data on the regional density of the different types of MA innervations.

126.11 SELECTIVE EFFECT OF KAINIC ACID ON AXONAL TRANSPORT OF ANATOMICAL TRACERS. D.A. Hopkins, T.R. King*, M.A. Morrison* and D.M. Nance. Dept. of Anatomy, Dalhousie University, Halifax, N.S., B3H 4H7, Canada.

The excitotoxin kainic acid (KA) has been reported to produce brain lesions via a selective effect on cell bodies relative to fibers of passage or axon terminals. We report here that KA in conjunction with neural tracers prevents both retrograde and anterograde transport by cell bodies but does not affect retrograde labeling of cell bodies with terminals in the injection site. Succinyl concanavalin-A (Con-A) or wheat germ agglutin-HRP (WGA-HRP) were injected bilaterally into the striatum, lateral septum, hippocampus or anterior thalamus of rats. Also, an infusion of 0.25-0.4 µl of KA (2 µg/µl) or saline was injected unilaterally into the above brain areas. After 2-3 days Con-A was visualized by immunocytochemistry using rabbit anti-Con-A and the ABC technique and WGA-HRP was visualized using the TMB procedure. Similar results were obtained with both tracers. After hippocampal injections, the anterograde labeling on the ipsilateral side of the lateral septal area was reduced or eliminated but there was no effect on retrograde labeling in the medial septal area. After lateral septal area injections anterograde labeling of the ipsilateral diagonal band was eliminated by KA injections but retrograde labeling of cells in the diagonal band and lateral hypothalamus was unaffected. After striatal injections anterograde labeling in the ipsilateral substantia nigra, pars reticulata, was almost entirely eliminated on the side of the brain which received the KA injection. Counts of retrogradely labeled cells in the substantia nigra, pars compacta, showed comparable numbers of labeled cells on the two sides of the brain. With anterior thalamic injections retrograde labeling in the mammillary nuclei and transnuclear labeling of axon collaterals in the dorsal tegmental nucleus were unaffected by KA. In other experiments, unilateral injections of KA into the lateral septum combined with bilateral injections of fast blue into the lateral hypothalamus eliminated the retrograde labeling of cells in the KA-injected lateral septum. KA exerts a selective effect on cell bodies located at the injection site relative to fibers of passage and axon terminals. Also, KA can be used to distinguish between direct anterograde projections and indirect collateral projections.

(Supported by MRC of Canada).

126.12 SIMULTANEOUS MACROSCOPIC, MICROSCOPIC AND ULTRASTRUCTURAL MARKING OF EXPERIMENTAL LESIONS WITH COLLOIDAL CARBON. L.C. Triarhou* and M. del Cerro (SPON: T.C. Theoharides). Center for Brain Research, University of Rochester Medical Center, Rochester, New York 14642.

A modern trend in neuroscience is to place minute lesions or microinjections into the nervous system for a variety of experimental purposes. Small size often makes localization of the lesions difficult during preparation of tissues for histopathology. We report the use of colloidal carbon as a lasting morphological marker for identifying injection sites at the macroscopic, microscopic and ultrastructural levels in the same specimen.

The colloidal carbon suspension was prepared by dialyzing India ink against distilled water for 48-72 hr. One drop of dialyzed ink was added to 1 ml of the solution to be injected. We used this procedure to mark the lesion sites in lysolecithin-induced demyelination and in cerebellar stab wounds. Following perfusion with aldehydes, visualization of the lesions could be readily performed a) macroscopically, without the need for microtomy, b) microscopically, without the need for histological staining, and c) ultrastructurally, without resorting to histochemical reactions. Since the amounts used were much smaller than those needed to block the reticuloendothelial system, colloidal carbon could be considered practically inert and serving merely to tatoo the lesions. The removal of the tracer took place slowly, primarily by the action of invading phagocytes. Lesions were still marked at 70 days postoperatively.

The applicability of the method is certainly not limited to neurobiology. Colloidal carbon may serve to trace lesions placed in other tissues as well. Thus, it may be effectively used as a multilevel marker in the general field of experimental morphology.

126.13 ALTERATIONS IN GLUCOSE-6-PHOSPHATASE (G6Pase) ACTIVITY AND GLYCOGEN WITHIN CIRCUMVENTRICULAR ORGANS OF SALT-STRESSED AND FASTED MICE. A.M. Cataldo* and R.D. Broadwell. (Spon: M. Salaman) Dept. Path., Univ. MD Med. Sch., Balt. MD.

Glycogen and localization of G6Pase activity were identified using ultrastructural cytochemical techniques applied to cells of the choroid plexus, median eminence and anterior and posterior lobes of the pituitary gland from control, salt-stressed, and fasted mice. G6Pase is a phosphohydrolase that converts glucose-6-phosphate to glucose. Cytochemical preparations of the tissues were similar to those described by us last year (Soc.Neurosci.Abstr. 9:298, 1983). Further identification of presumptive glycogen particles was provided by post-staining ultrathin sections with the periodic acid-thiocarbohydrazide-silver protein technique of Thiery (J. Microscopie 6:987, 1967). Presumptive glycogen particles were absent in tissue sections exposed to diastase, an α-amylase that digests glycogen. A subjective, qualitative assessment of glycogen concentration and G6Pase activity in tissues from experimental animals was rated as increased, decreased or no change (NC) compared to control tissues in which glycogen concentration and G6Pase activity were assessed as sparse (S), moderate (M) or prominent (P) from electron micrographs. A summary of the results is presented in the following table:

	Choroid Pl. Epithelium		Anterior Pituitary	
	Glycogen	G6Pase	Glycogen	G6Pase
Control	M	P	P	P
Salt	↑	↓	NC	NC
Fasted	↑	↓	↑	↓
	Posterior Pituitary		**Med. Emin. Ependyma**	
	Glycogen	G6Pase	Glycogen	G6Pase
Control	S	-	P	P
Salt	NC	↑	NC	NC
Fasted	NC	↑	↑	↓

With the exception of the posterior pituitary, glycogen particle concentration increased and G6Pase activity decreased in tissues sampled from fasted mice. A similar inverse relationship was evident in the salt-stressed choroid plexus. G6Pase activity became apparent in profiles of endoplasmic reticulum in axons and autophagic preterminal swellings in the posterior pituitary under salt-stressed and fasted conditions. Our results suggest that in the sampled cells glycogen storage and cytochemical activity of G6Pase may be interrelated; modulations in glycogen particle concentration and G6Pase activity may be a reflection of energy metabolism. Supported by NIH grant NS18030.

126.14 DIRECT AND INDIRECT ANALYSIS OF DENDRITIC DOMAINS OF IDENTIFIED PROJECTION NEURONS OF THE ACCESSORY OPTIC SYSTEM EMPLOYING TWO SITS MULTIPLE LABELING TECHNIQUES. L.C. Schmued*, R.A. Giolli, R.H.I. Blanks, Y. Torigoe*, and J.H. Fallon. (Spon: D.D.Williams). Dept. of Anatomy, Univ. Calif. Coll. Med., Irvine, CA 92717.

The fluorescent tracer SITS (4-Acetamido-4-Isothiocyanostilbene-2, 2'-Disulfonic Acid, Disodium) has been employed in two different modes and in conjunction with either an anterograde transynaptic or retrograde tracer to yield information about the dendritic distribution of neurons of the accessory optic system having specific identified axonal projections.

In the first experiment, SITS was used as a retrograde tracer injected into either the caudate nucleus or the prefrontal cortex. ^{3}H-adenosine which is capable of undergoing anterograde transneuronal transport was injected into the vitreous body of the eye. After a 4 day to 2 week survival period, the brains were sectioned and prepared for autoradiography. The existence of double labeled cells (silver grains plus blue fluorescence) in neurons of nuclei parabrachialis pigmentous and paranigralis and of the pars compacta, substantia nigra suggest that these neurons which project to the telencephalon also send dendrites into the medial terminal accessory optic nucleus where directionally and speed selective visual information is received monosynaptically.

In the second experiment SITS was administered systemically to rats whose oculomotor muscles had previously been injected with either of the fluorescent retrograde tracers propidium iodide (PI) or nuclear yellow (NY). SITS employed in this manner yields a rapid fluorescent Golgi-like impregnation which is fully compatible with other retrograde tracers. The existence of double labeled cells in the oculomotor complex allows one to directly observe the dendritic distribution of oculomotor neurons projecting to specific extraocular muscles. Particular attention was given to the smaller more peripheral neurons of the oculomotor nucleus which often send dendrites into the periaqueductal gray. The extensions of the dendrites of oculomotor neurons into adjacent periaqueductal gray provides for a direct, monosynaptic pathway linking retinal ganglion cells with oculomotor neurons through the accessory optic system.

SITS is used in an indirect and in a direct manner: Indirectly, as a retrograde marker, in combination with autoradiography, to reveal the presence of a heretofore unidentified accessory opti-mesotelencephalic pathway and, directly, as a stain to yield a "Golgi-type impregnation" of the dendritic processes of motoneurons of the oculomotor nucleus, whose identity as such has been determined by retrograde labeling from extraocular muscles with PI or NY.

(Supported by NIH grants EY03642 and NS 16017.)

126.15 MICROSCOPIC NETWORK ANALYSIS OF MAMMALIAN CNS MONOLAYER CULTURES VIA "NEURON-SPECIFIC" BODIAN-NISSL STAINING. M.H. Hightower*, D.I. Salerno* and G.W. Gross. Dept. of Biology, The Texas Woman's University, Denton, Texas 76204.

Bodian and Nissl staining techniques have been modified for use with dissociated mouse spinal cord cells cultured on coverslips. When both procedures are performed on the same culture, a population of cells with neuronal morphology costains and very little staining of the underlying cell carpet occurs. These results suggest that the classic histological techniques possess considerable specificity for neurons if proper fixation procedures are followed.

The combined Bodian/Nissl method enhances the visualization of somal regions and of the detailed fine process circuit. Neuronal 'monolayer' cultures are shown as shallow, three-dimensional networks with neurites extending through several focal planes. Mapping of afferent and efferent fibers in complex areas of the microcircuit can therefore not be easily achieved with conventional photography alone. Hand drawings of these areas made by observation of cells at different focal depths are essential for network analysis. These different views are then used to construct sets of transparent overlays which model the multidimensional character of the culture.

We are still facing the formidable problems of tight neurite bundling (cable formation) and of extensive contour-following (around somata) by afferent fibers and by the cell's own collaterals. This close association of fibers with neuronal outlines often makes it difficult to distinguish external fibers from internal fibrous elements in large volume cells. Furthermore, small volume cells are usually too heavily stained for detailed network mapping on the cell body. Despite this complexity, it appears that a systematic application of histology, immunohistochemistry, and subsequent scanning electron microscopy will provide information to reconstruct most of the microcircuitry with both drawings and three-dimensional modeling.

We are presently modifying the histological method for use on our multimicroelectrode surfaces (MMEPs) so that the electrophysiological activity in a microcircuit may be correlated with morphological analyses. We are also comparing statistically the specificity of these traditional neuronal stains with that of immunohistochemical methods.

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126.16 A MODIFICATION OF THE CARLSEN-DE OLMOS CUPRIC-SILVER IMPREGNATION METHOD FOR USE ON MOUNTED CRYOSTAT SECTIONS. D.A. Wheeler and S. Ritter. College of Veterinary Medicine, Washington State University, Pullman, WA 99164-6520.

The cupric-silver method (Carlsen, J. and de Olmos, J.S., Brain Research 208:426-431, 1981) is a particularly sensitive method for demonstrating both mechanically and chemically induced degeneration of neurons and their processes. However, this method has a disadvantage of requiring a long processing time per section. To reduce the total processing time, we modified the technique to enable bulk processing of cryostat sections mounted on slides.

Adhesion of sections to the slide is a major problem with reduced silver techniques. We have found that 1% gelatin-0.1% chrome alum subbed slides combined with 0.1% gelatin-0.01% chrome alum spreading solution has the best adhesion of any adhesive we have tried. The spreading solution is applied to the subbed slide immediately before lifting the cryostat section from the knife blade. After sections are mounted, they are not fixed on the slides since this causes a notable decrease in staining. Tissue preparation and staining are done according to the protocol of Carlsen and de Olmos (1981) with the following changes. Staining is done in white Tissue-Tek^{R} II staining dishes (VWR). These require a volume of only 170 ml of solution to stain 25 slides. Before processing, the staining dishes and racks are cleaned with 1 N HNO_3, rinsed with distilled water, soaked in 1% H_2O_2 for 1 min, and rinsed again. The H_2O_2 prevents the reduction of the silver on the vessel walls. Four baths of the reducing solution are used: 1 sec in the first, 5 sec in the second, 10 min in the third, and 15 min in the fourth. Creosote is not used. The slides are dehydrated to xylene and coverslipped with CoverBond^{TM}.

We have found this method to be valuable in processing large quantities of tissue for the demonstration of degeneration in the CNS caused by both mechanical and chemical lesions. An important advantage of this technique is that tissue from animals with different treatments can be processed in one batch. This affords a more accurate comparison of the actual treatment effects by eliminating the variability of the silver staining.

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126.17 IMPORTANCE OF ULTRASTRUCTURAL CYTOCHEMISTRY IN EVALUATING PATHOLOGIC HUMAN NERVE BIOPSIES. H.H. Kwan*, V. Askanas, W.K. Engel. Neuromuscular Center, USC School of Medicine, Los Angeles, CA 90017.

Ultrastructural cytochemistry contributed to the study of pathologic human muscle. However, the evaluation of a biopsied pathologic sural nerve is based mainly on light micropscopy of plastic-embedded material, conventional transmission electronmicroscopy (TEM) and limited light-microscopy histochemistry of fresh-frozen nerve. Numerous abnormalities, including a variety of abnormal inclusions in Schwann cells cytoplasm and axons, have been described in various neuropathies utilizing TEM; however, in most instances their precise identification remains unknown. The exact characterization of various abnormal inclusions in pathologic nerve biopsies is very useful for more precise diagnosis and for evaluation of their pathogenic mechanism. We have now demonstrated the application of cytochrome oxidase (CO) and acid phosphatase (AP) reactions to the ultrastructural evaluation of biopsied pathologic human sural nerves.

The reaction for CO activity is based upon the oxidative polymerization of 3, 3'-diaminobenzidine to an osmiophilic reaction product. The reaction product in nondroplet form is found in the intracristae spaces and between inner and outer mitochondrial membranes. In the normal human nerve, the CO reaction stains the mitochondria in the Schwann cell cytoplasm, and within the axons, the mitochondria appear small, having a rather dense matrix and sparse cristae. In the axons, mitochondria are oriented longitudinally and are small and round in cross-sections. In 3 patients with severly abnormal mitochondria in their muscle biopsies, CO revealed abnormal mitochondria in their sural nerves. The majority of mitochondria were greatly enlarged, had distorted or absent cristae, some appearing as empty shells. In most instances they could not be identified as mitochondria in TEM.

AP is practically not detectable in the Schwann cell cytoplasm of normal biopsied human nerves. However, in 2 patients with familial non-carnitine-deficient lipid neuro-myopathy there was intensive uniform staining in the subplasmalemmal region of the non-myelitic Schwann cell cytoplasm, without concomitant structure by TEM. In one patient with dysschwannian neuropathy and an increased amount of lipofuscin in the muscle, there was a strong, excessive acid-phosphatase staining in the cytoplasm, in membranous whorls and diffusely, in many Schwann cells.

The excellent ultrastructural preservation of the nerve, specificity of the reactions, and ease of application now permits CO and AP to become a part of routine evaluation of pathologic human nerves.

(Supported by a grant from Muscular Dystrophy Association.)

126.18 THE USE OF A NOVEL FIXATIVE AS AN ALTERNATIVE TO FORMALIN. H.M. Fenton* & J.M. Liebman (SPON: C. Boast). Neurosci. Res., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

The carcinogenic potential of formaldehyde vapors has recently been published (NIOSH Document, 4/15/81). These vapors are given off by formalin (a 10% solution of formaldehyde) that is widely used by neuroscientists to prepare brain tissue for sectioning and histological staining. Recently, a non-volatile tissue fixative, designated as Mirsky's Fixative (MF) (manufacturer: National Diagnostics, Somerville, NJ; patent pending) has been introduced as an alternative to formalin. We describe our experience with MF and the conditions under which this fixative may be a satisfactory substitute for formalin in preparing brain tissue.

Rats from intracranial self-stimulation experiments were used. Fifty-seven of these rats were transcardially perfused with MF and 86 with formalin. In addition, 28 others were perfused that had undergone extracellular single unit recording studies, with subsequent iontophoretic injection of fast green dye to mark recording sites. Brains were removed and three different methods were used to stain various sections: one of two Nissl stains (cresyl violet or neutral red) or a fiber tract stain (Weil's). All three stains yielded acceptable gross localization of electrode tracts regardless of the fixative, and sites marked by fast green could also be localized when a Nissl stain was used. The optimal concentration of MF was a 50:50 solution of MF:water. The MF tended to be more viscous than formalin, requiring slightly greater mechanical pressure to achieve a satisfactory transcardial perfusion in rats. A greater amount of de-fatting (xylene immersion) was required for brains prepared in MF, particularly when tissue was immersed in fixative more than one month. Storage of brains in MF for more than 2 to 3 months is not recommended. Provided that experimental procedures are modified to take these factors into account, MF can be a satisfactory substitute for formalin in preparing brain tissue for gross histological examination.

126.19 AUTORADIOGRAPHIC AND ELISA METHODS FOR VISUALIZING BINDING PATTERNS AND DETECTING ANTIBODIES WHICH RECOGNIZE BRAIN MEMBRANE ANTIGENS. R.J. Weber*, J.A. Danks*, J.B. O'Neill*, S. McLean*, J. Hill*, C.B. Pert. Section on Brain Biochemistry, National Institute of Mental Health, Bethesda, Maryland 20205.

Mice were immunized with synaptic-enriched membranes (lysed P2 fraction) from freshly dissected rat or human brain. An enzyme linked immunosorbent assay (ELISA) was developed for detecting mouse antibodies to brain. Human striatal membranes were attached to 96 well plates and serial dilutions of immune and preimmune sera assayed for anti-brain reactivity. Rabbit antibodies to mouse immunoglobulin (mIg) were used to detect mIg, followed by alkaline phosphatase conjugated goat antibodies to rabbit immunoglobulin and p-nitrophenyl phosphate as a colorimetric indicator, and analyzed spectrophotometrically. A one hundred fold difference in control versus immune sera was observed and dilutions of up to 1:2500 easily detected as positive. The minimal amount of antibody detected by this method is estimated to be between 0.1-1.0 microgram per ml. Antisera identified as positive by this method were screened for visualization of brain binding patterns.

Individual mouse serum samples were examined for their ability to bind to fresh frozen thaw-mounted horizontal sections of rat brain (Herkenham and Pert, J. Neuroscience 2: 1029-1049, 1982). Sections incubated in phosphate buffered saline containing a cocktail of peptidase inhibitors could be subjected to overnight incubation at 0°C with suprisingly perfect maintenance of tissue quality. Following incubation with various dilutions of antibodies, the primary antibody was visualized by ^{125}I secondary goat anti- mouse immunoglobulin (McLean, et al., Brain Res. 278: 255-257, 1983). Resulting patterns could be visualized on tritium-sensitive film in less than 24 hours. Individual mice showed varying patterns which were clearly distinguishable and were lacking in pre-immune mouse sera images. These patterns could then be compared with more defined patterns of varying neuroanatomical interest.

These methods should prove useful for detecting monoclonal antibodies to antigens whose patterns have been well studied (e.g. neuropeptide receptors). Visualization of patterns or detection of antibodies in sera from patients in whom anti-brain antibodies have been detected or suspected may also prove interesting.

MORPHOLOGY OF NEURONS AND GLIA II

127.1 ORGANIZATION OF ACTIN FILAMENTS IN DEVELOPING DENDRITIC SPINES OF THE RAT. J.A.Markham, E.Fifková and K.Cullen-Dockstader*. Dept. Psych.,Univ. Colorado,Boulder, CO 80309.

The organization of actin filaments within developing dendritic spines has been examined. Samples were taken from hippocampus, cerebellum and visual cortex at various postnatal days (P10-25) in order to observe spines at different stages of development. Previous work (Westrum et al., Cell Tiss.Res.208:171,'80) has stressed a role for microtubules in the maturation of the spine and the spine apparatus. Since actin filaments are difficult to preserve with regular fixation, a cytochemical label for actin, the S-1 fragment of myosin (Fifková, Delay, J.Cell Biol.95:345,'82) was employed to examine the role of actin filaments in spine formation. Since actin in nonmuscle cells is involved in various forms of cellular motility and in the maintenance of cell shape, it is likely to have similar functions in neurons. The results show actin filaments to be consistently present in both dendrites and maturing spines, where they form a network within the cytoplasm. Actin filaments are associated with the plasma membrane and postsynaptic density. They converge towards the spine apparatus and a number of them are associated with its sacs. Within the spine stalk, the filaments are oriented lengthwise. Overall, the organization of actin filaments seems to indicate a role both in the directing of materials into the spine as well as maintenance of spine shape during outgrowth and maturation. The transport of materials into the spine may involve both actin filaments and the microtubules, which are sometimes seen to curve from their parallel course in the dendrite towards the region of the future spine. Microtubules may direct materials necessary for spine outgrowth from the cell body to the area of the developing spine where actin filaments may direct transport into the spine itself. In the absence of other cytoskeletal elements, actin filaments are likely to determine the final shape of the spine and are probably responsible for the constriction of the spine neck following positioning of the spine apparatus within the spine head. Since the spine apparatus is known to contain Ca^{++} ions (Fifková, Markham, Delay, Brain Res.266:163,'83), it may serve to control the actin filament network during spine growth, as well as in mature spines, through regulation of cytoplasmic calcium concentration.

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127.2 ASSOCIATION OF THE ACTIN LATTICE WITH CYTOPLASMIC ORGANELLES AND THE PLASMA MEMBRANE IN DENDRITES AND DENDRITIC SPINES. E. Fifková, J.A. Markham, and K. Cullen-Dockstader*. Dept. Psych., Univ. of Colorado, Boulder, Colorado 80309.

Actin filaments in order to be functionally effective, must be in orderly organization and must be anchored to membranes. Actin filaments are asymmetric polymers with nonidentical ends which can be distinguished by a differential preference for the filament assembly. When reacted with the S-1 subfragments which attach themselves to the filament at an angle of 45° and so give rise to repeating arrowheads, the preferred end of filament assembly appears to be the barbed end, and the nonpreferred end the pointed one. Actin filaments are frequently associated through the barbed end with the plasma membrane and postsynaptic density (PSD). There may be also parallel associations when the filament is lengthwise positioned under the plasma membrane or PSD. End-on association, usually with the pointed end, have been also observed with the smooth endoplasmic reticulum and with the spine apparatus. On the other hand, pinocytotic and coated vesicles are most frequently associated with the barbed end of the filament. A frequent association may be also seen with dendritic microtubules via the microtubule associated proteins. Branching occurs very often within the actin networks, and a branch is attached with its pointed end to the parental filament via an actin capping protein which prevents the pointed end from a further growth. Any number of filaments may associate in bundles. Frequently there may be only two filaments running along each other in an opposite direction. Such an antiparallel orientation is thought to be involved in contractile activities, provided that both filaments are attached to the same myosin filament positioned between them. The actin networks of dendritic spines are by far denser than those of the dendrites. In spines, actin filaments anchored to the plasma membrane and PSD converge to the region of the spine apparatus, and some of them become associated with its sacs. A strikingly dense appearance of actin filaments is in the spine stalk where they are lengthwise oriented. In the absence of other cytoskeletal elements in dendritic spines, such an organization of actin may be important in controlling the shape and dimension of the spine as well as changes of these parameters during various functional states.

Supported by MH 27240.

127.3 CYTOPLASMIC STRUCTURE IN DROSOPHILA GANGLIONIC CONNECTIVE. G. Benshalom* and T.S. Reese. NINCDS, NIH, at the Marine Biological Laboratory, Woods Hole, MA 02543.

The structure of axons in the cervical connective of Drosophila was examined to determine what differences in cytoplasmic organization accompany their lack of neurofilaments. The cervical cuticle of Oregon flies was removed and the exposed connective between the head and thoracic ganglia was rapid frozen directly with a copper block cooled by liquid helium. Frozen specimens were freeze-substituted for 14-16 hours at -80°C in acetone containing 4% OsO_4, block stained with 0.1% hafnium chloride, and then thin sectioned. A basal lamina of filamentous material bisected interspaces up to 6 nm wide separating the axons from each other and from enveloping glial processes. This basement lamina covered most axons, especially those contacted by glial processes but it was thinner where axons contacted each other. Thus, an axonal basement lamina fills the spaces where K^+ accumulates during neural activity. The cytoplasm of axons contained many microtubules (27 nm average diameter) which typically had 12 rather than 13 protofilaments. The microtubules were evenly distributed through the cytoplasm but, as evident from longitudinal sections, they occurred in bundles defined by their congruent misalignments with the longitudinal axis of the axon. A thin rim of material next to the axolemma was characterized by a dense mesh of filamentous and granular material. The remaining cytoplasm consisted of a meshwork of short fine filaments (less than 6 nm in diameter) that were decorated with granular material; no other longitudinally oriented elements such as neurofilaments were evident. The fine filaments frequently contacted the microtubules, but no regular system of cross-bridges were evident. In every cross section, a few regions of the axoplasm were distinguished by a lower concentration of the filamentous and granular material. We could not determine how far these domains extended in a longitudinal direction though a few fortunate longitudinal sections showed that they might run for long distances up and down the axon and that they contained many of the axoplasmic organelles. Though the organization of axoplasm in this insect differs from that in vertebrates (eg., J Cell Biol. 94:667 1982), it may share with it segregation of organelles into special cytoplasmic domains. The significance of these domains for organelle movements and rapid axonal transport remains to be determined.

127.4 THE CYTOSKELETON OF NEURONS FROM THE COCKROACH. V.Aviv* and T.S.Reese. (SPON: J.S.McIntosh) NINCDS, NIH, Bethesda, MD 20205.

Microtubules are believed to be the major cytoskeletal element in insect neurons. In order to identify other cytoskeletal elements and to determine their distributions, we prepared cytoskeletons from cultured neurons from the cockroach Periplaneta Americana. Central nervous systems of embryos 16-21 days old were dissociated and cultured following a method developed by Chen and Levi-Montalcini (Arch.Ital.Biol., 108: 503). The isolated neurons were grown on formvar-coated gold grids treated with polyornithine. After 5 to 15 days in culture, when the neurons had developed distinct neuronal shapes, they were extracted with one of several detergent protocols (saponine, Triton, or polyoxyethylene in buffered EGTA) and then rapid-frozen. Freeze substitution and fixation with acrolein-tannic acid was done at -80°C, followed by dilute OsO_4 fixation at -55°C, and subsequent fixation and staining with glutaraldehyde, uranyl acetate, and hafnium chloride. Whole mounts were critical point dried with dessicated liquid CO_2 and then carbon coated for examination in an intermediate voltage electron microscope. At least two types of filaments survived the extraction and preparation for electron microscopy. One type, which was more frequent in neurites, had a diameter greater than 20 nm and a length greater than 2 um. These filaments were not in bundles but were parallel to the long axis of neurites. Their size and staining pattern indicated that they might be microtubules. The other type of filament had a diameter of approximately 5 nm and an average length of 0.2 um. A meshwork of these microfilaments was found throughout the cell but was more prominant in the cell body and growth cones. No intermediate filaments were recognized. Examination of the neuronal cytoskeletons by light microscopy after labeling them with rhodamine-phallacidine revealed meshworks of fluorescent filaments that were also more prominant in the cell body and growth cones. Because the labelled filaments had the same distribution as the microfilaments seen in the whole mounts, the microfilaments are tentatively identified as actin. Thus, actin filaments and microtubules appear to be major stable structural elements in different parts of these insect neurons, but other important structural elements could have been removed by the detergent extractions.

127.5 COMPUTER MORPHOMETRIC ANALYSIS OF NEURONAL DENDRITIC BRANCHING. F.R. Amthor. Dept. of Physiological Optics, University of Alabama in Birmingham, Birmingham, Alabama 35294

Retinal ganglion cells are a particularly good subject for quantitative morphometric analysis due to the basically two dimensional structure of the dendritic tree. This two dimensional structure reduces the complexity of the geometrical analysis, and allows the direct correlation of physiological properties, such as receptive field size, with anatomical properties, such as dendritic field size.

The precise meaning of various quantitative anatomical attributes, such as average branch angle or total dendritic length, has been heretofore primarily speculative. Quantitative computer methods have revealed more concrete physiological/anatomical relationships with the analysis of physiologically identified and intracellularly stained retinal ganglion cells, in conjunction with those obtained by extracellular staining.

Three basic types of analysis have been undertaken on both intracellularly and extracellularly stained rabbit retinal ganglion cells. (1) Three dimensional reconstructions have shown significant differences in the manner of bistratification between direction-selective cells and orientation-selective cells, with orientation selective cells having dendrites of primarily low order in one sublamina of the IPL, primarily high order in the other, versus the more symmetric distribution in direction-selective cells. (2) Concentric ganglion cell types with weak versus strong inhibitory surrounds appear to correspond to previously delineated anatomical classes which vary significantly in quantitative attributes that measure branch angles and dendritic curvature. Values of retroflexive curvature in direction-selective cells are the largest of all ganglion cell types. (3) Several complex "dendritic field" attributes related to dendritic order and hierarchy, and local dendritic density have also been computed. Some ganglion cells with apparently complex and overlapping dendritic branching actually show regions exclusive to each primary dendrite system. Some ganglion cell types show hot spot regions of dendritic density. The meaning of these is, as yet, unclear.

Supported by USPS Grants EY05070, EY02207 and EY03039 (CORE).

127.6 EXPRESSION OF MOSAICISM IN SPINAL CORDS OF JIMPY HETEROZYGOUS FEMALES. W.P. Bartlett* and R.P. Skoff, (SPON:J. Benjamins) Dept. of Anatomy, Wayne State Univ., Detroit, MI 48201

Jimpy is a sex-linked recessive gene which produces severe hypomyelination throughout the CNS of hemizygous male mice. In female carriers, mosaicism of the jimpy (jp) gene has been demonstrated biochemically in their brains during early myelination (Benjamins et al, J. Neurochem., 42:487, 1984). Patches of myelinated and unmyelinated tissue have been described in the optic nerve but are apparently lacking in other CNS areas (Skoff et al, Brain Res., 212:175, 1981). To determine the manner of expression of the jp gene in other CNS regions, the spinal cords of carriers were examined using light and electron microscopy.

Females were identified as carriers of the jp gene by the presence of patches in the optic nerve. The ventral column of spinal cords from 14 and 30 day heterozygous females (Ta jp/++) were compared to normal littermates (Ta+/++) and non-littermate, non-jp controls (Ta+/++, Ta+/Ta+). The percentages of surface area occupied by axoplasm, myelin sheaths and neuropil were determined with a point counting method with low power EMs. At 14 days the surface area of axoplasm in the mosaic cords is not decreased compared to controls. However the surface area of myelin is reduced 25-30%. The area occupied by perikarya and processes is increased 35-40% in the mosaic. With high power EMs, the area of axoplasm and surrounding myelin sheath was digitized using the Bioquant image analysis system. The surface area of myelin around individual axons in the mosaic is decreased by 10-15%. In contrast to two week old mice, the spinal cords of 30 day old mosaics show myelin is reduced no more than 10%. Our results indicate that myelin is reduced in spinal cords of 14 day old mosaics but that significant compensation has occurred by 30 days. This retardation preferentially affects myelination, sparing neuronal development. This finding contrasts with most other dysmyelinating models, in which both axonal and myelin differentiation are affected. Thus, the female mosaic provides an unique model to analyze oligodendrocyte plasticity.

In addition to the reduction of myelin, the relationship of the axon and surrounding sheath is often abnormal. Myelin free segments of axons, up to 100 microns, extend from internode to internode. An individual axon of constant diameter may have internodes of myelin of considerably differer thickness. These observations, taken together with the patches in the optic nerve, indicate that the neuron is not th site of the mutation. Supported by NIH NS 18883 and 18898.

127.7 COMPUTER IDENTIFICATION OF TH-CONTAINING NEURONS IN RAT BRAIN: AN ARTIFICIAL INTELLIGENCE APPROACH TO CELL MAPPING. L. Tucker*, H. Cornejo* and D.J. Reis (SPON: T. Kingan). Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Computer-based information processing systems are powerful tools for neuroanatomical analysis (e.g., Benno et al., Brain Res. 246: 225-236, 1982). Such systems are limited in their ability to perform morphometric analysis of tissue sections automatically, in failing to account for unevenly stained or fragmented cells and complex neuropil. Thus, an operator must manually outline cell bodies to segment the image (i.e., divide into regions) for analysis, relying upon his knowledge of particular neuronal types. We have designed a system which, using Artificial Intelligence (AI) techniques, performs automatic segmentation, identification, and analysis of cell images.

"Information" about neurons was incorporated into the system in the form of model rules such as "nucleus must be surrounded by cytoplasm." These rules were codified in semi-autonomous "expert" tasks designed to simultaneously search for and "recognize" neurons within a digitized image. Information encoded in model rules was used, therefore, to drive the search strategy and segment the image through a process of successive approximations. Resulting regions were matched with appropriate model elements and labeled ("background," "cytoplasm," "nucleus") such that adjacent labels were compatible. Once identification and refinement of regions in the image was accomplished, "recognized" cells were mapped and statistics on size, shape and density of staining obtained.

The system was tested using six images of single neurons in substantia nigra stained for tyrosine hydroxylase. The borders and component parts of an unevenly stained neuron were located by the system and defined within a field which contained non-neuronal fragments of various sizes, gray intensities and shapes. Records were made of the sequence of events during refinement by the system, showing that background artifacts were quickly eliminated, and actual cellular regions established. Processing time was primarily devoted to refining borders of the cell and component parts. Resulting segmented images were compared with those obtained by manual segmentation: the system correctly labeled 97.1 $\pm$ 0.79 percent of the regions in the six images, requiring 53.3 $\pm$ 0.72 iterations. We conclude that AI techniques may be beneficial in automating segmentation of neuronal images. For more complex images in which cells are in contact or overlap, additional rules are needed.

127.8 USE OF A NATURALLY PRODUCED BASEMENT MEMBRANE-LIKE EXTRACELLULAR MATRIX FOR THE GROWTH AND CHARACTERIZATION OF NEUROGLIA IN CULTURE. H. Ovadia* and O. Abramsky, Dept. of Neurology, Hadassah University Hospital, Jerusalem, Israel.

In previous studies we have demonstrated the successful in vitro interaction of isolated rat and human oligodendrocytes (ODG) with a naturally produced basement membrane-like extracellular matrix (ECM). ECM, secreted by bovine corneal endothelial cells, contain characteristic components of basement membranes such as collagen, laminin, fibronectin and heparan sulfate. ECM has the ability to enhance growth and cellular differentiation of ODG. Differentiation and proliferation of rat ODG are affected mainly through the interaction of cells with laminin in conjunction with other ECM components. This physiological substratum was further used to grow and maintain neuroglial cells sprouting out from brain slices without the use of enzymes for tissue dissociation. Hippocampal organs were dissected from brain of young rats (60-80 gr body weight), sliced to small pieces of tissue (1 mm^2) and incubated in a CO_2 incubator at 37°C. Within 3 days, neuroglial cells start to sprout around and beneath the brain slices. The cells undergo several mitoses and create a confluent monolayer. After two weeks in culture, the cells are immunologically characterized by specific antisera, anti Galactocerebroside (GalC) for the identification of ODG and anti-glial fibrillary acidic protein (GFAP) for the identification of astrocytes. Most of the cells were stained with anti GFAP and appeared as type 1 and type 2 astrocytes. Our attention was focused mainly on ODG and we found that such cultures yielded only 1% of GalC-positive cells. ODG obtained from whole brain by use of trypsin and percoll gradient were found cells with several elongated dendrites projecting from the cell soma. ODG obtained from hippocampus, without the use of trypsin, showed a different morphology where the cell soma is surrounded with a diffuse cytoplasm expressing GalC on its surface and appearing as a cytoplasmic-like oligodendrocyte. The culture also contained elongated and parallel fine fibers stained with anti GalC resembling myelin membrane. The described cell culture of neuroglia would allow characterization of ODG contained within distinct brain areas and would eliminate the detrimental effect of enzymes on cell membranes.

127.9 DEVELOPMENT OF ASTROGLIA IN THE MOUSE RETINAL AXON PATHWAY. P. Bovolenta, R.K.H. Liem, and C.A. Mason. Dept. Pharmacology, N.Y.U. Sch. Med., New York, NY 10016.

We have shown that during astroglial development in mouse cerebellum, stellate-shaped cells in axon tracts express glial filament protein (GF) at the end of the first postnatal week, after axonal outgrowth has occurred. Initially a "natural gliosis" takes place in which GF-positive cells are more numerous than in mature brain and GF expression is at a maximum (Dev. Biol. 102:248). It is not known whether this pattern is common to astroglial cells in other regions of the CNS. We are now studying astroglial development in the visual system where axonal outgrowth, guidance and topographic relationships are under study for many species. Sections of embryonic and postnatal mouse optic nerve, chiasm and tract were immunostained with antisera against two cytoskeletal proteins, GF and vimentin (Vim). Five findings have emerged: (1) In optic nerve, during the major phase of axon outgrowth (embryonic day (E) 11-17), neither GF nor Vim is expressed by the neuroepithelial cells through which retinal axons grow. (2) GF appears at E17-postnatal day (P) 5, in a wave from the eyes toward the optic tract. Within the nerve GF is first expressed in cells along the pial boundary. GF-positive cells appear in the middle of the nerve when axonal growth cones are no longer observed (E18-PO). (3) "Gliosis" occurs from P5-7, when GF expression is at maximum, particularly in the optic nerve. (4) Vim is expressed simultaneous with GF, in contrast to cerebellum where radial glia express Vim before GF. (5) The morphology and organization of astroglial cells differ with respect to position in the pathway, both during development and in the mature brain. In the optic nerve at birth GF-positive cells are oriented parallel to axons but from P7 to adulthood are multipolar. In the optic tract from P5 to adulthood, GF-positive cells are sparse and always have processes oriented parallel to axons.

Our results indicate that as in cerebellum, astrocytes in the retinal axon pathway express GF after axonal outgrowth. However, the developmental stages astrocytes follow may be specific to each brain region or fiber pathway, and the final shape and disposition of astrocytes may be influenced by the organization of axons. (Supported by NIH grants EY 03849 (RKHL) and NS 16951 (CAM).

127.10 MORPHOLOGY OF ASTROCYTES GROWN IN OCULO ON THE INTACT IRIS. H. Björklund* and D. Dahl. Department of Histology, Karolinska Institutet, Stockholm, Sweden, and West Roxbury VA Medical Center and Harvard Medical School, Boston, MA, USA.

We have studied the survival and morphology of glial fibrillary acidic protein (GFA)-positive astrocytes growing in oculo using two experimental designs. Injection into the anterior eye chamber of adult recipients of a cell suspension prepared from cortex cerebri of 10 day old rat pups gave rise to multiple GFA-positive astrocytic islets of various sizes as well as to scattered individual cells on the host iris. Several morphologically distinct cell types were observed. These included small epitheloid cells, cells with a large cytoplasm and a restricted number of relatively thick processes, and highly differentiated, typically star-shaped astrocytes with small cell bodies and numerous branching processes. This morphological variation as well as other characteristics in terms of GFA-fluorescence, such as a strong perinuclear fluorescence in less well differentiated cells and a high general fluorescence in more developed cells, is in line with that reported for rodent astrocytes in vitro. No differences in terms of cell morphology were noted between irides from animals injected with cell suspensions 10 or 42 days earlier. Instead well differentiated cells were usually seen growing individually or in less cell dense areas while more undifferentiated astrocytes were often seen in areas with a high cellular density. Cells from suspensions prepared from fetal cortex cerebri readily aggregated into large transplants comparable in size to solid intraocular cortex cerebri grafts. In contrast, only in one out of 30 irides from eyes injected with suspensions prepared from young adults were any viable GFA-positive astrocytes recovered.

When irides with mature intraocular CNS grafts were stretch-prepared as whole mounts, a thin halo of GFA-positive cells and fibers were seen emanating from both cortex cerebri and brain stem grafts. The morphology of the individual astrocytes was comparable to that seen after injection of a cell suspension. Thus, a large variation in cell morphologies were noted. In contrast, the grafts themselves contained star-shaped, well differentiated astrocytes. Obviously astrocytes develop more normally within solid grafts than in the outgrowth zone surrounding the grafts.

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127.11 COMPUTER IMAGE ANALYSIS OF RAT GFA-POSITIVE ASTROCYTES FROM ADOLESCENCE TO SENESCENCE Eriksdotter-Nilsson, M.[1]*, Björklund, H.[1]*, Dahl, D.[2], Rose, G.[3] and Olson, L.[1]*
[1]Dept. of Histology, Karolinska Institute, Stockholm, Sweden, [2]West Roxbury VA Medical Center and Harvard Medical School, Boston, MA, and [3]Dept. of Pharmacology, UCHSC, and Medical Research, VAMC, Denver, CO

The objective of the study was to determine the morphometry of GFA-positive astrocytes during maturation and aging in the rat. Using an antiserum against glial fibrillary acidic protein (GFA), smears of unfixed brain tissue were used to visualize individual astrocytes from cortex cerebri, cerebellum and the hippocampal formation. Computerized image analysis was used to determine cell area and perimeter. Strongly fluorescent spider-shaped cells with a distinct negative-staining nucleus were seen in smears from all three brain regions, although the total number of GFA-positive cells was low in cortex cerebri. While cerebellar and hippocampal cells had numerous long processes, astrocytes in cortex cerebri had only a few short processes. The postnatal development of hippocampal astrocytes was studied in detail in male Sprague-Dawley rats. Cell growth, as evidenced by increased area and perimeter, was most prominent during the second postnatal month, with both parameters increasing approximately 45%. Although the growth rate declined thereafter, cells continued to enlarge for several additional months; after 7.5 months of age both parameters were approximately 40% higher than at 2 months of age.

Male Fisher 344 rats, 4 to 30 months of age, were studied to determine astrocytic changes during senescence. During this period both cell area and cell perimeter values increased by 60%. Interestingly, the increase in cell size was uniform over this time and, using linear regression analysis, was significant at the $p<0.001$ level. Large increases in size of GFA-positive astrocytes were also noted in cortex cerebri and cerebellum between 6 weeks and 18 months in female rats.

In conclusion, the growth of GFA-positive astrocytes is a process which is prominent for several months postnatally. Following this phase, there is a slower increase of cell size which seems to continue throughout life. (Supported by the Swedish Medical Research Council, USPHS grant AG04418 and the Veterans Administration.)

127.12 INCREASED GLIAL CELL DEATH IN JIMPY MOUSE OPTIC NERVE AND SPINAL CORD. P.E. Knapp* and R.P. Skoff, Dept. of Anatomy, Wayne State Univ. Sch. of Med., Detroit, MI 48201

Jimpy (jp) is a murine sex-linked recessive trait which manifests itself in severe CNS hypomyelination. Affected animals die by 30 days of age. Previous work (Skoff, R.P., Brain Res., 248:19, 1982) has shown that the number of glia in jp optic nerve (ON) and spinal cord (SC) which incorporate (^{3}H)-thymidine, an index of proliferation, is dramatically elevated while total glial cell numbers do not increase. Several explanations may be offered for this phenomenon, the most likely being that a large percentage of glia die during development. We now present quantitative evidence of extensive glial death in ON and SC white matter of jp animals.

SC and ON from jp animals and normal littermate controls 4-20 days old were examined using 1 micron plastic sections stained with Toluidine Blue. Cells classified as dying were clearly pyknotic with grossly condensed, abnormal cytoplasm. Their nuclei were usually extremely dense with little contrast between heterochromatin and euchromatin. Percentages of dying cells are presented in the table below.

		4D.	7D.	12D.	16D.	20D.
SC	Ctrl	0.60	1.21	1.61	1.04	1.05
	Jp	1.45*	3.84**	8.82**	8.82**	5.44**
ON	Ctrl	0.26	0.70	0.64	0.89	0.23
	Jp	0.29	1.83	5.96*	5.13*	5.09*

*$p < 0.05$; **$p < 0.005$

The percent of dying cells in white matter of jp SC is significantly higher than in controls at all ages. Glial death in jp ON does not differ from normal until after 7 days.

Fine structure of dying cells reveals that nuclear degeneration usually occurs prior to cytoplasmic changes. Our studies have not yet uncovered cells in the early stages of degeneration with astroglial filaments or inclusions typical of microglial cells. Rather, the cytoplasm contains stacks of Golgi apparatus and elongate ER characteristic of oligodendrocytes. Processes of dying cells are occasionally seen adjacent to axons which they appear to ensheath. We conclude that a large percentage of the dying cells seen at the light level are oligodendrocytes and/or oligodendrocyte precursors. This idea is supported by the relatively late onset of glial death in jp ON, since oligodendroglia proliferate and begin to myelinate in ON later than they do in SC. Selective proliferation and death of oligodendroglia in jp animals suggests a fundamental defect in oligodendrocyte differentiation which may interfere with and limit myelin sheath formation.

Supported by NIH NS 15338.

127.13 EFFECTS OF PLOIDY ON AXON CALIBER AND DENDRITIC BRANCHING IN 2 STRAINS OF XENOPUS. B.G. Szaro*and R. Tompkins (SPON: A. A. Gerall). L.N.N., NICHD, NIH, Bethesda, MD 20205, and Dept. of Biology, Tulane Univ., New Orleans, LA 70118.

Although overall size, and individual organ size and shape of autotetraploid (4N) Xenopus laevis are normal, they consist of fewer cells of twice the volume of the same basic cell types found in diploid (2N) frogs. We have exploited these differences to investigate the effects of increased gene dosage and decreased cell density on the morphology of neuritic processes in the CNS. Invertebrate neurons of different ploidy and vertebrate motorneurons with different size somas show a rough correlation of axon caliber with soma diameter, but the possibility remains that these effects on axon caliber are primarily a function of specific cell type. We compared, at the light microscope level, the cross sectional areas of the large myelinated axons in the optic nerves of 4N and 2N frogs of similar size. Optic nerves from 2N and 4N frogs were stained by soaking the nerves in 2% osmium tetroxide. The nerves were embedded in epon-araldite, and semi thin sections were cut approximately one third the distance from the eye to the chiasm. Non-overlapping central fields of view (2500 to 3000 sq microns in size) were photographed under phase contrast optics (linear resolution, 0.27 microns) and printed at a magnification of 3700X. The axon cross-sections were traced on a digitizing tablet attached to a microcomputer, which then calculated the axon areas. The distributions of axon cross sectional areas (2163 2N axons; 2188 4N axons) were highly skewed, and qualitatively unimodal (2N: 0.1 to 0.15 sq microns; 4N: 0.20 to 0.25 sq microns). When transformed logarithmically these distributions were symmetrical, centered about their respective geometric means. The 95% confidence interval (student's t-test) for the ratio of the geometric means of the cross sectional areas of 4N versus 2N axons was 1.91 to 2.23. Thus the cross sectional areas and the volumes of these axons are directly proportional to gene dosage. In addition, analysis of golgi-stained dendrites in homologous cell types from other regions of the brain revealed that the total volume of dendritic processes is also affected by ploidy, but that the specific algorithm employed to achieve this increase (i.e. number or length of individual branches) varies. Thus neuronal morphology is affected by quantitative differences in gene dosage as well as by the qualitative differences in gene expression assumed to occur between different cell types. Supported by NIH 5F32 NS-06948 and NSF BNS-8216681.

127.14 DENDRITIC BUNDLES IN THE MURINE BARREL FIELD: AN ANALYSIS BASED UPON A MONOCLONAL ANTIBODY TO MAP-2.(SPON: R.S. Williams). M.I. Escobar*, H. Pimienta*, M. Jacobson*, K.S. Kosik, J.E. Crandall, and V.S. Caviness, Jr.
Southard Lab., E.K. Shriver Ctr., Waltham and Ralph-Lowell Lab., McLean Hosp., Belmont, MA

Microtubule associated protein-2 (MAP2) is selectively associated with CNS neurons and within these cells it is concentrated in dendrites. A monoclonal antibody (5F9), monospecific for the MAP2 doublet on gel blots, stains darkly the apical dendrites of murine neocortical pyramidal neurons and stains moderately the apical dendrites of the polymorphic neurons in layer VI when reacted with peroxidase conjugated secondary antibody. Other dendrites stain lightly. The present study is an analysis of the patterns of fasciculation of MAP-2 immunoreactive apical dendrites with respect to barrels of the posterior medial barrel subfield in murine SI neocortex.

The apical dendrites of all pyramidal cells, on the one hand, and the polymorphic neurons of layer VI on the other, display two separate fascicular systems. These systems have complementary domains which overlap in layers V and IV. The apical dendrites that arise in layer VI generally do not exceed 1 micron in diameter and are gathered into fascicles of 4 or more. The stained dendrites appear to ramify within the hollows of barrels in layer IV. Only exceptionally can the apical processes arising in layer VI be followed above layer IV. The apical dendrites of pyramidal cells of layer V, generally of a diameter exceeding 2 microns, ascend radially without deflection through the barrels either singly or in fascicles of 3 or more. The great majority of these fascicles are concentrated within the septa between barrel walls or ascend through the walls of the barrels themselves. Apical dendrites of pyramidal cells located in layers III and II above the barrels are recruited into these same fascicles, increasing their dendritic content several fold. In tangential sections through the supragranular layers, the fascicles form a fine-grained honeycomb pattern that does not approximate barrel contours. The present observations suggest that the distribution of pyramidal neurons of layer V, but not those of supragranular layers, forms a pattern approximating that of the barrel contours.

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127.15 SURVIVAL AND DIFFERENTIATION OF ST40 NEURONS ISOLATED FROM CHICK CILIARY GANGLION IN CULTURE. G. Crean, M. Ogawa* and G. Pilar, Physiology Section, Biological Sciences Group, The University of Connecticut, Storrs, CT 06268.

In studies from other labs, it was found to be difficult to culture for long term freshly dissociated ST40 (after period of normal cell death) neurons isolated from the ciliary ganglion. We found that when cytosine arabinoside (1 - 10 μM) was added to the culture medium to control non-neuronal proliferation, neuronal survival for 2-3 weeks was obtained. Neurons grew extensive processes and made morphological interneuronal synapses after 2-3 days in culture. Such cells also exhibited a high affinity Na^+-Ch uptake mechanism, synthesized Ach (B. Gray and J. Tuttle, this volume), and were also able to synthesize cholinesterase, such synthesis occurring around the periphery of the cell where RER is abundant. By immunocytochemistry it was shown that most of these neurons showed reactivity to leu- and met enkephalins. These observations indicate that older cultured neurons express their cholinergic and peptidergic characteristics similar to the mature in vivo state and qualitatively better than those removed at ST35.

Surprisingly, the interneuronal synapses disappeared at later times, and abundant microglia cells were observed to engulf synaptic endings, possibly mediating this synapse elimination. However, many cell processes with the characteristics of growing tips (abundant large dense core vesicles and smooth endoplasmic reticulum) were observed to abut on ganglion cells. It is possible that these ST40 cells mature sufficiently to develop a mechanism to eliminate interganglionic synapses (which do not exist in vivo). Alternatively the ST40 microglia in culture may be hyperactive in this respect. In contrast ST35 cultures show a maintenance of synapses.

Finally, we showed that these cells appear to compete for survival with non-neuronal cells, since survival or at least the occurrence of phenotypically differentiated neurons was enhanced by reducing the population of ST40 non-neuronal cells. In addition when ST40 cells were isolated on a density gradient, and seeded onto ST35 non-neuronal cells they also survived. Thus ST40 non-neuronal cells inhibit neuronal survival, possibly by competing with them for trophic or mechanical support. (Supported by NS10338, U.S. Army Research Office.)

127.16 DISTRIBUTION OF CROSS SECTIONAL AREA AT DENDRITIC BIFURCATIONS: A PRIMARY DETERMINANT OF BRANCHING PATTERNS. D. E. Hillman and M. Chujo, Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

Three principal factors compose neuronal dendritic arbors: size, branching patterns and orientation. Each can be represented by fundamental parameters that together are useful in defining neuronal arbors. The pattern of arbor branching can be defined exclusive of size or orientation by ordering of segments according to the number of terminals that are distal to each segment. Fundamental parameters of arbor size are related to segment cross sectional area and length.

This study was to determine which parameters of size may be involved in the generation of branching pattern. Various parameters of cross sectional area were tested in models and measured on dendritic arbors of Purkinje cells and pyramidal cells for their effect on branching pattern. These models were defined as cross sectional area of processes arising at the soma and then divided into paired daughter segments until a limiting terminal size was reached. Analysis of various parameters of size (diameter for stem, terminals, daughter segments and arbor taper, distally or proximally) in models revealed that distribution of cross sectional area between the daughter segments of bifurcations was the primary factor altering the type of branching pattern. Equal distribution resulted in minimum number of levels in the longest path of bifurcation to reach the limiting terminal diameter. Unequal bifurcation of cross sectional area (3:1 ratio) yielded much greater number of branch levels in the longest path to reach the terminal size. Display with standard length and orientation parameters gave a bushy arbor for the former and slender arbors for the latter. Although the extent of the branch pattern was altered by the size parameters, the basic pattern of branching was not altered.

Analysis of dendritic arbors by three-dimensional reconstruction revealed that branching patterns correlated with distribution of cross sectional area to the daughter segments as found in the models. Purkinje cells had a combination of near equal and unequal distribution of cross sectional area in the proximal part of the arbor while the apical dendrite of pyramidal cells had a uniformly unequal distribution in this zone. Both had near equal distributions in the apical zone. Supported by USPHS grant NS13742 from NINCDS.

127.17 ELECTROTONIC STRUCTURE AND SPECIFIC MEMBRANE PROPERTIES OF FROG DORSAL ROOT GANGLION NEURONS MAINTAINED IN VITRO. R. López* and F.J. Alvarez-Leefmans. Department of Pharmacology and Toxicology, CINVESTAV del IPN, Ap. Postal 14-740, México 07000 D.F.

The passive membrane properties of frog dorsal root ganglion (DRG) neurons have been studied. Spinal ganglia excised from Rana montezumae were maintained "in vitro" (7-10°C), superfused with a continuous flow of Ringer. Neurons were impalled with microelectrodes filled with 4M potassium acetate (60-90 MΩ), connected to a conventional bridge circuit. Constant current pulses were injected into the cells. There was usually a linear range in the I-V curve between resting potential and more hyperpolarized or depolarized potentials. Voltage transients were induced within this linear range by imposing a depolarizing current step. The resulting charging curves from 5 cells were analyzed following Rall's model (Biophys J. 9: 1483-1508, 1969). The "peeling" technique was used to determine the membrane time constant (τ_o) and its coefficient Co, as well as the first equalizing time constant τ_1 and its coefficient (C_1). The mean electrotonic length (L) of the cells was 1.1±0.2 (±S.E.; range 0.7 to 1.5). The mean ratio (ρ) of input conductance of the process to that of the soma was 1.1±0.3 (±S.E.; range 0.4 to 1.5). The mean membrane time constant τ_o was 4.8±0.8 ms (±S.E.; range 3.5 to 7.3 ms). The first equalizing time constant, τ_1, had a mean of 0.9±0.2 ms (±S.E.; range 0.7 to 1.5 ms). Assuming that the specific membrane capacitance (Cm) was 1μF/cm^2, the mean value of the specific membrane resistance (Rm) was 4760±815 Ω·cm^2 (±S.E.; range 3500 to 7300 Ω·cm^2). It is concluded that the electrotonic structure of DRG neurones is not that of an ideal sphere as previously thought (Ito. Jpn J. Physiol. 7: 297-323, 1957; Ransom et al., J. Neurophysiol 40: 1132-1150, 1977). DRG neurones can be represented as a lumped soma compartment attached to a uniform cylinder. Our results agree with those of Brown et al (J. Neurophysiol 45: 1-15, 1981) obtained from cultured DRG neurones from mouse fetuses, of 12-14 days gestational age.

(R.L. was supported by a CONACYT fellowship).

127.18 DIFFERENTIATION CHARACTERISTICS OF HUMAN NEUROBLASTOMA CELLS IN CULTURE. M. Gupta[1,2], M.F.D. Notter[2], S.Y. Felten[2] and D.M. Gash[2]. Dept. of Ob/Gyn[1] and Anatomy[2], University of Rochester School of Medicine, Rochester, NY, 14642.

Neuroblastoma cell lines have many advantages for the investigation of neuronal differentiation, biochemistry, and electrophysiology. The present study was carried out to determine the morphological characteristics of the undifferentiated and differentiated neuroblastoma. Monolayer cultures of a human neuroblastoma (IMR-32 clone) were grown in Eagle's minimum essential medium supplemented with 10% fetal calf serum at 37°C. After 48 hours of culture, the cells were either treated with mitomycin C and 5-BrdU or Prostaglandin E_1 and cAMP to cause neuronal differentiation. Untreated cells served as controls. Three days later, all the cultures were processed for acetylcholinesterase staining (AChE), scanning and transmission electron microscopy and High Performance Liquid Chromatography (HPLC). Mitomycin c/BrdU and PGE_1/cAMP treatment caused development of long neurites. The treated cells showed increased AChE staining compared to the controls. Scanning EM demonstrated that the differentiated cells contained long neurites, varicosed processes and growth cones, while transmission EM showed that these cells contained a large number of neurosecretory granules in their cytoplasm and neurites. Specialized cell contacts were also observed between the treated cells. The HPLC assay revealed that differentiated and undifferentiated cells contain large amounts of serotonin and comparatively small amounts of epinephrine, norepinephrine, and dopamine.

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128.1 IMMUNOHISTOCHEMICAL LOCALIZATION OF NEUROPEPTIDE Y IN BASAL FOREBRAIN AND UPPER BRAIN STEM OF MONKEY. Y. Smith, L. Kerkerian*, G. Pelletier and A. Parent. Lab. of Neurobiology, Fac. Med., Laval Univ., and MRC Group in Molecular Endocrinology, CHUL, Québec, Canada.

The distribution of neuropeptide Y (NPY) in the brain of the squirrel monkey (Saimiri sciureus) was studied by means of the indirect immunofluorescence method. The antibodies used, which were raised in rabbit against porcine NPY, did not show any significant crossreactivity with related peptides including peptide YY and avian pancreatic polypeptide.

In the upper brainstem of squirrel monkey a dense NPY terminal field is seen in lateral parabrachial area and in interpeduncular nucleus. It appears that the innervation of the latter structure originates from NPY-containing cell bodies located in lateral habenula. A moderate number of NPY fibers also occurs in periaqueductal gray of midbrain tegmentum. The substantia nigra (SN) appears mostly devoid of NPY immunoreactivity whereas the ventral tegmental area contains a few reactive fibers. In the hypothalamus the medial preoptic area and the nuclei arcuatus, suprachiasmaticus and paraventricularis receive a strikingly dense NPY innervation. In addition, numerous NPY-positive cell bodies are found within the dorsomedial half of nucleus supraopticus but very few are seen in paraventricularis nucleus. A large number of NPY-reactive cell bodies are also present in nucleus arcuatus. In the telencephalon NPY cells abound mostly in cortex and striatum, but some are also found in amygdala (particularly nucleus basalis and centralis), claustrum, and in bed nuclei of stria terminalis and anterior commissure. Intensely reactive network of NPY fibers are also present in all of these structures. In striatum the numerous, fine and unvaricosed NPY fibers are slightly more abundant in caudate nucleus than in putamen. The NPY cell bodies, however, appears rather uniformely distributed in striatum. These cells vary in shape from small and globular (max. diam.: 18-20 um) to large and fusiform (max. diam.: 27-30 um), with a few thin processes. The globus pallidus (GP) stands out in sharp contrast against the striatum since it is mostly devoid of NPY fibers and terminals. The fact that the two major recipient structures of striatal outflow (SN and GP) do not receive significant NPY input suggests that the striatal NPY-containing neurons are intrinsically organized.

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128.2 LOCALIZATION AND DISTRIBUTION OF NEUROPEPTIDE Y (NPY)-LIKE IMMUNOREACTIVITY IN THE GUINEA PIG HEART. C. Sternini* and N. Brecha. Center for Ulcer Research and Education, VA Wadsworth Medical Center and UCLA School of Medicine, Los Angeles, California 90073, U.S.A.

NPY-like immunoreactivity has been reported in the central and peripheral nervous system. In the present investigation the localization and distribution of NPY-like immunoreactivity was studied in the heart of normal, 6-hydroxydopamine (6-OHDA) and capsaicin treated guinea pigs. The heart and great vessels of the heart were fixed in a paraformaldehyde solution, sectioned with a cryostat and processed by either the indirect immunofluorescence or peroxidase-antiperoxidase techniques, using antisera directed to NPY or tyrosine hydroxylase (TH). Specificity was assessed by incubating tissue sections with primary antiserum absorbed with 1uM synthetic NPY.

Specific NPY-like immunoreactivity was localized to varicose processes distributed to the atrial and ventricular walls, interatrial and interventricular septa, cardiac valves, papillary muscles and auricles. A particularly rich NPY-like innervation was present in the region of the sinu-atrial and atrio-ventricular nodes, around coronary vessels and at the media-adventitia junction of the great vessels of the heart. Immunoreactive processes were present around nodal ganglion cells, but NPY-like positive somata were not observed. An identical immunoreactive pattern to that observed with NPY antiserum was seen in adjacent tissue sections incubated with TH antisera. Double-label studies using the elution-restaining technique demonstrated the presence of NPY- and TH-like immunoreactivity in the same processes. NPY- and TH-like immunoreactivity were markedly reduced, by about the same magnitude, after treatment with the sympathetic neurotoxin 6-OHDA, but were unaltered after capsaicin treatment.

These observations provide evidence that NPY- and TH-like immunoreactivity are present in the same processes and that the majority if not all of the NPY-like immunoreactivity in the guinea pig heart is derived from extrinsic sympathetic sources. These results suggest that NPY or a closely related substance may play a role in the regulation of cardiovascular functions, perhaps acting in concert with catecholamines.

128.3 EFFECTS OF NEONATALLY-ADMINISTERED CAPSAICIN ON BOMBESIN-LIKE IMMUNOREACTIVITY(BLI) IN RAT SPINAL CORD, MEDULLA, AND THALAMUS. M. W. Decker*, G. Bissette, A. C. Towle*, R. A. Mueller, J. M. Lauder, and C. B. Nemeroff (SPON: P. Loosen). Univ. of North Carolina School of Medicine, Chapel Hill, NC 27514 and Duke Univ. Medical Center, Durham, NC 27710.

The effects of intracranially-injected bombesin (BOM) on a variety of physiologic and behavioral measures, including food consumption, temperature regulation and locomotion, the heterogeneous distribution of BOM receptors in brain, and the preferential localization of BLI in synaptosomes suggest that a BOM-like peptide may be a neuroregulator. One area particularly rich in BLI in the rat CNS is the substantia gelatinosa of the spinal cord. The BLI in this region is substantially reduced by rhizotomy, implicating primary afferents as the source (Panula et al., Regul. Peptides 4 (1982) 275). Because neonatal capsaicin treatment destroys many peptide-containing primary afferents, we studied the effects of capsaicin on BLI concentrations in the CNS using radioimmunoassay procedures.

Adult, male rats that had been treated at 5 days of age with capsaicin (50mg/kg, sc) displayed not only significant depletions of somatostatin (SRIF-LI ↓35.7%, $p<.05$) and substance P (SPLI ↓90.5%, $p<.01$) as is previously reported (Nagy, Handbook Psychopharm. 15(1982)185), but also a small (16.4%), statistically significant ($p<.01$) reduction of BLI in the cervical spinal cord. A similar depletion (21.5%) of BLI was noted in the medulla. SPLI was reduced by 44.3% in the medulla ($p<.05$), whereas SRIF-LI was unchanged. Neurotensin-like immunoreactivity was not depleted in either brain region, and none of these peptide concentrations were changed in the thalamus.

Because capsaicin induces release of SRIF (Gamse et al., N-S Arch. Pharmacol. 316(1981)38) and SP (Gamse et al., Life Sci. 25(1979)629) but not BOM (Moody et al. Life Sci. 29 (1981)2273) from spinal cord slices, these results suggest that capsaicin's neurotoxicity may not solely be related to its ability to induce neuroregulator release. Alternatively, these results suggest that a small portion of BLI in spinal afferents is co-localized with other neuropeptides. Interestingly, some workers have observed co-localization of BOM and SP in certain dorsal root ganglion cells (Fuxe et al., Neurosci. Lett. 37(1983)17), but this has not been confirmed by others (Panula et al., J. Neurosci. 3(1983) 2021).

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128.4 **IMMUNOHISTOCHEMICAL STUDIES USING ANTIBODIES TO AVIAN PANCREACTIC POLYPEPTIDE IN THE GASTROINTESTINAL TRACT OF MONKEY AND RAT.** Y-N. Wang, A.C. Church and R.J. Wyatt. Adult Psychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032

Using immunohistochemical techniques in a previous study, we found that Avian Pancreatic Polypeptide (APP) is distributed in neuronal structures in the stomach and duodenum of dog, guinea pig and rat (Wang et al., Gastroenterology, 84:1345, 1983). In the present study, we report that APP, a member of pancreatic polypeptide family, is located in neuronal structures of the gastrointestinal tract of the monkey and rat. Esophagus, stomach, duodenum, jejunum, ileum and colon were fixed in a paraformaldehyde solution, washed and subsequently sectioned with a cryostat (10 um). Tissue sections were incubated in antiserum directed to APP and processed according to immunohistochemical techniques. Specificity was established by incubating tissues in antiserum previously absorbed with 10 uM synthetic APP. APP-like immunoreactive nerve cells and fibers are distributed in all parts of the gastrointestinal tract with the greatest number of immunoreactive nerve cells and fibers in the duodenum. APP-like immunoreactive nerve cell bodies are present in myenteric plexus and submucous layer. Immunoreactive nerve fibers are located in myenteric plexus, circular and longitudinal muscle layers, muscularis mucosa, submucous and mucous layers, and surrounding vascular elements in the mucous and submucous layers. The results suggest that: 1) APP is distributed in the neuronal structures of all parts of the gastrointestinal tract in the monkey and rat. 2) APP as a neurotransmitter or neuromodulator may play a role in the regulation of gastrointestinal function, and in addition may have some influence on gastrointestinal vasculature.

The authors are grateful to Dr. J.G. Kimmel for generously providing the APP antiserum.

128.5 AUTORADIOGRAPHIC VISUALIZATION OF A NOVEL PEPTIDE BINDING SITE IN RAT BRAIN USING THE SUBSTANCE P ANALOG, ELEDOISIN. J.A. Danks*, R.B. Rothman, M. Herkenham, M.A. Cascieri, G.G. Chicchi*, T. Liang, and C.B. Pert (SPON: J. Donoghue). Section on Brain Biochemistry, NSB, Laboratory of Neurophysiology, National Institute of Mental Health, Bethesda, Maryland 20205, and Department of Biochemical Endocrinology, Merck Institute for Therapeutic Research, Rahway, New Jersey 07605.

In previous studies we reported the autoradiographic distribution of SP (substance P) binding sites in rat brain using Bolton Hunter conjugated SP, ^{125}I-BHSP (Rothman, et al. Brain Research, in press). Based upon the absence of detectable high affinity binding sites in the SN (substantia nigra), we hypothesized that the SN might possess a low affinity SP binding site not detectable with ^{125}I-BHSP. The finding that ^{125}I-BHSP and ^{125}I-BHED (Bolton Hunter conjugated eledoisin) might label distinct binding sites (Cascieri and Liang, Life Sciences, in press), led us to pharmacologically characterize and autoradiographically visualize the binding site labeled by ^{125}I-BHED in rat brain.

The binding of ^{125}I-BHSP and ^{125}I-ED to slide mounted sections of molded minced rat brain proceeded essentially as previously described (Rothman, et al., ibid). The assay took place for 90 min. at 25°C in 50 mM TRIS-HCl, pH 7.4, containing 50 ug/ml chymostatin, 5 mg/ml BSA, and 5 mM $MnCl_2$ (^{125}I-BHSP) or 1 mM $MnCl_2$ (^{125}I-BHED). ED and SP displaced ^{125}I-BHSP binding with IC50's of 0.2 nM and 19 nM respectively. The IC50 of SP vs ^{125}I-BHED was greater than 2500 nM, while the IC50 and ED vs ^{125}I-BHED was 5.6 nM. Substance P free acid was inactive at 2500 nM.

^{125}I-BHSP and ^{125}I-BHED binding sites were visualized at the level of the SN. Adjacent 20 uM sections were incubated with the tracers as described above. ^{125}I-BHED sparsely labeled the SN (35% above background, but densely labeled the IPN (interpeduncular nucleus). ^{125}I-BHSP sparsely labeled the IPN, and did not label the SN at all. Additionally, the cortical layering pattern observed using the two ligands were strikingly different.

These data strongly suggest that ^{125}I-BHSP and ^{125}I-BHED label pharmacologically and anatomically distinct binding sites in rat brain, and support the classification of SP receptors proposed by Iversen and associates (Sandberg and Iversen, J. Med. Chem., 25:1009-1015, 1982).

128.6 BOMBESIN IMMUNOREACTIVITY IN THE RAT BRAIN: LOCALIZATION BY IMMUNOHISTOCHEMISTRY. Clive W. Coen. Section of Neurosurgery, Yale University School of Medicine, New Haven, CT 06510.

Bombesin, a tetradecapeptide originally isolated from frog skin, produces marked effects on thermoregulation, glucoregulation and pituitary, adrenomedullary and gastric secretions when infused at various intracranial sites (Tache & Brown, TINS 5: 431, 1982). Although the precise amino-acid sequence of mammalian brain bombesin remains to be established, rat brain has been shown to contain not only high-affinity bombesin binding sites but also, following HPLC fractionation, a single major peak of immunoreactive bombesin coeluting with the synthetic peptide (Moody et al., Peptides 3: 559, 1982).

In this study bombesin immunoreactivity has been identified in the central nervous system of rats by means of the PAP method. Normal and colchicine treated rats were perfused with 4% paraformaldehyde. Serial sections (50um) were cut coronally or parasagitally through the brain and spinal cord using a vibratome. Because of the homology between substance P and bombesin at the C-terminal, the primary antiserum was routinely preadsorbed with the former peptide. Every third or sixth section in the series was incubated with primary antiserum preadsorbed with both substance P and bombesin; no immunoreactive staining occurred under these circumstances.

In colchicine treated rats bombesin immunoreactive somata are visible in the bed nucleus of the stria terminalis, throughout the ventrolateral component of the suprachiasmatic nucleus, in the lateral hypothalamic area, in the parvocellular division of the paraventricular nucleus, in the medial amygdaloid nucleus, in the periventricular area ventral to the posterior commissure, around the medial border of the medial geniculate nucleus, in the ventrolateral medulla and in the dorsolateral region of the nucleus of the solitary tract. Areas with a dense concentration of immunoreactive fibres include the dorsomedial region of the nucleus accumbens, the medial preoptic area, the suprachiasmatic nucleus and the area immediately dorsal to it, the lateral hypothalamic area, the dorsomedial hypothalamic nucleus, the central and medial amygdaloid nuclei, the substantia nigra pars compacta, the perirhinal cortex, the central grey, the interpeduncular nucleus, the dorsal raphe nucleus, the parabrachial nucleus, the locus coeruleus, the nucleus of the solitary tract, the ventrolateral medulla, the area postrema and laminae I and II of the spinal cord.

These results indicate that a bombesin-like peptide may be involved in a multiplicity of neuronal systems. Further investigation of the connectivity within these systems will clarify the various functions of this peptide.

128.7 ORGANIZATION OF THYROTROPIN-RELEASING HORMONE (TRH) IMMUNOREACTIVITY IN THE HUMAN SPINAL CORD. R.M. Lechan, L.S. Adelman, S. Forte* and I.M.D. Jackson. Endocrine Division, Department of Medicine and Department of Neuropathology, Tufts-New England Medical Center, Boston, MA 02111 and Rhode Island Hospital, Providence, R.I. 02902.

The distribution of TRH in the human spinal cord was studied using a highly specific antibody to TRH and the indirect peroxidase-antiperoxidase technique. Three adult, human spinal cords were obtained within 3 to 20 hours of death, divided into 30 spinal segments (C_1-C_8, T_1-T_{12}, L_1-L_5, S_1-S_5) and fixed by immersion in 5% acrolein in 0.1 M Sorensen's phosphate buffer, pH 7.2 for 2 hrs at room temperature. 50-70 um transverse sections of each segment were cut on a vibratome and the reaction product developed with diaminobenzadine in the presence of H_2O_2.

Immunoreactive TRH was found exclusively in neuronal processes at all levels of the spinal cord, predominantly in lamina IX of the ventral gray and in the intermediolateral column. All motor groups in lamina IX were innervated by axons containing TRH, which frequently outlined the cell soma and first order dendrites of α-motoneurons. The highest density of terminal fields was observed in the ventrolateral motor nuclei in the cervical and lumbar cord and the lowest density in the dorsolateral and retrodorsolateral groups. The intermediolateral column was intensely immunostained, particularly between C_8-T_3 and T_{10}-T_{11} and innervated by fibers coursing in the lateral funiculus and along the lateral border of the dorsal gray. Some of these fibers extended medially through the intermediate gray toward the central canal. Immunoreaction product was also present in the region of preganglionic parasympathetic neurons in the sacral cord.

These studies demonstrate that the distribution of TRH in the human spinal cord bears a remarkable similarity to the distribution described in the rat and monkey spinal cord and indicate that in man TRH may play an important role in the autonomic nervous system through effects on sympathetic and parasympathetic preganglionic neurons and on motor function through effects on α-motoneurons.

128.8 NEUROPEPTIDE NEURONAL EFFERENTS FROM THE CENTRAL NUCLEUS OF THE AMYGDALA TO THE PARABRACHIAL NUCLEI. M. Moga* and T.S. Gray (SPON: J. Trimble), Dept. Anat., Loyola Univ. Stritch Sch. Med., Maywood, IL 60153.

The central nucleus of the amygdala (CNA) and the parabrachial nucleus (PBN) are included within a group of brain regions thought to participate in regulation of autonomic behaviors. Both the CNA and PBN also are known to contain many various neuropeptide-containing neurons. We have examined the organization of somatostatin (SS), neurotensin (NT) and corticotropin-releasing factor (CRF) neuronal efferents of the CNA to the PBN.

The subjects of the study were male Long-Evans rats weighing 150-250 g. All animals were injected with 50 nl of Fast Blue tracer into the parabrachial nuclei. 48h prior to sacrifice all animals were prepared with intracerebroventricular injections of colchicine. Post-Fast Blue-injection survival periods of 8-10d were used. Animals were perfused transcardially with 4.0% phosphate buffered paraformaldehyde. The brains were cut into 20μ coronal sections and processed immunocytochemically using antibodies to SS, NT or CRF (ImmunoNuclear Corp). SS, NT and CRF antibodies were visulized using rhodamine-conjugated antirabbit immunogammaglobulin secondary antibody.

Injections centered on the lateral PBN with some spread to the medial PBN (three animals) produced maximal retrograde label in the CNA. Fast Blue labeled cells were distributed throughout the rostral-caudal extent of the CNA and within the anterior group of intercalated cell masses (IC). Most retrogradely-labeled neurons within the CNA were located in its caudal half within the lateral, lateral capsular and ventral subdivisions. Many retrogradely labeled cells within the lateral CNA also were immunoreactive to SS, NT and CRF.

The results provide evidence for a SS, NT and CRF neuronal pathway from the lateral CNA to the parabrachial nucleus. The present study also demonstrates a previously undescribed axonal efferent from the anterior intercalated cell group of the amygdala to the parabrachial nucleus. Studies are in progress to study the neuropeptide character of the IC-PBN projection and to determine if other peptidergic neuronal types participate in the CNA-PBN projection.

(Supported by a Potts Foundation grant and NIH grant NS 20041-01 to T.S. Gray)

128.9 NEUROPEPTIDE "Y" INNERVATION OF HYPOTHALAMIC AND AMYGDALA NEURONS PROJECTING TO THE NUCLEUS TRACTUS SOLITARIUS/DORSAL VAGAL NUCLEI. D.J. Magnuson, T.L. O'Donohue and T.S. Gray. (SPON: L.D. Van De Kar) Dept. Anat., Loyola Univ. Stritch Sch. of Med., Maywood, IL 60153, and Experimental Therapeutics, NINCDS, NIH, Bethesda, MD 20205

In this investigation neuropeptide Y (NPY) terminal fields were studied in the paraventricular (PVN), periventricular (PERI), arcuate (AR), and lateral (LH) hypothalamic nuclei, the bed nucleus of the stria terminalis (BNST) and the central nucleus of the amygdala (CNA). The neuronal subpopulations within these regions that project to the vagal autonomic nuclei were examined in relationship to NPY terminal immunoreactivity.

The subjects were 150-250 g male Long-Evans rats. All animals were prepared with 50µl injections of Fast Blue retrograde tracer into the caudal dorsomedial medulla. After 7-10 d survival periods, animals were transcardially perfused with 4.0% phosphate buffered paraformaldehyde. (Animals in this study were not pretreated with colchicine). Brains were cut into 20µ coronal sections with a vibratome. They were processed immunocytochemically using a primary antibody generated against NPY and secondary antibody to rhodamine or FITC conjugated antirabbit immunogammaglobulin. Sections were mounted on slides and examined using an Olympus BH-2 microscope equipped with an epifluorescence attachment.

NPY fibers and terminals were observed within the PVN, PERI, ARC, LH, lateral BNST, and medial CNA. NPY-terminal labeling was especially dense within paravocellular regions of the PVN and along the ventricle in PERI. In all regions examined NPY-immunoreactive terminals appeared to contact perikarya and/or dendrites of retrogradely fluorescent (Fast Blue) labeled neurons. The results of this study indicate that one function of NPY neurons is to modulate amygdala and hypothalamic outflow to the vagal autonomic nuclei of the medulla. The source of these NPY-terminals is the subject of future studies. (Supported by NIH Grant NS 20041-01 to T.S.G.)

128.10 PRO-OPIOMELANOCORTIN AND NEUROPEPTIDE Y PROJECTIONS FROM ARCUATE AND PERI-ARCUATE HYPOTHALAMIC AREAS TO THE NUCLEUS TRACTUS SOLITARIOUS-DORSAL VAGAL COMPLEX. T.S. Gray, T.L. O'Donohue, S.J. Watson and D.J. Magnuson.* Dept. Anat., Loyola Stritch Sch. Med., Maywood, IL 60153, Mental Health Res. Inst., Univ. Mich., Ann Arbor, MI 48109, and Exp. Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20205

In the present study we have examined the distribution of pro-opiomelanocortin (POMC) and neuropeptide Y (NPY) cell bodies within basal hypothalamic regions that project to the vagal complex in the lower medulla. To this end, we have employed the combined methods of fluorescent retrograde tracer and immunofluorescence. Injections (50nl) of Fast Blue were made into the vagal complex of the caudal medulla in 150-250g male Long-Evans rats. All animals were prepared with intracerebroventricular injections of colchicine (100µg/10µl saline) 48h prior to sacrifice. Post-tracer-injection survival periods were 9-12d following which all animals were perfused with 4.0% paraformaldehyde in phosphate buffer. Coronal brain sections were cut at 20µm and alternate sections were processed immunocytochemically using primary antibodies to ACTH, α-MSH, and NPY. The primary antibodies were visualized using rhodamine-conjugated antirabbit immunogammaglobulin secondary antibody. Most POMC-immunoreactive neurons in the medial basal hypothalamus were located within the lateral half of the ARC and extended laterally along the base of the hypothalamus. Also, as previously reported, additional groups of α-MSH-immunoreactive cells were observed with the hypothalamus. Numerous ACTH and α-MSH-immunofluorescent neurons that were labeled with Fast Blue were observed. These "double-labeled" cells were usually located laterally within the arcuate nucleus (ARC) or just outside the ARC along the base of the hypothalamus. NPY-immunofluorescent cells were located medial to POMC neurons within the ARC adjacent to the third ventricle. Fast Blue retrograde tracer was not observed within NPY cells in the arcuate nucleus. However, a scattering of NPY-immunofluorescent and Fast Blue double-labeled cells was observed within the dorsomedial hypothalamic nucleus. The results demonstrate that POMC cells in lateral arcuate regions and NPY neurons in the dorsomedial hypothalamus send axons to the vagal autonomic nuclei.

(Supported by NIH Grant NS 20041-01 to T.S. Gray)

128.11 IDENTIFICATION OF NEUROPEPTIDES IN ONUF'S NUCLEUS IN THE CAT. S.L. Erdman*, M. Kawatani, K.B. Thor, R. Eskay* and W.C. deGroat. Dept. Pharm. and Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Motoneurons which innervate the external anal and urethral sphincter muscles are located in a circumscribed region of lumbosacral ventral horn termed Onuf's nucleus (ON). A previous report described a dense collection of enkephalinergic terminals in ON. The present immunohistochemical experiments were undertaken to determine whether other neuropeptides might also be localized in ON.

ON was identified by retrograde transport of fluorescent dye (fast blue) applied to the pudendal nerve. The origin of peptidergic terminals to ON was studied by surgical interruption of bulbospinal and/or primary afferent inputs to the sacral spinal cord.

Leucine-enkephalin (LE), methionine-enkephalin (ME), somatostatin (SS), and substance P (SP) were always present in ON of normal cats, whereas vasoactive intestinal polypeptide (VIP) and dynorphin A 1-8 (DYN) were seen only in 30% of the animals. Cholecystokinin was not present in ON.

LE, ME and SS exhibited similar distributions in ON in normal, deafferented (10-30 days) and chronic spinal cats (8-10 months, T13 transection) indicating the source of the terminals was intrinsic to the spinal cord. These terminals were most highly concentrated with the confines of ON and along the longitudinal dendritic bundles, but they were also distributed laterally, dorsally and dorsomedially from ON in a pattern similar to the transverse dendritic projections of ON neurons.

In normal animals SP terminals were present in ON in a density similar to that in other motor nuclei. However, chronic spinal transection eliminated SP from the other motor nuclei in 100% of the lesioned animals but eliminated SP in ON in only 50% of the animals, indicating two possible sources for the SP terminals. VIP and DYN were not eliminated in chronic spinal animals.

These results indicate that the peptidergic input to ON is very similar to the peptide distribution in the sacral parasympathetic nucleus, but quite distinct from the peptidergic input to other lumbosacral motor nuclei. Since sacral preganglionic neurons (SPGN) contain LE and SS and are closely linked functionally with ON neurons it is possible that some of the peptidergic inputs to ON are derived from axon collaterals of SPGN.

128.12 IMMUNOCYTOCHEMICAL LOCALIZATION OF NEUROTENSIN IN RAT SPINAL CORD FOLLOWING CERVICAL GANGLIONECTOMY. E. Rossitch,Jr.*, J. Ovelmen-Levitt, and B.S. Nashold,Jr. (SPON:I. Diamond). Div. of Neurosurgery, Duke U. Med. Ctr.,Durham, N.C. 27710.

Neurotensin (NT) is a tridecapeptide heterogenously distributed in the brain and gut of mammals. In the rat spinal cord NT terminals and cell bodies have been localized in the interneurones of the Substantia Gelatinosa by immunohistochemical methods. Antinociception is one of the multiple central effects of NT. In fact, NT is more potent than morphine on a molar basis, and produces an analgesia that is not reversed by naloxone. In this study, we examine the NT localization in the rat spinal cord following dorsal root ganglionectomy. A total of 9 Sprague-Dawley rats were included in this study. Four rats underwent C5-T2 unilateral dorsal root ganglionectomies and were sacrificed approximatly 150 days following surgery. One rat had the identical operation, but was sacrificed three days after surgery. The remaining four rats served as controls. All rats were perfused via aortic cannulation with PBS, 4% paraformaldehyde in 0.1 M phosphate buffer, and 5% sucrose in 4% paraformaldehyde buffer. 40 micron vibratome sections of cervical cord were incubated overnight at 4° C in NT antiserum diluted 1:2000 in 1% goat serum/PBS. The secondary phase was performed using the Hsu technique. The sections were then s stained with DAB and subsequently mounted on slides. Control sections were prepared using antiserum preabsorbed with NT. Terminals and fibers were seen in laminas I,II, and III of all rats. Cell bodies were fewer in number and confined to laminas II and III. Qualitatively, there were no differences observed between the controls, the acute deafferented or the chronic deafferented groups.

128.13 **ULTRASTRUCTURAL LOCALIZATION AND AFFERENT SOURCES OF NEUROTENSIN IN THE PARABRACHIAL REGION OF THE RAT.** T.A. Milner and V.M. Pickel, Lab. of Neurobiology, Dept. Neurology, Cornell Univ. Med. Coll., New York, NY 10021

The ultrastructural localization of neurotensin (NT) and afferent pathways contributing to the immunoreactivity for the peptide were examined in the rat parabrachial region (PBR). Rabbit antisera to NT (courtesy of R.J. Miller or commercially obtained from Immunotech, Inc.) were localized by the peroxidase-antiperoxidase (PAP) method. Sections were prepared for electron microscopy from the ventrolateral PBR of adult male rats with and without ventricular infusion of colchicine (100 µg in 7.5 µl). In all animals neurotensin-like immunoreactivity (NTLI) was detected primarily in axon terminals. The labeled terminals were 0.4-1.2 µm in diameter and contained a few large dense core and many small clear vesicles. The synaptic junctions formed by these terminals were asymmetric contacts on dendrites. In addition, NTLI was detected in myelinated and unmyelinated axons (0.1-0.8 µm in diameter) and in a few small (4-6 µm in diameter) perikarya. In the perikarya, NTLI filled a thin rim of cytoplasm and extended into proximal dendrites (0.5-1.6 µm in diameter).

The relative sparsity of perikarya showing NTLI even following intraventricular colchicine and the extensive terminal labeling within the PBR suggested that most of the NTLI was derived from extrinsic neurons. The location of these neurons was established by combining retrograde transport of wheat germ agglutin-conjugated horseradish peroxidase (WGA-HRP) with the immunocytochemical localization of NT (Rye et al., 1984). The black granular reaction product of retrogradely transported WGA-HRP was evident in regions known to project to the PBR. In certain perikarya the black granules were detected in combination with the brown PAP reaction for NT, whereas other cells had only NTLI. Perikarya containing retrogradely transported WGA-HRP and NTLI were found predominantly in the ipsi- and contralateral nucleus of the solitary tract. Moreover, a few doubly labeled cells were observed in the ipsi- and contralateral locus coeruleus, the ipsilateral ventrolateral reticular formation, and in the ipsilateral paraventricular and lateral hypothalamic nuclei. We conclude that NT is a putative transmitter or modulator within a number of pathways to the PBR and that afferents containing NT act primarily through axodendritic synapses with intrinsic neurons.

(Supported by grants HL18974 and NS689-83).

128.14 COEXISTENCE OF NEUROPEPTIDE Y- AND FMRFAMIDE-LIKE IMMUNOREACTIVITIES IN L6 AND S1 SPINAL CORD SEGMENTS OF THE RAT. C.A. Sasek[1], R.P. Elde[1], T. Hokfelt*[2] and L. Terenius*[3]. [1]Dept. of Anatomy, Univ. of Minnesota, Mpls, MN 55455. [2]Dept. of Histology, Karolinska Institutet, Stockholm, Sweden. [3]Dept. of Pharmacology, Uppsala University, Uppsala, Sweden.

In a previous study we described staining with FMRFamide (FMRF) antiserum in autonomic regions of L6 and S1 of the rat spinal cord and hypothesized that the immunoreactivity was due to recognition of an NPY-like peptide by the FMRF antiserum. We based this on the absence of extractable FMRF in the rat CNS, the cross reactivity of FMRF antiserum with synthetic NPY and the presence of extractable NPY in the rat CNS. The present experiments were undertaken to further test this hypothesis by describing the distribution of NPY in L6 and S1, comparing it to the distribution of FMRF and by determining if NPY and FMRF coexist in neurons in the dorsal gray commissure (DGC).

Tissue from colchicine treated male rats was processed in two ways. For distribution studies tissue was processed according to the PAP technique on 50um sections with anti-NPY as the primary antiserum. For coexistence studies serial 5um sections were processed for immunofluorescence. Adjacent sections were incubated with either anti-NPY or anti-FMRF as the primary antiserum. The number of immunoreactive cells/section was counted and each section was photographed. The sections were then restained with the opposite antiserum, the number of cells/section was recounted and the sections were rephotographed.

It was found that NPY- and FMRF-like immunoreactive fibers were similarly distributed in the dorsal horn, DGC, sacral parasympathetic nucleus, lateral spinal nucleus and the dorsolateral and dorsomedial ventral horn nuclei. By comparing micrographs of adjacent sections for coexistence, it was found that every cell in the DGC that contained NPY immunoreactivity also contained FMRF immunoreactivity. This was further verified when no additional cells were identified after restaining with the opposite antiserum.

These results suggest that anti-FMRF does indeed recognize NPY in the rat CNS. However, in some regions of L6 and S1 NPY immunoreactivity was found in neurons that did not stain with anti-FMRF. Since anti-FMRF strongly reacts with synthetic NPY, this suggests that some neurons process NPY-like peptides to a form unrecognized by anti-FMRF.

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128.15 SUBSTANCE P IN THE HUMAN SYMPATHETIC GANGLIA: IMMUNOHISTOCHEMICAL LOCALIZATION AND IMPLICATION IN THE CIRCULATORY REGULATION. M. Del Fiacco*, M.C. Levanti*, S. Falchi* and R. Montisci* (SPON: G. Di Chiara). Istituto di Anatomia Umana Normale and Istituto di Patologia Chirurgica, University of Cagliari, Italy.

Strong experimental evidence obtained on laboratory animals, suggesting that the undecapeptide substance P may act as a neurotransmitter or neuromodulator in the autonomic transmission, prompted this study on the presence of substance P-like immunoreactive nerve structures in the human sympathetic ganglia and the possible physiological significance of the peptide on the circulatory regulation.

Specimens of the cervical and lumbar segments of the paravertebral sympathetic ganglia were obtained at surgery from patients affected by either arteriosclerosis obliterans of the lower extremities or Buerger's desease. The indirect immunohistochemical technique revealed the presence of substance P-like immunoreactive nerve fibres running through the ganglia either isolated or in thin bundles. Images likely to represent sites of synaptic contacts with the ganglionic neurones were also detectable. Some of the principal ganglionic cells appeared wrapped up in a thick tridimensional nest of strongly immunoreactive nerve terminals. If substance P-containing fibres in the human sympathetic ganglia have a transmitter or modulatory role on postganglionic neurones, this peptide is likely to be involved in disorders characterized by altered vascular tone due to altered autonomic response.

In the rabbit paravertebral sympathetic ganglia substance P-like immunoreactive nerve fibres and terminals were also detected at immunohistochemistry. In order to examine the significance of the peptide on the circulatory regulation, local injections of substance P and substance P analogs with agonist and/or antagonist effects were performed in the lumbar segment of the rabbit paravertebral sympathetic ganglia. The circulatory modifications induced by these compounds in the posterior limb will be reported as valued with a plethysmographic technique.

128.16 LOCALIZATION OF NPY-LIKE IMMUNOREACTIVITY IN THE CAT'S CENTRAL NERVOUS SYSTEM. P.Wahle*and K.Albus, (SPON:B.B.Lee). MPI für Biophysikalische Chemie , Dept.of Neurobiology, 3400 Göttingen - FRG.

The 36-residue neuropeptide Y (NPY) was recently isolated from porcine brain (Tatemoto,K.,Proc. Natl.Acad.Sci.USA,79:5485,1982).Using a polyclonal antiserum(dil.1:700-1000)against NPY(Allen,Y.S. et al.,Science,221:877,1983)immunohistochemistry was carried out using the PAP-method. Intense NPY-like immunoreactivity in neuronal somata and axons was found in the n.caudatus, n.accumbens, bed nucl. of stria terminalis, lateral septum,putamen,amygdala, neocortex(layer II+III,V+VI) and neocortical white matter. Less intense staining was seen in the hippocampus(dentate gyrus,subiculum) and the claustrum. Intensly stained fibres and terminals were also seen in the ventral hypothalamus(arcuate- and VMH-region),dorsal hypothalamus(n.paraventricula - ris),in the medial preoptic area and the periaequaductal grey.NPY-neurones in the neocortex occured most consistently in structures bordering the anterior and posterior S. rhinicus, but were also seen in larger numbers in all other neocortical areas. In the neocortex,the neocortical white matter and the n.caudatus NPY-neurones were not homogeneously distributed but tended to form clusters, which, in the neocortex appeared not to be constrained by areal boundaries. NPY-like IR was found predominantly in multipolar neurones (without dendritic spines) having round to triangular somata.A few pyramidal(in the neocortex)and spindle-like(in the neocortical white matter)neurones were also found. No bipolar cell types have been identified in the neocortex. Mean soma diameter of NPY - neurones were between 15 and 20 µm , the largest somata found so far belonging to few irregular distributed neurones in the upper layers of the neocortex. Our findings on the localisation of NPY-IR in the cat's brain are largely in agreement with reports on localisation in the rat(Allen,Y.S. et al.,Science,221:877,1983) and human(Adrian,T.E. et al.,Nature,306:584,1983)brain. Some discrepancies might be explained by species differences, or by the fact, that we have so far investigated only animals not treated with colchicine. We thank Dr. J.M.Polak for providing us with NPY-antiserum.

128.17 NEUROTENSIN-LIKE IMMUNOREACTIVITY IN VERTEBRATE PARASYMPATHETIC POSTGANGLIONIC NEURONS. D.S. Neel*, R.L. Parsons and D.E. Cochrane*. (SPON: G. Webb). Dept. of Anatomy & Neurobiology, Univ. of Vermont, Burlington, VT 05405, and Dept. of Biology, Tufts Univ., Medford, MA 02155.

The tridecapeptide neurotensin has been localized in the central nervous system of a number of vertebrate species (1). However, the mode of action of neurotensin is still not established. Neurotensin immunoreactive fibers also have been found in mammalian cardiac muscle where application of neurotensin produces a positive inotropic and chronotropic effect (2,3,4). We have recently found neurotensin-like immunoreactivity in postganglionic neurons of the cardiac parasympathetic ganglion of the mudpuppy, Necturus maculosus. Whole mounts of the parasympathetic cardiac ganglion, sinus venosus, and atrium were prepared for immunocytochemistry using the method of Costa, Buffa, Furness, and Solcia (5). Immunoreactivity to neurotensin was localized in nerve cell bodies and fibers located in the connective tissue sheet extending from the sinus venosus to the posterior wall of the pericardial cavity. A network of immunoreactive fibers also was observed coursing through the sinus venosus and atrial muscle. Additional evidence for the presence of a neurotensin-like peptide in the cardiac ganglion was also obtained using a radioimmunoassay (6). Samples of tissue extracts from isolated cardiac ganglion reacted strongly with HC-8 antisera which is directed towards the COOH-terminal region of neurotensin. Since in this ganglion the postganglionic fibers make synaptic contact with each other as well as with cardiac muscle (7), the presence of neurotensin-like immunoreactivity in this ganglion makes it a very good model system for analyzing neurotensin's mechanism of action.

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128.18 ELECTRON-MICROSCOPIC IDENTIFICATION OF PROLACTIN-LIKE IMMUNOREACTIVITY IN MEDIAL BASAL HYPOTHALAMUS. M. Nishizuka, B.D. Shivers*, D.W. Pfaff. The Rockefeller University, New York, NY 10021, and Cs. Lèránth*, Yale University School of Medicine, New Haven, CT 06510.

Prolactin-like immunoreactivity has been found in the medial basal hypothalamus at the light microscopic level in hypophysectomized rats (Shivers et al., Abstracts, Society for Neuroscience, 9: 1018, 1983), and is synthesized in the rat hypothalamus (Harlan et al., this meeting). We have observed prolactin-like immunoreactive neurons using pre-embedding immunocytochemistry at the electron-microscopic level.

Young, adult female rats (Sprague-Dawley) were treated with colchicine intraventricularly 1 day before use. They were transcardially perfused with a mixture of paraformaldehyde, glutaraldehyde and picric acid in a phosphate buffer, and the tissue was prepared according to Lèránth and Fehèr (Neuroscience 10: 947, 1983). Prolactin-like immunoreactivity (Rabbit anti-rat prolactin, National Hormone and Pituitary Program) was detected with the avidin-biotinylated peroxidase complex (ABC) method (Hsu et al., J. Histochem. Cytochem. 29: 577, 1981).

Reaction product was discovered in medial basal hypothalamic neurons, which had typical large nucleoli and received axo-somatic synapses. In the cytoplasm, reaction product was distinctly granular. Immunoreactive neurons were usually surrounded by non-reactive cells. Reaction product was also seen in dendrites, some of which had spines. These dendrites received typical vesicle-containing, pre-synaptic contacts. Some axons in the hypothalamus also contained reaction product, and were usually surrounded by non-reactive axons. Immunoreactivity was found in synaptic terminals, intrinsic to the medial basal hypothalamus.

Prolactin-like immunopositive neurons in the hypothalamus send projections to the midbrain, and may participate in the control of a female reproductive behavior (Harlan et al., Science 219: 1451, 1983). Thus, prolactin-like immunoreactive terminals in the midbrain will be of particular interest.

PEPTIDES: ANATOMICAL LOCALIZATION III

129.1 IMMUNOCYTOCHEMICAL STUDIES OF THE DEVELOPING HYPOTHALAMO-NEUROHYPOPHYSEAL SYSTEM OF THE CHICK EMBRYO. V. Tennyson, A. Hou-Yu,* G. Nilaver and E. Zimmerman. Depts. Anatomy and Cell Biology, Pathology (Neuropathology) and Neurology, Columbia University, College of Physicians and Surgeons, New York, NY 10032.

The neurosecretory neurons of the chicken were studied using a monoclonal antibody which cross reacts with arginine vasotocin and mesotocin. The second antibody was rabbit anti-mouse IgG conjugated to HRP. In the adult, the most rostral neurons are located in a diffuse arrangement laterally, medially, and ventrally near the pia in the preoptic area. Caudal to the crossing of the anterior commissure (AC), they extend into the anterior hypothalamus where there are prominent groups of periventricular neurons with axons that arch ventrolaterally, as well as groups of lateral and dorsal neurons. There is a distinct tract that bifurcates into the zona interna and externa in the median eminence. The former ends in the neural lobe, which is lobulated. This distribution of neurons has been established by embryonic day 17 (E 17), but the neurons are smaller, stain less intensely, and are more diffusely arranged.

The earliest most distinct immunoreactive neurons we have seen so far are located along the dorsolateral hypothalamic subependymal zone of the 3rd ventricle at the rostral level of the optic chiasm (OC) at E 9. Faintly stained neurons with wispy processes extend rostrally into the anterior hypothalamus, caudal to the AC. Some neurons have migrated laterally and have axons that arch ventrolaterally. Immunoreactive axons are located on the external surface of the median eminence and form the external zone dorsal to the anterior pituitary. By E 10, the stained fibers approach the neural lobe but do not enter it. By E 12, a broad band of staining is present in the median eminence, which probably includes fibers of both the internal and external zone. Between E 12 and E 16, the numbers of neurons increase as does their staining intensity, but few if any neurons are found rostral to the AC in the preoptic region until E 17.

Immunostained neurons develop first in the midportion of the hypothalamus and migrate rostrally, but have not established all of the cell groups found in the adult until the late fetal period. The external zone of the median eminence is evident before the internal zone. The neural lobe appears to be innervated between E 11 and E 12.

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129.2 THE HISTOCHEMISTRY OF THE PTERYGOPALATINE GANGLION AND HARDERIAN GLAND OF THE CHICKEN. B. Walcott, K. T. Keyser*, P. Sibony*, and H. J. Karten. Depts. of Anatomical Sciences, Psychiatry and Neurology, School of Medicine, SUNY, Stony Brook, N.Y. 11794.

The Harderian gland of birds is the major lacrimal gland of the orbit and is densely innervated by the autonomic nervous system. There is extensive acetylcholinesterase reactivity throughout the gland and the glyoxylic acid procedure revealed many varicose catecholinergic fibers among the plasma cells in the medulla of the gland. The gland exhibited both Vasointestinal Peptide (VIP) and Substance-P-like (SP)immunoreactivity that appeared to be in fibers. The pterygopalatinum ganglion lies on the superior anterior margin of the gland close to the N. ophthalmicus. It is a diffuse structure with large cell bodies in the ganglion as well as scattered along the radix autonomica. The glyoxylic acid method showed that there were many catecholinergic fibers that passed through the ganglion but these were not seen to arborize within it. The N. ophthalmicus showed no positive reactivity while the radix autonomica did. Immunohistochemistry of the ganglion revealed that there was extensive anti-SP immunoreactivity which was in fibers and often appeared to form varicosities near cell bodies. Anti-VIP immunoreactivity was found within many of the cell bodies of the ganglion and fibers of small nerve bundles. Some fibers appeared to join the N. ophthalmicus. In the adjacent gland tissue there was also extensive immunoreactivity to antisera against SP and VIP. The staining appeared to be localized to fibers and was most dense among the plasma cells that constitute the bulk of the medulla of the gland. We are currently examining the retrograde transport of HRP from the gland in order to determine whether the neurons of the ganglion were possibly the source of the VIP immunoreactive fibers seen within the gland. Supported by NS 19350 (BW) and NEI 02146 (HJK).

129.3 IMMUNOCYTOCHEMICAL LOCALIZATION OF C-TERMINAL GLYCOPROTEIN FRAGMENT OF PRO-PRESSOPHYSIN IN THE MEDIAL BED NUCLEUS OF THE STRIA TERMINALIS, MEDIAL AMYGDALOID NUCLEUS, DORSOMEDIAL HYPOTHALAMUS AND LOCUS COERULEUS OF THE RAT. A.R.Caffé*, F.W.van Leeuwen*, N.G.Seidah** and M.Chrétien** (SPON: ENA). *Netherlands Institute for Brain Research, Amsterdam; **Clinical Research Institute of Montreal, Canada.

Lately it has become clear that vasopressin (VP)-immunoreactive cell bodies are more widely distributed over the rat brain than had previously been thought. In addition to the paraventricular, suprachiasmatic and supraoptic nuclei, VP-immunoreactive cell bodies were found in the bed nucleus of the stria terminalis (BST), medial amygdaloid nucleus (AME), dorsomedial hypothalamus (DMH) and locus coeruleus (1,4). These cell groups were also observed after incubation with an antibody directed against another part of the vasopressin precursor molecule: neurophysin (kindly provided by Dr. A.G. Robinson, University of Pittsburgh). In addition it was proven that the immunoreactivities of anti-VP and anti-neurophysin are independent: they do not react with a single epitope present on vasopressin or neurophysin (1). Recently the complete genomic DNA-sequence of the rat VP precursor was determined (3). The rat VP precursor contains, in addition to VP and neurophysin, also a glycoprotein part consisting of 39 amino acids. This protein was called C-terminal glycoprotein fragment of pro-pressophysin (CPP). An antiserum directed against the human CPP appeared to cross-react also with rat CPP (2).

In the present study the anti-CPP was used in order to extend the similarity in immunoreactivities in the hypothalamic magnocellular nuclei and the recently described VP areas (BST, AME, DMH and LC).

After incubation with anti-CPP, following the PAP method indeed staining in all areas was obtained in cell bodies, confirming the idea of a similar molecular primary amino acid sequence. Present research is directed towards the independence of the CPP-immunoreactivity in comparison to those of VP and neurophysin.

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129.4 SEX STEROID EFFECTS ON THE VASOPRESSIN INNERVATION OF THE ADULT RAT BRAIN; G.J.de Vries*, W.Duetz*, R.M.Buijs*, F.W. van Leeuwen and R.A.Caffé* (SPON: ENA). Netherlands Institute for Brain Research, IJdijk 28, 1095 KJ Amsterdam, The Netherlands.

Vasopressin (VP) fibers have been reported to innervate various areas in the brain. These fibers seem to be derived mainly from the paraventricular (PVN) and suprachiasmatic nucleus (SCN) and from the bed nucleus of the stria terminalis (BST). Of some areas (e.g. the ventral hippocampus and the medial amygdaloid nucleus) the source of the VP innervation is still unknown, although it may be derived from the VP cell bodies in the medial amygdaloid nucleus (De Vries, G.J. and Buijs, R.M., *Brain Res., 273:*307-317, 1983).

When after gonadectomy the brain of adult male and female rats were processed immunocytochemically for the presence of VP, no obvious changes were detected in the projections of the PVN and SCN. All other VP pathways, however, showed a gradual decrease in the number of immunoreactive VP fibers. This decrease lasted over a period of 15 weeks till hardly any fibers could be found. The original fiber density could be restored by testosterone replacement therapy in male rats within four weeks (De Vries, G.J., Buijs, R.M. and Sluiter, A.A., *Brain Res., 298:* 141-145, 1984). Parallel to the hormonal effects on the VP projections, similar endocrine manipulations caused no detectable changes in the VP cell bodies of the VPN and SCN. The VP cell bodies of the BST and medial amygdaloid nucleus, however, disappeared after gonadectomy and reappeared after testosterone treatment.

To explore whether androgen or estrogen receptors are involved in the testosterone effects on the BST projections, long-term castrated male rats were treated with estradiol (E), dihydrotestosterone (DHT; which, in contrast with testosteron, cannot be converted into an estrogen) or with both steroids together. When, after 4 weeks of treatment, the brains were processed immunocytochemically for the presence of VP, DHT alone appeared to be without any effect. E had restored the original fiber density, but the staining of the individual fibers was weaker than in normal males. Only the combination of E and DHT led to the full restoration of the original type of innervation. Therefore both androgen and estrogen receptors seem to be involved, which might contribute to the sexual dimorphism found in the BST projections to the lateral septum and lateral habenular nucleus, which appear to be denser in male than in female rats (De Vries, G. J., Buijs, R.M. and Swaab, D.F., *Brain Res., 218:* 67-76, 1981).

129.5 A STUDY OF AFFERENT PROJECTIONS TO THE RAT INTERPEDUNCULAR NUCLEUS. G. S. Hamill and D. M. Jacobowitz. Laboratory of Clinical Science, NIMH, Bethesda, MD 20205.

The distribution of afferent projections to the interpeduncular nucleus (IPN) was determined in male rats by retrograde transport of a fluorescent dye, "fast blue," microinjected into the IPN followed by intraventricular colchicine 48 hrs prior to perfusion. Serial frozen sections cut coronally throughout the brain were examined for "fast blue" fluorescence, substance P (SP) or leu-enkephalin (L-ENK) immunofluorescence or both simultaneously, using the appropriate filters.

In the rostral septum, labeled neurons containing "fast blue" were distributed throughout the rostrocaudal extent of the nucleus of the diagonal band, concentrated in the horizontal limbs of the nucleus. A small number of labeled cells were distributed along the medial margin of the nucleus accumbens, in the medial forebrain bundle, and in the bed nucleus of the stria terminalis. Additional sparse forebrain afferents included the lateral and medial septal nuclei, claustrum, medial preoptic, paraventricular, dorsomedial and posterior hypothalamic nuclei and medial mammillary nuclei.

The most intensely labeled cells projecting to IPN were concentrated throughout the entire rostrocaudal extent of the medial habenular nuclei, with virtually every cell labeled. In addition, a small number of labeled medial habenular cells, located dorsomedially, also revealed SP immunofluorescence. A moderate number of "fast blue" labeled cells were present along the medial and lateral margins of the lateral habenula.

Of the hindbrain afferents projecting to IPN, the most intensely labeled neurons were present in the "nucleus incertus," a circumscribed dorsal cap region overlying the dorsal tegmental nucleus, as described in the cat (Berman, U. Wisc. Press, 1968). Many labeled cells in the medial aspect of this nucleus further revealed L-ENK immunofluorescence. Other brainstem afferents included the dorsal and central raphe, dorsolateral tegmental nuclei and locus coeruleus.

This study demonstrates that IPN receives a wide variety of afferents and reveals the presence of a SP and L-ENK projection from the medial habenula and nucleus incertus, respectively.

129.6 DIFFERENTIAL PATTERNS OF PEPTIDERGIC IMMUNOREACTIVITY IN THE HUMAN SPINAL CORD. N.C. de Lanerolle & C.W. Coen. Section of Neurosurgery, Yale Univ. Sch. Med., New Haven, CT 06510.

The distribution of angiotensin II (ANG), thyrotropin releasing hormone (TRH), cholecystokinin (CCK), neurophysin (NPH), neuropeptide Y (NPY), vasoactive intestinal polypeptide (VIP), neurotensin (NT), met-enkephalin (ENK), and substance P (SP) immunoreactivity was studied at all levels in human spinal cord fixed by immersion in 5% acrolein and stained by the PAP method. The distribution of the peptides in fibers and/or terminals was as follows:

Area	ANG	TRH	CCK	NPH	NPY	VIP	NT	ENK	SP
I	3S		1	2	1	1		2	4
IIo	3		2	2	2	1	1	3	3
IIi	3		2	2	1	1	2	3	2
III-IV	1		2	1			1	1	2
Va	3S	1	2	1	2	2	2	2	2
Vb		1	2				1		2
VIIa	1S	1	1		1		1	1	1
VIIb	3	3	3S	3	3		1	2	2
VIIc	2	2S	2	1		1	1	1	1
VIId		1	2	2	1			1	2
IXa	3S	3S	2S		1	1	1	2S	2S*
IXb	3S	3S	1S					1S	2S
X	1	1	1	1	1	1	1	1	1*

[I = Marginal zone; IIo & IIi = outer & inner zones of substantia gelatinosa; III-IV = N. proprius; Va = N. reticularis (lateral); Vb = N. reticularis (medial); VIIa = N. dorsalis of Clarke; VIIb = N. intermediolateralis; VIIc = N. intermediomedialis; VIId = N. cornucommissuralis dorsalis; IXa = N. motorius lateralis; IXb = N. motorius medialis; X = lamina X; S = fibers outlining somata and/or dendrites; 1 to 4 is a rating scale denoting a subjective estimate of the relative abundance of immunoreactivity; * = small stained neurons.]

The various regions of the spinal gray are differentially innervated by the peptides studied. VIP was scant throughout the spinal cord except in the sacral region (omitted from table for this peptide). Although the other peptides, with the exception of TRH, were readily identified in both the dorsal and lateral horns, there was greater variation in the presence of peptides in the ventral horn. ANG-, TRH-, CCK-, ENK-, and SP-like immunoreactivity was relatively intense in the ventral horn, often apposed to motoneurons and/or their dendrites. Only a few scattered NT and NPY fibers were seen among motoneurons.

(Supported by ALSSOA)

129.7 LOCALIZATION OF LHRH IN THE FEMALE HUMAN HYPOTHALAMUS: AN IMMUNOCYTOCHEMICAL ANALYSIS. G.P. Kozlowski, W.L. Dees*, J.C. Porter and C.R. Parker, Jr.*. Depts. of Physiology and Obstetrics and Gynecology, Univ. of Tex. Hlth. Sci. Ctr. at Dallas, Dallas, Texas 75235

Few immunocytochemical studies of luteinizing hormone releasing hormone (LHRH) have been done on the human brain. Using radioimmunoassay (RIA) for LHRH in the adult human brain, Parker et al., (Br. Res. Bull. 5: 307, 1980) showed that LHRH was concentrated in medial basal hypothalamic tissue (1.14 ng/mg protein). Later, it was found (Parker and Porter, J. Clin Endoc. Metab. 58: 488, 1984) that the hypothalamic content of LHRH in women was related to age and reproductive status. In this study, we examined the LHRH-containing system of neurons and fibers of the female human hypothalamus. Brains were obtained at autopsy from 6 human females of various ages (13, 18, 38, 49, 61 and 75 years). The interval between death and fixation of the tissue varied from 2 to 20 hrs. The hypothalamus was removed, divided into 3 parts, and fixed by immersion in Zamboni's fluid for 14 days and then washed until the picric acid was removed. Serial sections (100 µm thick) were made using a vibrating microtome and incubated in anti-LHRH serum (WP-1), diluted 1:700 for 24 hrs as previously described (Kozlowski and Dees, J. Histochem. Cytochem. 32: 83, 1984) for use in the peroxidase-antiperoxidase (PAP) technique. Intense staining of cells and fibers was seen in every hypothalamus regardless of the postmortem interval prior to fixation. Scattered LHRH cell bodies were found in the preoptic, anterior, and medial-basal hypothalamus. Unlike the rat, numerous cell bodies were found in the tuberal region of the human. Several LHRH neurons were found in the median eminence. The cell bodies were unipolar, bipolar and multipolar. There were LHRH fibers associated with the ventricular ependyma. LHRH fibers also made contact with other LHRH cell bodies and fibers suggesting a morphological basis for integration of function. It appears that the number of LHRH cell bodies and fibers is related to the age of the individual brain. The greatest number of LHRH perikaya and fibers were present in the youngest brain and the fewest were present in the oldest brain. (Supported by grants AA06014 and AG00306).

129.8 THE USE OF CRYOPROTECTANT TO MAINTAIN LONG-TERM PEPTIDE IMMUNOREACTIVITY AND TISSUE MORPHOLOGY IN FREELY FLOATING SECTIONS. G.E. Hoffman-Small, R.E. Watson and S.J. Wiegand. Dept. Anatomy, Univ. Rochester, Rochester, NY 14642.

The use of freely floating sections cut on a cryostat or a vibrating microtome is the preferred procedure for maintaining immunoreactivity (IR) of peptides in brain. However, a major disadvantage of this procedure is the necessity of initiating the tissue reaction immediately after the sections are cut. Occasionally, experimental protocols demand that animals from different groups be sacrificed simultaneously and that extensive regions of the brain be examined. While the time needed to section large amounts of tissue can impose limits on the size of the experiment, the major limiting factor is the subsequent immunocytochemical (ICC) processing of many groups of sections, involving numerous rinses and incubations. Yet often it is important to compare groups of sections processed under identical conditions. Thus, a means by which tissue can be stored for a long time if necessary with neither a diminution of IR nor morphological integrity was sought.

A similar problem had been encountered in HRP tract-tracing studies and solved with an ethylene glycol based cryoprotectant solution (deOlmos, J.S., et al., JCN 181:213, 1978). Since many of the considerations relating to HRP stability are similar to those for peptide stability, the use of cryoprotectant was a logical choice for improvement of IR upon tissue storage. Therefore, after fixation and sectioning, rat brain tissue was rinsed twice in PBS, immersed in cryoprotectant or PBS, and processed immediately or stored for 2 to 12 weeks. Sections stored in cryoprotectant were maintained at -15°C, while sections stored in PBS were maintained at 4°C. At the appropriate times, sections were processed for LHRH and substance P IR.

Storage for only a few days in PBS resulted in tissue which was more fragile upon handling and which had increased background staining. In contrast, sections stored for up to 3 months in cryoprotectant had no observable losses of peptide IR, were more resistant to damage and typically remained intact throughout the staining procedure. Thus, storage of tissue in cryoprotectant is a means by which sections can be preserved for future ICC reactions at the discretion of the investigator.

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129.9 THE SEXUALLY DIMORPHIC VASOPRESSINERGIC FIBER DENSITY IN THE MEDIAL PREOPTIC NUCLEUS ORIGINATES IN THE SUPRACHIASMATIC NUCLEUS. Robert E. Watson, Jr., Stanley J. Wiegand, Richard W. Clough, Celia D. Sladek and Gloria Hoffman-Small. Department of Anatomy, University of Rochester, Rochester, N.Y. 14642.

The medial preoptic nucleus (MPN), situated adjacent to the rostral pole of the third ventricle, exhibits a cytoarchitectonic sexual dimorphism in a number of species. In the rat, the MPN is slightly larger and more densely cellular in the female than in the male (Bleier, et al., JCN 212:118, 1982). Also, the MPN is indispensible for the maintenance of phasic gonadotropin secretion in the female (Wiegand, et al., Neuroendo. 31:147, 1980). While completing a study of the peptidergic components of this structure (Watson, et al., Soc. Neurosci. Abstr. 9:454, 1983), it became apparent that the density of vasopressin (VP) and rat neurophysin II immunoreactive (ir) fibers was considerably more in the male, compared with the female.

To investigate whether the sex difference in VP-ir fiber density reflected a difference in the VP content, a RIA study was conducted, using the punch technique. Five cycling female rats, in proestrus, and 5 age-matched males were decapitated between 0930-1000 h. and the brains rapidly removed and frozen on dry ice. In a cryostat, 300 um thick sections were cut, and a 1 mm diameter punch centered over the rostral pole of the third ventricle and including the MPN was removed and assayed for VP by a single antibody RIA (Sladek, Endo. 101:411, 1977) with a sensitivity of 1 pg. per tube. Alternate 20 um sections were taken, stained with cresyl violet, and used to verify the position of the punches by comparison with thick sections which were retained on slides. The data showed a statistically significant ($p < 0.05$) increased VP content in the male MPN, versus that in the female. The next series of experiments was aimed at identifying the source of the VP input to the MPN. Since the suprachiasmatic nucleus (SCN) contains a large population of VP neurons and gives rise to a rostrally directed projection to the OVLT (Hoorneman and Buijs, Brain Res. 243:235, 1982), it seemed likely that it was the source of the VP input to the MPN. Indeed, small electrolytic lesions of the SCN effectively eliminated the VP-ir in the MPN, as well as in the OVLT. Lesions which involved the ventral SCN, sparing the dorsally-situated VP population, were without effect. These data support the notion that the MPN may serve as an important integration region through which input conveyed by SCN VP neurons exerts an influence upon estrous cyclicity.

Supported by NIEHS ES 07026 and NINCDS 16107.

129.10 THE DISTRIBUTION OF DYNORPHIN AND PROENKEPHALIN (BAM-22-P) IMMUNOREACTIVITY WITHIN THE CENTRAL NUCLEUS OF THE RAT AMYGDALA. A. Zardetto-Smith*, S.J. Watson and T.S. Gray, (SPON: R. Wurster) Dept. Anat., Loyola Stritch Sch. Med., Maywood, IL 60153 and Mental Health Res. Inst., Univ. Mich., Ann Arbor, MI 48109.

Previous studies have demonstrated the presence of dynorphin and proenkephalin immunoreactivity within the central nucleus of the amygdala (CNA). In order to better understand the function of these two endogenous opioid substances within the CNA, we have undertaken an immunocytochemical study comparing the distribution of dynorphin and proenkephalin neurons within subregions of the CNA.

The subjects of this study were 150-250g male Long-Evans rats. The brains of both intact animals and animals that had been pretreated with intracerebroventricular injections of colchicine were fixed with 4.0% paraformaldehyde. Brains were cut at 30µm using a vibratome and the sections were processed using the avidin-biotin immunoperoxidase technique. Dynorphin (DYN) immunoreactivity was visualized using an antibody generated against dynorphin A. Proenkephalin (P-ENK) immunoreactivity was visualized using an antibody generated against an adrenal enkephalin precurser fragment, BAM-22-P. Previous studies demonstrated that these two antibodies do not cross-react with each other and are discriminators of enkephalinergic and dynorphinergic systems with the brain.

Numerous DYN and P-ENK immunoreactive cell bodies were observed within the CNA of both intact and colchicine-pretreated animals. However, more immunoreactive cell bodies were observed within brain sections of colchicine-treated animals. DYN cell bodies were much fewer in number compared to P-ENK cell bodies and mostly were limited to the lateral and ventral subdivision of the CNA. P-ENK cell bodies, fibers and presumed-terminals were seen within all subdivisions of the CNA, but were more densely distributed within the lateral, lateral capsular and ventral subdivisions. DYN fibers and presumed-terminals appeared evenly distributed over all subdivisions of the CNA but were less dense than P-ENK terminal staining. The results indicate a much more extensive innervation of the CNA by P-ENK than DYN. The overlap of DYN and P-ENK cells within the lateral and ventral subdivisions suggests the possibility of coexistence of DYN and P-ENK within neurons of the CNA.

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129.11 WITHDRAWN

129.12 NEUROPEPTIDE Y INNERVATION OF THE RAT PARAVENTRICULAR AND SUPRAOPTIC NUCLEI. J.A. Olschowka. Dept. of Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642

Neuropeptide Y (NPY), a 36 amino acid peptide, is one member of a family of peptides which also includes pancreatic polypeptide and peptide YY. Previously we and others have demonstrated a widespread distribution for a NPY-like peptide throughout the CNS and PNS (Peptides, 2:309, 1981 and 3:569, 1982). Foremost among the areas of the CNS innervated by NPY neurons are central autonomic nuclei i.e. bed nucleus of the stria terminalis, paraventricular nucleus (PVN), parabrachial nuclei, nucleus tractus solitarius, etc. The present study is the first of several which will examine the interaction of NPY neurons with immunohistochemically-defined neurons within these nuclei.

To determine the distribution of NPY-like fibers in the PVN and supraoptic nuclei (SON), male rats were given colchicine (100 ug, intracisternal), perfused 2 days later and processed for the indirect immunohistochemical procedure. Serial cryostat sections of the PVN and SON were stained first for NPY, photographed and then the antiserum was removed by elution with acidified $KMnO_4$. The sections were then restained for rat neurophysin (for both vasopressin and oxytocin) and photographed. Alternatively, adjacent sections were stained singly for NPY and neurophysin. The innervation density of NPY fibers was assigned to a 0 to 4+ scale.

Within the SON, NPY fibers were relatively diffuse (1+) and appeared to form pericellular baskets around the magnocellular neurons. A more dense innervation (2+) was observed in the ventral glial lamina along the pial surface, an area filled with the dendrites of magnocellular neurons. Within the PVN, NPY fibers (2+) were again observed forming pericellular baskets around the neurophysin-stained magnocellular cells. However, the parvocellular (3+) and periventricular (4+) areas were much more densely innervated. These results suggest that NPY fibers may have varying degrees of interaction with both magnocellular (vasopressin and oxytocin) and parvocellular (eg. CRF, dopamine, somatostatin, neurotensin) neurons. However, these interactions should be regarded as tentative pending verification at the electron microscopic level.

129.13 MICROTOPOGRAPHIC DISTRIBUTION OF NEUROTENSIN AND CATECHOLAMINE CONCENTRATIONS AT THE LEVEL OF INDIVIDUAL RAT BRAIN NUCLEI. C.M. Anderson*, G. Bissette, C.B. Nemeroff and C.D. Kilts* (Spon: H.K.H. Brodie) Duke Univ. Med. Center, Durham, NC 27710.

Considerable evidence is concordant with the hypothesis that neurotensin (NT)-containing and dopamine (DA)-containing elements interact within the central nervous system (CNS), including their colocalization in some neurons. Histochemical techniques have demonstrated a heterogeneous distribution of NT cells and fibers in the rat CNS. We sought to determine if the quantitative resolution afforded by the combination of radioimmunoassay procedures for NT, micropunch dissection techniques and catecholamine (CA) quantition by online trace enrichment HPLC would indicate a distribution of NT which paralleled that of DA or norepinephrine (NE). The brains from twelve male Sprague-Dawley rats were sliced in a coronal plane (300 μm) and 28 discrete nuclei microdissected from each brain which unpooled tissue samples assayed for NT or CA. The 28 brain nuclei sampled corresponded to the cells of origin and terminal projections of the mesostriatal, mesocortical and mesolimbic DA systems as well as the cell body regions of other neurotransmitters.

The nature and strength of the correlation between DA and NT concentrations was DA system dependent, being positively correlated ($R=0.65$, $p<0.01$) in terminal areas of the mesolimbic DA system (amygdaloid and septal nuclei, hippocampal areas, bed nucleus of stria terminalis) but not in terminations of the mesostriatal (caudate, accumbens, globus pallidus) or mesocortical (medial prefrontal, cingulate, piriform, entorhinal) systems. NT was found in high concentration in cell body regions (substantia nigra, ventral tegmental area, locus coeruleus and dorsal and medial raphe) and was correlated ($R=0.75$, $p<0.01$) with the NE but not DA concentrations. NT was also found in high concentrations in the eight amygdaloid nuclei examined and was positively correlated with both the NE and DA concentrations of these nuclei. The central amygdaloid nucleus contained the highest concentration of NT (4.1 ng/mg protein) of all the brain nuclei examined and as proposed by Roberts et al. (Neurosci. 7:99, 1982) may represent a pivotal point of modulation by DA of the peptidergic outflow of the limbic system. These results suggest that DA-NT interactions may be more functionally significant in the mesolimbic DA system and that cell bodies, particularly those with highly arborized and collateralized projections, may represent a focal point of regulation by NT, perhaps in concert with NE. (Supported by NIMHMH-39415).

129.14 IMMUNOCYTOCHEMICAL LOCALIZATION OF LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) IN THE NERVUS TERMINALIS (NT) OF THE FETAL RAT. M. Schwanzel-Fukuda, J.I. Morrell, D.W. Pfaff. Rockefeller University, New York, N.Y. 10021.

Localization of LHRH was carried out on Bouin's-fixed paraffin embedded fetal rat tissues using unlabeled antibody enzyme procedures. At 15 (but not at 13 or 14) days of gestation LHRH was detected in ganglion cells of the NT in each of 10 brains from 4 litters. LHRH was not seen in any other area of the brain at this age. LHRH cells in the 15 day old fetus were round or fusiform with a clear nucleus and a single centrally placed nucleolus. These cells were found adjacent to non-immunoreactive cells in NT ganglia 1) rostral to the anlage of the olfactory bulb, 2) in the "ganglion terminale" medial and caudal to the base of the developing olfactory bulb, 3) singly or in clusters interspersed among more medial fibers of the olfactory nerves, 4) in ganglia dorsal to the vomeronasal organ and 5) in the anterior part of the nasal septum. In addition LHRH cells were seen medially in the olfactory sulcus extending from the ventral surface of the brain to the septal region. These cells were found in close proximity to the numerous blood vessels which course through the nasal tissues and along the ventral surface of the telencephalon or penetrate the substance of the forebrain. At 17 days, the number of LHRH cells associated with the NT greatly increased over that seen in the 15 day fetus, throughout the peripheral, intracranial and central projections of this nerve in 9 brains from 3 litters examined. In addition, LHRH positive cells were seen in the nucleus and tract of the diagonal band and in the medial septal nucleus. At this age (but not at 15 days) immunoreactive ß-LH was localized in gonadotrophs of the anterior pituitary. The 19 day old fetal brain showed a broader distribution of LHRH-positive cells and reactive processes were now seen in both the organum vasculosum of the lamina terminalis and in the median eminence (ME). Our data show that from 15 to 19 days in the fetal rat the LHRH system of the NT is the principal source of LHRH. The presence of this hormone in the NT early in gestation in the rat (and guinea pig: Schwanzel-Fukuda et al Brain Res. Bull. 7, 1981) prior to detection in the OVLT or ME, or to the appearance of LH in gonadotrophs suggests possible influence of this system on maturation of the CNS-pituitary-gonadal axis earlier in development than has been previously considered and through a route other than primary portal plexus of the ME.
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129.15 PEPTIDERGIC TRIGEMINAL AFFERENTS PROJECT TO THE LATERAL SOLITARY NUCLEUS AT THE LEVEL OF THE OBEX. E.H. South and R.C. Ritter. WOI Regional Program in Veterinary Medicine, University of Idaho, Moscow, ID, 83843 and Dept. of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA, 99164-6520.

Examination of serial sections of the rat brain stained for substance P-like immunoreactivity (SPLI), using peroxidase-antiperoxidase technique, revealed discrete fiber bundles connecting the dorsal portion of the caudal spinal trigeminal nucleus (nST) with the lateral solitary nucleus. In coronal sections the SPLI-containing fibers appeared as two or three well defined bundles which coursed medially from the dorsomedial edge of caudal nST toward the lateral solitary nucleus, subjacent to the solitary tract. These SPLI-containing fiber bundles were restricted to a region that extended from 0.3mm caudal to the obex to 0.4mm rostral to the obex. Examination of both coronal and saggital sections indicated that the fibers arborized abruptly in the lateral solitary nucleus. The bundles were not observed in sections stained using antisera prepared against somatostatin, however, in an occasional rat brain prepared with antisera against cholecystokinin, a few fibers containing cholecystokinin-like immunoreactivity were seen extending from the dorsomedial caudal nST toward the lateral solitary nucleus at the level of the obex.

The SPLI-containing fibers were not present in rats pretreated with intraperitoneal injections of capsaicin, a neurotoxin which damages small diameter primary afferent neurons. Furthermore, after unilateral transection of the trigeminal nerve proximal to the Gasserian ganglion, the SPLI-containing fibers ipsilateral to the cut vanished. Finally, unilateral nodose ganglionectomy of the vagus nerve did not eliminate the SPLI-containing fiber bundles.

We believe these SPLI-containing "trigeminosolitary" fibers are primary afferent neurons of trigeminal origin. These sensory neurons appear to terminate in the lateral solitary nucleus at the level of the obex. Although the structures innervated by the peripheral terminals of these neurons are not known, Jacquin et al. (J. Comp. Neurol. 215: 397-420, 1983), using transganglionically transported horseradish peroxidase, have reported that the mandibular branch of the trigeminal nerve supplies primary afferent terminals to the lateral solitary nucleus at the level of the obex. Such fibers might mediate reflex visceral responses to oral stimuli.

129.16 DISTRIBUTION OF CHOLECYSTOKININ-LIKE IMMUNOREACTIVITY IN THE RAT MAIN OLFACTORY BULB. K. B. Seroogy, C. M. Gall and N. Brecha. Dept. of Anatomy, University of California, Irvine, CA 92717 and CURE, VA Wadsworth Medical Center, Los Angeles, CA 90073.

Previous RIA studies have demonstrated a high concentration of cholecystokinin-like immunoreactivity (CCK-I) within the olfactory bulb. In the present study, immunocytochemical techniques were used to study the distribution of CCK-I perikarya and processes within the rat main olfactory bulb. Adult albino rats were processed according to either the peroxidase antiperoxidase or the indirect immunofluorescence technique using two different antisera to CCK-8.

A complex collection of CCK-I perikarya and fibers was localized within the superficial 1/2 to 1/3 of the external plexiform layer (EPL). Based on somal size and morphology, there appeared to be three different subpopulations of CCK-positive neurons in this region. The vast majority were medium-sized round or fusiform shaped somata distributed in the outer 1/3 of the EPL and sometimes in the deep periglomerular region. Thick processes from these neurons extended diagonally to the glomeruli, rarely branching within a glomerulus. These immunoreactive neurons most likely correspond to intrinsic superficial short-axon cells. A less numerous population of large triangular or multipolar neurons was observed mainly in the superficial 1/2 of the EPL and very rarely in middle or deep EPL. These neurons exhibited thick ascending processes which extended between and around glomeruli. The cells in this group may correspond to tufted cells of the EPL. The third population of CCK-I somata were small, round cells in the periglomerular region. Processes of these neurons encircled and occasionally entered the glomeruli. These least observed CCK-I neurons are most probably periglomerular cells.

A moderately dense band of CCK-I puncta was observed in the internal plexiform layer, with less dense bands of immunoreactivity distributed between striations of unlabeled cell bodies in the granule cell layer. Finally, fine, sometimes varicose CCK-I fibers were occasionally observed within glomeruli.

The results of this study indicate that the distribution of CCK-I in the rat main olfactory bulb is distinct from that of other peptides and putative neurotransmitters in this structure. There do, however, appear to be some areas of overlap; most notably with tyrosine hydroxylase, enkephalin and GAD immunoreactivity in periglomerular cells and with tyrosine hydroxylase and substance P immunoreactivity in tufted cells. Thus, the possibility of colocalization of CCK with one or several of these other neuroactive substances exists. (Supported by NSF grant BNS82-00319 and an Alfred P. Sloan Fellowship to C.M.G.)

129.17 THE HETEROGENEITY OF PEPTIDE-IMMUNOREACTIVE NEURONS IN THE RAT CENTRAL NUCLEUS OF THE AMYGDALA (CNA). M.D. Cassell[1],* T.S. Gray[2], and J.Z. Kiss[3] (SPON: A.E. Applebaum). Dept. of Anatomy, Univ. of Iowa, Iowa City, IA[1], Dept. of Anatomy, Loyola U.M.C., Maywood, IL[2] and Neuroendocrine Unit, NIH, Bethesda, MD.[3]

Several studies have demonstrated that neuropeptide-containing neurons in the CNA are distributed with little reference to its proposed cytoarchitectonically- and morphologically-based subdivisions. To address the question of whether peptidergic neurons in the CNA represent morphologically homogeneous populations, we have compared the morphology of CNA neurons identified in Golgi preparations with neurons observed in sections processed by the Sternberger-PAP immunocytochemical method. Material was obtained from 9 rat brains stained by a modified rapid Golgi method and 56 brains stained for immunoreactivity to neurotensin (NT), somatostatin (SS), met-enkephalin (ENK), corticotrophin releasing factor (CRF), vasoactive intestinal polypeptide (VIP) and substance P (SP). 34 of the immunoreacted brains had received intraventricular colchicine prior to sacrifice. DAB was used as the immunocytochemical chromagen. NT, SS, ENK and CRF neurons in the lateral subdivision (CL) were identified as being similar to the principal CL neuron - a large (12-18μ), spiny neuron - though fewer spines were observed on the immunoreactive neurons. A large (15-20μ), spiny pyramiform cell type, previously unreported, was identified in CL and NT and CRF neurons of this form were observed. Two other CL neuron types, a medium-sized, sparsely spinous neuron and a small (8-10μ), aspinous neuron, were not identified in immunoreactive preparations. In the medial subdivision (CM), SP, NT, CRF & SS neurons resembled the characteristic large, sparsely spinous neurons. ENK and NT neurons in the lateral capsular division (CLC) resembled the characteristic medium-sized spiny neurons. SS and VIP neurons scattered throughout CM and CL were identical to similarily distributed bipolar, aspinous neurons. The data indicate that peptide-specific populations of neurons in the CNA are heterogeneous but their individual morphologies are characteristic of the subdivisions in which they are located. The morphological heterogeneity of CNA peptidergic neurons may reflect differences in efferent projections and/or interactions with highly differentiated terminal fields.

129.18 OPTIC TECTUM OF TELEOSTS: IMMUNOHISTOCHEMICAL STUDIES. R.M. Kriebel, L.F. May* and K.E. Miller. Dept. Anatomy & Neurobiology, The University of Vermont College of Medicine, Burlington, VT 05405.

The optic tectum of nonmammalian species is especially noteworthy due to the remarkable segregation of neurons and fiber systems into discrete laminar patterns. Many investigators have utilized this organization to advantage in studying central processing of visual information and these principles have often been extended to working hypotheses on other sensory systems. It has been shown (J.C.N. 212:188, 1982) that several criteria for laminar delineation have been utilized, and in certain studies biologically active peptides present within this nucleus also demonstrate a specific lamination shown immunohistochemically. In addition to the anuran optic tectum, the teleost optic tectum also is a laminated structure and has been utilized in many investigations especially on regeneration of specific synaptic inputs. Although not as highly laminated as the anuran, the teleost optic tectum has multiple laminae with many of the afferents segregated into these laminae. As in the anurans, it might be suggested that the optic tectum of fishes may have sublamination when specific neurochemical markers are localized. These studies were undertaken to compare the peptidergic localization between teleost and anuran optic tecta and to compare the distribution in two species of fishes, C. auratus and P. latipinna. Immunohistochemical localization of substance P, LHRH, CRF, somatostatin (SS), enkephalins, VIP, CCK, 5-HT, and tyrosine hydroxylase (TH) was examined in the optic tecta of 20 fish using the peroxidase-antiperoxidase staining method. Twenty micron thick frozen sections were incubated free-floating for 24-36 hours in each antisera, and adjacent sections counterstained with cresyl violet. TH localization was seen throughout the optic tectum in a diffuse pattern similar to histofluorescence studies for catecholamines. Labelled immunoreactive profiles for peptide localization were seen in the stratum griseum centrale and the stratum album centrale primarily. The present studies showed that most of the peptidergic fibers were localized within the pre-existing laminar delineation of teleostean tecta. Modification of laminar restrictions has been shown in anuran optic tecta following primary afferent removal. It will be of interest to determine if rearrangement of peptide localization occurs in teleost optic tectum after lesioning experiments.

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129.19 PEPTIDERGIC AFFERENTS TO THE CAUDAL NEUROSECRETORY COMPLEX: LHRH LOCALIZATION. K.E. Miller, R.L. Parsons, and R.M. Kriebel. Dept. Anatomy & Neurobiology, The University of Vermont College of Medicine, Burlington, VT 05405.

The presence of a peptidergic nucleus in the rostral midbrain which projects to caudal spinal cord levels and the caudal neurosecretory complex (CNC) of fishes has been suggested. These studies utilized combined HRP-EM tracing methods to draw this conclusion. The present study was undertaken to examine immunocytochemically the peptidergic innervation of the CNC using antisera against several peptides and correlating these findings with peptide localization in the projection nuclei at brain stem levels. The brains and spinal cords of Poecilia sphenops and latipinna were fixed in phosphate buffered 4% paraformaldehyde and subsequently frozen sectioned at 15 microns. Sections were processed for immunocytochemical localization according to the unlabelled antibody method of Sternberger. Primary antisera included: LHRH, 1:500 (from G. Kozlowski and Immunonuclear); somatostatin, 1:500 (Peninsula Labs.); CRF, 1:1000 (Immunonuclear); substance P, 1:500 (Immunonuclear). Absorption controls for the specificity of the immunoreactivity were produced by incubating purified peptide with its respective antisera for 24 hours before incubating sections in the antisera. In the CNC immunoreactive staining was not seen with the antisera to substance P and somatostatin. Small diameter collateral branches from the processes of CRF immunoreactive CNC neurons were observed in proximity to other CRF positive neurons. Larger diameter varicose fibers which were LHRH positive were found in the CNC neuropil. LHRH immunoreactive profiles surrounded many of the CNC neuroendocrine neurons. Neuronal perikarya stained for LHRH were not seen in the caudal spinal cord. At brain stem levels, projection nuclei to the CNC have been shown in medulla and mesencephalon. Fibers were observed throughout the brain stem using antisera against LHRH, substance P, and somatostatin. Specific attention was focused on the neurons of the dorsal tegmental magnocellular nucleus (DTMN) due to previous studies suggesting its peptidergic nature and projection to the CNC. Large LHRH immunoreactive neurons were present within the DTMN. The present study suggests that an LHRH-like descending projection to the caudal neurosecretory complex originates from neurons in the mesencephalic DTMN.

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129.20 MONOCLONAL ANTIBODIES REVEAL WIDESPREAD SOMATOSTATIN SYSTEMS IN THE BRAIN. S.R. Vincent, A.M.J. Buchan*, C.H.S. McIntosh* and J.C. Brown*. Depts. of Psychiatry and Physiology, Univ. of British Columbia, Vancouver, B.C., V6T 1W5, Canada.

Somatostatin has been found in a variety of central and peripheral neurons and endocrine cells. We have used three monoclonal antibodies raised against synthetic cyclic somatostatin in immunohistochemical studies. With these specific, high-avidity antibodies, we have been able to detect many previously undescribed somatostatin systems.

Many periglomerular cells in the olfactory bulb and large neurons in the anterior olfactory nucleus were stained. The neocortex contained somatostatin-immunoreactive neurons throughout layers II-VI and many cells were also found scattered in the striatum, nucleus accumbens and amygdala. Somatostatin neurons were most common in the stratum oriens of the hippocampus and in the dentate hilus, with terminal fields in the stratum lacunosum-moleculare and the outer molecular layer. A cell group was also present in the lateral septum.

Hypothalamic periventricular cells were intensely stained as were terminals in the median eminence. Many somatostatin neurons were also present in the arcuate nucleus, the zona incerta and the lateral hypothalamus. Entopeduncular neurons were stained intensely, and terminals were present in the lateral habenula, suggesting the existence of a pallido-habenular somatostatin pathway.

Neurons of the tegmental reticular nucleus of the pons, which project to the cerebellar cortex, displayed somatostatin immunoreactivity. In addition, a great many of the large neurons of the granule layer of the cerebellar cortex were positively stained, indicating that Golgi cells may use somatostatin as a transmitter. A subpopulation of Purkinje cells in the paraflocculus was also somatostatin-positive, raising the possibility of GABA-somatostatin coexistence in the cerebellum. Somatostatin neurons were also present in the ventral cochlear nucleus, the ventral nucleus of the lateral lemniscus and throughout the inferior colliculus, suggesting an important role for this peptide in central auditory pathways.

Somatostatin may be involved in other sensory pathways. Somatostatin-positive neurons were present in nucleus gracilus and cuneatus, and the substantia gelatinosa of the medulla and spinal cord contained many small neurons and a dense terminal field.

These results illustrate that these new monoclonal antibodies are powerful tools for studies of somatostatin cells.

TRANSMITTER CYTOCHEMISTRY AND IMMUNOHISTOCHEMISTRY

130.1 LOCALIZATION OF ADENOSINE DEAMINASE-CONTAINING NEURAL SYSTEMS IN THE RAT CNS: POSSIBLE RELATION TO ADENOSINE NEUROTRANSMISSION. J.I. Nagy and J.D. Geiger*. Dept. of Physiology, Univ. of Manitoba, Winnipeg, Man., Canada, R3E 0W3.

The enzyme adenosine deaminase (ADA) is responsible for the conversion of adenosine to inosine through a deamination reaction. The aim of the present work was to determine whether there is an anatomical and neurochemical relationship between the occurrence of ADA in the CNS and the putative neurotransmitter role of adenosine. Currently, behavioral, electrophysiological and biochemical evidence supports the notion that deamination of adenosine may be involved, though perhaps not in an obligatory fashion, at some stage in the processes which govern the neuroregulatory activity of adenosine.

The regional distribution of ADA in rat CNS was investigated by *in vitro* enzyme assay methods and by immunohistochemical techniques using specific antibodies directed against ADA. The results of these studies were compared with the distribution pattern of nucleoside uptake sites in the CNS as measured autoradiographically using the ligand ^{3}H-nitrobenzylthioinosine (^{3}H-NBI) which labels these sites.

A strikingly heterogeneous distribution of ADA was found using both the enzyme assay and immunohistochemical approach. ADA-immunoreactivity was confined to neurons located in discrete brain structures which included the hypothalamus, superior colliculus and septum. The axons eminating from these neurons were also immunoreactive for ADA and could be followed throughout the brain to their respective areas of termination. The projection areas of the ADA-containing hypothalamic neurons were particularly diverse and included many cortical and subcortical structures. The brain regions containing ADA-positive neurons and those exhibiting the greatest density of ADA-positive axons also had the highest grain density in autoradiographic profiles of tissue sections incubated with ^{3}H-NBI.

These results demonstrate the existence of specific neural systems which contain high levels of both ADA and nucleoside uptake sites. Adenosine, perhaps in conjunction with other neurotransmitters, may be released by these systems to serve as a transmitter agent or in some other neuromodulatory function.

130.2 IMMUNOCYTOCHEMICAL LOCALIZATION OF THE GABA-SYNTHESIZING AND THE TAURINE-SYNTHESIZING ENZYMES IN THE RAT RETINA. J.-Y. Wu, C.T.Lin* and G.X. Song*. Dept. of Physiology, Penn State Univ., Hershey Med. Ctr., Hershey, PA 17033.

GABA and taurine are among the most abundant amino acids in the mammalian retina and both have been suggested as important putative retinal neurotransmitters. GABA and taurine are synthesized by L-glutamate decarboxylase (GAD) and L-cysteinesulfinic acid decarboxylase (CSAD), respectively. Although GAD and CSAD are quite similar in terms of their properties, they have been shown to be two distinctly different molecules (Wu, Proc. Natl. Acad. Sci. U.S.A. 79:4270-4274, 1982). Furthermore, the specific antibodies against GAD and CSAD have been obtained and used extensively for immunocytochemical studies. Previously we have shown that GAD is most concentrated in the inner plexiform layer (IPL). A moderate staining was observed in the inner nuclear layer (INL) and the ganglion cell layer (GCL). No staining was observed in the other layers. CSAD was found to be present in all layers with the strongest staining in the IPL (Lin, Li and Wu, Brain Res. 270:273-283, 1983). Now we have shown that at EM levels, GAD reaction product was seen only in some amacrine cells and their terminals, but CSAD reaction product was found in some photoreceptor cells (including rod and cone), bipolar, amacrine and ganglion cells and their processes. The GAD-positive amacrine terminals have been found to make synaptic contact with other unstained bipolar terminals, amacrine terminals, and ganglion cell dendrites. Most of the GAD-positive terminals are presynaptic. Occasionally, the synaptic contacts between two GAD-positive amacrine terminals or between GAD-positive amacrine terminals and GAD-negative ganglion cell body were also observed. The CSAD-positive terminals include amacrine terminals which make synaptic contact with other terminals and bipolar terminals which make synaptic contact with some CSAD-positive as well as CSAD-negative amacrine terminals. Both CSAD-positive amacrine and bipolar terminals are mostly presynaptic to other CSAD-negative terminals. It is concluded that only a fraction of amacrine cells in the rat retina may use GABA as neurotransmitter. The presence of CSAD in some amacrine, bipolar, photoreceptor and ganglion cells in the rat retina further support the notion that taurine may have some important retinal functions, such as neurotransmitter or neuromodulator. (Supported in part by NIH grants EY 05397 and NS 20978, 20922.)

130.3 CROSS INNERVATION OF GLUTAMIC ACID DECARBOXYLASE (GAD) AND TYROSINE HYDROXYLASE (TH) IMMUNOREACTIVE NEURONS IN THE RAT ARCUATE NUCLEUS (AN) SHOWN BY A FERRITIN-AVIDIN-D AND PAP DOUBLE LABEL PRE-EMBEDDING ELECTRON MICROSCOPIC IMMUNOSTAINING METHOD. C. Leranth*, H. Sakamoto*, N.J. MacLusky*, M. Shanabrough* and F. Naftolin* (Spon.: M.F. Eckenhoff). Dept. of Ob/Gyn, Yale University School of Medicine, New Haven, CT 06510

The presence of GAD (Tappaz,M. et al. Neuroscience 9: 271, 1983) and TH (Baker,H. et al. J. Neuroscience 3:832, 1983) containing neurons in the AN has been reported without evidence regarding their interactions. We have now developed a Ferritin/PAP double labeling method to study the synaptic connections of GAD and TH neurons in the same freeze-thaw treated Vibrotome section.

	Procedure 1	Procedure 2	Procedure 3
1.	R-a-TH	S-a-GAD	R-a-TH + S-a-GAD
2.	B-S-a-R-IgG	B-R-a-S-IgG	B-S-a-R-IgG
3.	F-A-D	F-A-D	ADH + B-P
4.	Fixative	Fixative	B-R-a-S-IgG
5.	S-a-GAD	R-a-TH	A-F-D
6.	B-R-a-S-IgG	B-S-a-R-IgG	DAB
7.	ADH + B-P	ADH + B-P	
8.	DAB	DAB	

Abbreviations: R-a-TH = Rabbit-anti-TH (Joh,T.); S-a-GAD = Sheep-anti-GAD (Kopin,I.); B-S-a-R-IgG = Biotinylated Sheep-anti-Rabbit-IgG; B-R-a-S-IgG = Biotinylated Rabbit-anti-Sheep-IgG; F-A-D = Ferritin-Avidin-D; ADH + B-P = Avidin DH + Biotinylated Peroxidase; DAB = Diamino Benzidine reaction.

Results: All 3 procedures showed the same results: Axons immunoreactive for TH synapse with TH and GAD neurons, and GAD positive axons have synaptic connection with GAD and TH reactive neurons.

Conclusions: (1) All 3 procedures are suitable for simultaneous immunostaining using antibodies raised in different species; (2) TH and GAD immunopositive neurons have reciprocal innervations; (3) Since GAD (Leranth,C., et al. in prep.) and TH (Chan-Palay,V. et al. Brain Res. in press) neurons and the majority of such axons are intrinsic to AN, the present morphological evidence suggests functional interactions between these two dopamine and GABA-ergic systems in the AN. (C.L. and H.S. are Mellon Fndn. Fellows, supported by HD13587 to F.N.)

130.4 Double pre-embedding ultrastructural immunocytochemistry with intensified colloidal gold and peroxidase: Dopamine neurons in the dorsomedial hypothalamus receive GABA synapses. Anthony N. van den Pol. Sect. Neurosurgery, Yale Univ. Sch. Med., New Haven, Ct. 06510.

Dopamine neurons, identified with tyrosine hydroxylase (TH) antiserum, are found throughout the hypothalamus. High densities of GABA terminals, identified with glutamic acid decarboxylase (GAD) antiserum, are found in the same regions where dopamine neurons are located. To determine whether the GAD immunoreactive axons terminate directly on TH immunoreactive neurons, a new double immunocytochemical staining procedure was used. After incubation in rabbit TH antiserum, vibratome sections were stained with a secondary antibody of colloidal gold adsorbed IgG, which was subsequently treated with a heavy metal intensification methodology. The intensification increased the size of the colloidal gold particles by several orders of magnitude, allowing gold immunostained cells, dendrites and axons, not visible before intensification, to be identified both with light and electron microscopy. The intensification also covered the IgGs with a heavy metal sphere, reducing further cross-reaction with the second set of immunoreagents. Gold labeled TH immunoreactive neurons were found in the arcuate, periventricular, anterior, and posterior hypothalamus and preoptic area. After incubation in normal rabbit serum, terminals containing GAD were stained with sheep GAD antiserum, followed by biotinilated rabbit anti-sheep IgG, and subsequently with avidin-biotin peroxidase. In the dorsomedial hypothalamus, large TH immunoreactive gold labeled cells with extensive dendritic ramifications were found. These received GAD immunoreactive peroxidase-labeled synapses on perikarya and dendrites. The particulate intensified gold label was easily differentiated from the diffuse peroxidase label. Controls with antibody deletion, substitution of normal or pre-immune serum for the primary antiserum, or deletion of the secondary antisera were negative. A different double immunostaining methodology (van den Pol,Q.J.Ex.Physiol.69:1-33,'84) using post-Epon embedding immunogold staining is compatible with the one described here, allowing the simultaneous ultrastructural localization of three neurotransmitter- related antigens. Combinations of immunocytochemical procedures for ultrastructural identification of both pre- and postsynaptic neurons will facilitate the precise characterization of synaptic relationships in the central nervous system.
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130.5 NEW MARKERS FOR THE ULTRASTRUCTURAL QUANTITATIVE IMMUNOCYTOCHEMICAL LOCALIZATION OF NEUROTRANSMITTERS AND NEUROMODULATORS IN POSTEMBEDDED NERVOUS TISSUE. L.M. Partlow and L.J. Stensaas. Veterans Admin. Medical Cntr and the Depts of Pharmacology & Physiology, Univ, of Utah, Salt Lake City, UT 84132

The inability to quantitatively assess the ultrastructural distribution of biomolecules presently constitutes a major impediment to research in neurobiology. Difficulties with techniques now available could theoretically be overcome by use of analytical electron microscopy (AEM) in conjunction with a set of electron-dense particulate markers each of which would contain a large number of metal atoms of a single kind. Since AEM combines the resolving power of electron microscopy with the quantitative analytical capabilities of energy dispersive X-ray analysis, this would theoretically allow quantitative ultrastructural mapping of the distribution of multiple specific cellular constituents in a tissue section or on the surface of a cell.

Marker particles meeting all of the following criteria have been synthesized for four metals (gold, palladium, iridium and bismuth). (1) Particles must have an average diameter $\lesssim$150 Angstroms. (2) Particles must be relatively homogenous in both size and shape. (3) Individual markers must have sufficient electron density to be readily visualizable by standard electron microscopy. (4) Each type of particle must emit X-rays at at least one energy level which does not overlap with any other emission originating from either the background or other marker particles. (5) Individual particles must be separately quantifiable (i.e., each must produce a detectable number of characteristic X-rays). (6) Individual particles must be associable with molecules (e.g., antibodies, lectins, ligands) which confer marker specificity.

If each type of metal marker particle is coated with adsorbed protein, it will bind specifically to complementary tissue sites. When such preparations are examined via AEM, the incidence of markers in different regions of the cell can be quantified. In addition, AEM makes it possible to selectively visualize only those particles containing specific marker atoms.

Use of combinations of marker particles in conjunction with AEM might make possible the simultaneous quantitative co-localization of multiple neurotransmitters and neuromodulators. Such experiments are in progress. (Supported by a grant from the Veterans Administration.)

130.6 IMMUNOHISTOCHEMICAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE (ChAt) IN RAT AND CAT USING POLYCLONAL ANTISERA. B.K. Hartman, C. Cozzari*, S. Kalmbach*, and S. Hendry, Dept. Psychiatry and Div. Exp. Neurol., Washington Univ. Sch. of Med., St. Louis, MO. 63110

Purified preparations of ChAt contain two forms of the enzyme, the larger form ChAt-A and the smaller form ChAt-B. The B form exhibits at least one major antigenic determinant which is masked in the A form. Rabbit anti-serum to ChAt-B purified from bovine caudate n. (Cozzari and Hartman, J. Biol. Chem. 258:10013-10019, 1983) was used for localization of ChAt employing nickel-cobalt enhanced peroxidase anti-peroxidase at the light and electron microscopic level. The specificity of antiserum has been demonstrated by standard immunochemical methods and by SDS immunoblot against crude extract from caudate n. ChAt localization was carried out in rat and cat. In both species, intense specific immunoperoxidase staining was visable in known cholinergic neurons such as those located in the cranial motor nuclei, the nucleus basalis, and the habenular complex. ChAt immunoreactivity in the caudate nucleus was observed only in the population of large neurons. In the rat neocortex, a small number of non-pyramidal cells were positive for ChAt. The staining filled the perikarya and the dendritic arborizations of cholinergic neurons. The axons of the cholinergic neurons were clearly visualized and the terminal plexuses were intensely stained in septum, cerebral cortex, hippocampus, amygdaloid complex, interpeduncular n. and the neuro-muscular junctions. At the electron microscopic level cholinergic cell bodies, axons, and terminals were stained in both species. Neurons thought not to be cholinergic such as pyramidal cells in the neocortex and hippocampus, and Purkinje cells showed no immunoreactivity. When antiserum prepared to ChAt-A was utilized for localization the cholinergic axons and terminals were not visualized.

These results indicate that most of the antigenic sites common to the A and B forms of ChAt are not accessible for immuno-reaction in cholinergic axons and terminals in situ. Localization in these compartments require antibodies directed against the determinent exposed only in the B form of ChAt, suggesting that ChAt-B may represent the transported form of the enzyme. These results may also explain why many monoclonal antibodies to ChAt give weak or no reaction in axons and terminals, since only clones directed against that specific determinant would stain these structures. (Supported by NS-12311 and MH-70451).

130.7 CHOLECYSTOKININ-IMMUNOREACTIVE CELLS FORM SYMMETRICAL SYNAPTIC CONNECTIONS WITH PYRAMIDAL AND NON PYRAMIDAL NEURONS IN THE HIPPOCAMPUS. M.G. Nunzi, Gorio A., Milan F.*, T. Freund*°, P. Polato*, P. Somogyi*°, A.D. Smith °. Fidia Research Laboratories, Dept. of Cytopharmacology, 35031 Abano Terme, Italy. "University, Dept. of Pharmacology, Oxford OX1 3QT, Great Bretain. °Semmelweis University, 1st Dept. of Anatomy, Budapest IX, Hungary.

We have investigated distribution, ultrastructural characteristics and synaptic connections of cholecystokinin (CCK) containing cells in both rat and cat hippocampus, by means of the immunoperoxidase technique and correlated light and electron microscopy. The CCK immunoreactive neurons were shown to have a rather widespread distribution in all hippocampal fields and layers and to exhibit variable soma size and shape and dendritic arborization pattern. The perikarya of the immunostained neurons are characterized by large, indentated nuclei, well developed rough endoplasmic reticulum and Golgi apparatus. Many CCK-positive cells receive synaptic input upon soma and dendrites from CCK-labelled boutons. The soma and dendrites of CCK-positive cells also receive unreactive both symmetrical and asymmetrical synapses. Two types of CCK-positive cells were observed sending axons collaterals onto the soma of pyramidal neurons. The axon of one of the immunoreactive cells profusely arborizes in the pyramidal layer, forming pericellular nets of synaptic boutons onto the soma of pyramidal neurons. All the CCK-positive boutons make symmetrical synaptic contacts. Synaptic relationship was observed between CCK-positive cells of the stratum lacunosum-moleculare. In addition, it was found a kind of CCK-positive neuron characterized by a thin dendritic process endowed with strongly immunoreactive swellings establishing symmetrical synapses with some profiles identifyed as collaterals of apical dendrites of pyramidal neurons. These findings provide evidence of the complexity of CCK connections in the hippocampal circuitry. The data are consistent with the view that CCK and GABA could coexist in the same neuron.

130.8 NADPH DIAPHORASE IN THE RAT BRAIN, MACAQUE STRIATE CORTEX AND THE MAMMALIAN RETINA. J.H. Sandell, Psychology Dept., Rm. E25-634, M.I.T., Cambridge, MA 02139.

The distribution of the enzyme NADPH diaphorase was investigated in the CNS of the rat, in macaque striate cortex and in the retinae of many species. Several histochemical techniques reveal these cells in Golgi-like detail.

In the rat, NADPH diaphorase cells were found in: lamina X of the spinal cord, the n. ambiguus, the cranial motor nerve nuclei, the pontine tegmentum (throughout the caudal cholinergic cell column), the dorsal raphe, in a dorsolateral wedge of tectal central grey matter, in the superficial grey layer of the superior colliculus, in the interpeduncular nucleus, in the lateral margin of the ventral lateral geniculate body, in the interstitial magnocellular nucleus of the posterior commissure, in the hypothalamic paraventricular and supraoptic nuclei, in the neostriatum and in the cholinergic nuclei of the basal forebrain. Positive cells were also scattered throughout the neocortex. No individually labelled cells were seen in the cerebellum, locus coeruleus, substantia nigra, dLGN, hippocampus or globus pallidus.

In macaque striate cortex, NADPH diaphorase-positive cells were found primarily in layers II-III, VI and white matter. They often had heavily beaded processes that could be followed laterally for up to 1 mm. Background diaphorase activity in striate cortex matched cytochrome oxidase activity, and following monocular damage, ocular dominance stripes were visible in layer IVc when the tissue was treated to reveal either enzyme.

In the retina of the rat, rabbit, cat, marmoset, owl and rhesus monkey and the human, NADPH diaphorase cells were always found at the inner margin of the inner nuclear layer. They were large (~12 um), sparse (<2000/rat retina) and sent processes into the inner plexiform layer, suggesting that diaphorase is found in amacrine cells.

Vincent and colleagues (JCN, 217:252-263, 1983) have described perfect colocalization of NADPH diaphorase with APP and somatostatin in the rodent striatum, however this relationship is not maintained elsewhere in the brain. The overall pattern of NADPH diaphorase activity does not match the published distribution of any putative neurotransmitter yet described. Nonetheless, the unique, discrete distribution of this substance suggests that it may have a specialized role in neural function.

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130.9 PROBLEMS ASSOCIATED WITH GENERATION AND SPECIFICITY TESTING OF ANTISERA RAISED AGAINST SMALL MOLECULES. J.E. Madl*, A.J. Beitz and A.A. Larson. Dept. Veterinary Biology, University of Minnesota, St. Paul, MN 55108

Antisera against small molecules are usually raised by immunizing animals with conjugates of the small molecule bound to carrier proteins. However commerically available carrier proteins may contain significant amounts of small molecule contaminants that could cause unwanted immunoreactivity. This problem, in addition to a variability in the efficiency of conjugation for different small molecules may be important in the production of antisera and in the testing of specificity using absorption controls, i.e. decreasing staining by incubation of antisera with a small molecule-protein conjugate. Our results indicate that keyhole limpet hemocyanin (KLH) contained 45 µg/g of tryptophan (TR). When six rabbits were immunized with KLH conjugated using carbodiimide (ECDI) to either pipecolic acid (PA), TR, tryptamine (TA) or indoleacetic acid (IAA), similar tissue staining patterns were obtained. Assessment of immunoreactivity of these antisera using ELISA revealed similar reactivities of these antisera to TR, TA, serotonin (5HT), IAA and PA, with strongest reactivity to TR. Other major problems were encountered when attempts were made to evaluate specificity of the antisera. KLH conjugates absorbed out immunocytochemical tissue staining of antisera raised against non-indoleamines, thus negating absorption controls as definitive indicators of specificity. This nonspecific binding of antisera was confirmed for TR, TA and 5HT conjugates of KLH and thyroglobulin using ELISA. Furthermore, sepharose beads coupled to d-TR or l-TR bind the peroxidase-antiperoxidase complex non-specifically either with or without primary rabbit antisera. Beads coupled to glutamic acid, tyrosine or aspartic acid did not show such nonspecific binding. A further complication in our evaluation of the antisera was the strong nonspecific staining pattern produced by using 1-5% glutaraldehyde perfusion and postfixation regardless of the primary antisera employed in the staining procedure. These results will be discussed in terms of using appropriate modifications of these procedures to determine specificity of antisera to small molecules. Supported by NIH grants NS17407 and DE06682 and NSF grant BNS-8311214.

130.10 IMMUNOHISTOCHEMICAL LOCALIZATION OF GABA IN THE AREA POSTREMA OF THE RAT AND CAT B.W. Newton and B. Maley. Dept. of Anatomy, Univ. of Kentucky Med. Ctr., Lexington, KY 40536.

The area postrema (AP) in the rat and cat is a midline and bilateral structure respectively that is situated on the dorsal surface of the brainstem at the level of the obex. It is a well vascularized organ that lies outside of the blood-brain barrier and is the proposed chemoreceptive trigger zone for the emetic center of vomiting species e.g., the cat, while in non-vomiting species e.g., the rat, the AP has been associated with the acquisition of taste aversion learning. In order to study the distribution of GABA-like immunoreactivity (GABA-LI) in the AP, eight cats and ten rats were sacrificed by vascular perfusion transcardially with 4% paraformaldehyde-0.3% glutaraldehyde in phosphate buffer. The brainstems were cut at 50um on a vibratome and processed using the peroxidase, antiperoxidase technique. All antibodies were diluted in phosphate buffered saline/3% normal sheep serum with 0.3% Triton X-100 added to facilitate antibody penetration.

In the rat AP GABA-LI was present in dense to very dense accumulations of puncta and occasional varicose fibers. The numbers of puncta, were greatest at rostral levels and slightly less in the ventral portion of the AP. GABA-LI cell bodies appeared as small unipolar or bipolar cells distributed throughout the rostro-caudal extent of the AP, however, most of the immunostained cells were located at intermediate levels.

In the cat AP GABA-LI was present as dense to very dense accumulations of puncta and fibers. The medial border of the AP possessed very dense amounts of GABA-LI, while the lateral border possessed mainly dense immunostaining. The rest of the cat AP consisted of a patchwork of dense and very dense amounts of immunostaining, and compared to the AP of the rat, the cat AP had less overall GABA-LI. Low numbers of GABA-LI cell bodies were present throughout the rostro-caudal extent of the cat AP with the majority of the cell bodies, consisting of large and small neuron classes, located in small groups at intermediate levels. In both species the GABA-LI cell bodies were immunostained without the use of colchicine. The large amount of GABA-LI in the AP of the rat and the cat suggests that GABA may be acting as a neurohumoral agent, and may be involved in modulating the emetic response of the cat and taste aversion learning in the rat. This work was supported by NIH grant 1R23HL30702 to B.M.

130.11 LOCALIZATION OF GLUTAMIC ACID DECARBOXYLASE (GAD)-POSITIVE CELLS IN THE HYPOTHALAMUS OF THE RAT. J.A. Finkelstein, M.L. Tappaz*, W.W. Blessing, W.H. Oertel* and J.O. Willoughby, Centre for Neuroscience, Flinders University of South Australia 5042, and INSERM U 171, Department de Medicine Experimentale, Universite Claude Bernard, Lyon, France.

Intrahypothalamic microinjections of gamma-aminobutyric acid and its agonist muscimol, stimulate growth hormone and prolactin secretion in rats. As a basis for further studies determining anatomical structures through which these effects might be mediated, we have examined the localization of hypothalamic cells which contain GAD.

The brains of male Porton rats were fixed by perfusion with a mixture of picric acid and formaldehyde. Tissue was sectioned immediately on a vibratome at 50 um, washed in Tris buffer and incubated in 20% normal rabbit serum for one hour. The sections were then incubated overnight in sheep anti-rat GAD antibody (1:20,000) in Tris buffer, ph 7.6, containing 1% normal rabbit serum. Sections were further processed by the avidin-biotin-HRP method. Some rats were treated with an intrahypothalamic injection of colchicine (5 ug in 0.5 ul), 48 to 72 hours prior to sacrifice. Sections incubated with normal sheep serum did not reveal any positive cells.

Immunoreactive cells were seen in both normal and colchicine pretreated rats but more cells were observed after local colchicine pretreatment. GAD-positive cells were found in many hypothalamic regions including medial and lateral preoptic areas, anterior, dorsal and lateral hypothalamic areas, the suprachiasmatic, the arcuate, the supramamillary and the caudal magnocellular nuclei. Labelled cells were seen in the zona incerta continuous with the labelled cells of the reticular thalamic nucleus. In contrast, there were few of no GAD-positive cells in the paraventricular, ventromedial, supraoptic, ventral premamillary nuclei and the compact central portion of the dorsomedial nucleus. These findings confirm and extend those of Tappaz, Oertel, Paut and Pujol, (Neuroscience 9:271, 1983) and Vincent, Hokfelt and Wu, (Neuroendocrinology 34:117, 1982).

The presence of GAD-positive cells throughout the hypothalamus suggests they may influence both neuroendocrine and other hypothalamic regulatory functions.

Supported by grants from the National Health & Medical Research Council of Australia.

130.12 IMMUNOCYTOCHEMICAL STUDY OF CHOLINE ACETYLTRANSFERASE IN WILD TYPE AND MUTANT DROSOPHILA MELANOGASTER. K. Ikeda, P.M.Salvaterra, G. Crawford and D.A. Matthews. Div. of Neurosciences, Beckman Res. Inst. of the City of Hope, Duarte, CA 91010.

Antibody specific for Drosophila choline acetyltransferase (ChAT) has been produced and its binding to the enzyme found to be at or near the acetyl-CoA binding site (Crawford et al., J. Biol. Chem., 257:3853-3856, 1982). The present study demonstrates (1) the identification of neurons containing ChAT and (2) the temperature sensitive inactivation of the immunological reaction in a mutant. Materials used were Canton-S (wild-type) flies and two alleles of the temperature sensitive ChAT mutant, cha^{ts}, in which ChAT activity is reduced by exposure to high temperature. All flies were kept at 18°C (permissive temperature) except during experimental exposure to 30°C (restrictive temperature) for various periods.

Antibody (1G4) was conjugated with horseradish peroxidase (HRP). This 1G4-HRP conjugate was applied on cryosectioned cephalic ganglia and visualized by 3,3'-diaminobenzidine (DAB)-H_2O_2 reaction. Among many neurons stained in wild type, three synaptic layers in the medulla which showed distinct stain are taken as subjects for this study.

In Canton-S, these three layers stain distinctly at both 18° and 30°. In cha^{ts1} at 18°C the stain appeared on the same layers as that of Canton-S, but with somewhat lower density. In cha^{ts2} at 18°C the density of the stain was even lower. The densities of the stain in these mutants were further decreased after exposure to 30°C, dependent on the period of exposure. The decrement in stain of the specimens obtained after 24 hrs exposure to 30°C was clearly recognizable in both cha^{ts1} and cha^{ts2}. The stain was visually unrecognizable after 80 hours incubation at 30°C in cha^{ts1}, while after 36 hours in cha^{ts2}. The stained structures in the medulla are apparently terminals of the laminar neurons. Further support for this observation comes from α-bungarotoxin (α-BTX) conjugated with HRP and visualized by the DAB-H_2O_2 reaction. Three layers of stained structures in the medulla overlapped with the layers revealed by 1G4. The exposure to high temperature did not show any effect on the stain obtained by α-BTX-HRP in either allele. These results show that the immunoreaction of 1G4 identifies ChAT containing neurons and their reaction is reduced when ChAT is inactivated in the mutant. Supported by USPHS NIH grants NS18858 and NS19482.

130.13 BIOCHEMICAL AND IMMUNOHISTOCHEMICAL EVALUATION OF THE SPECIFICITY OF 5-HYDROXYTRYPTAMINE ANTIBODY. LIGHT AND ELECTRON MICROSCOPY STUDIES OF THE NODOSE GANGLION AND BRAIN STEM OF THE CAT. G. Chazal*, H. Bras*, and J.J. Puizillout*, (SPON: M. Law) Department of Anatomy, University of California, San Francisco, School of Medicine, San Francisco CA 94143; Inserm U6, 280 Bd. Ste Marguerite, 13009 Marseille, France

Previous studies from our laboratory (Gaudin-Chazal et al., Neurosci. Lett. 33:169-172, 1982) have revealed the presence of serotonin-like immunoreactivity in the nodose ganglion of the cat by light microscopy. In order to test the specificity of the antibody, we have performed comparative quantitative analysis of the titers, specificity and sensitivity of antibodies (AB) to 5-hydroxytryptamine (5-HT) in vitro using radioimmunoassay (RIA) procedures and in situ by competitive immunohistochemical (IHC) studies in the brain stem of the cat. 5-HT antibodies were raised in rabbits following injections of 5-HT-bovine serumalbumin, linked via paraformaldehyde. The in vitro tests were based on the competitive binding properties of the AB with (^{3}H) 5-HT. The IHC procedure was performed using the peroxidase-antiperoxidase (PAP) method for both light and electron microscopy. For light microscopy, the brain stem sections were embedded in paraffin. It is important to note that for the IHC PAP techniques no pharmacological treatments, detergents or proteolytic enzymes were used.

RIA tests show that the AB recognizes the ethylamine CH2-CH2-NH2 chain. IHC labeling could be obtained with AB dilutions of up to 1:3000. The extinction IHC assays revealed good specificity for 5-HT as cross reactivity was estimated at only 0.1%. to tryptamine. These tests indicate that the AB is specific for 5-HT.

Using the PAP method, the antibody was found to label neurons of the nuclei of the dorsal raphe, which have been shown in other species to contain 5-HT. Using electron microscopy, we found a large number of 5-HT positive neurons of varying soma size and containing deeply indented nuclei. 5-HT positive dendrites, myelinated axons and a small population of synaptic terminals were also present. In the nodose ganglion, the fine structure of 5-HT reactive cells will be described.

It is concluded that the reaction product seen by light and electron microscopy does represent 5-HT in neurons of the nodose ganglion and brain stem.

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130.14 INTRINSIC 5HT-IMMUNOREACTIVE NEURONS IN THE SPINAL CORD OF THE FETAL NON-HUMAN PRIMATE. R. M. Bowker. Dept. of VCAPP, College of Veterinary Medicine, Washington State University, Pullman, Wash. 99164.

Serotonin-immunoreactive neurons have been localized in the spinal cords of normal submammalian animals (Ritchie and Leonard, 1984) and of primates, but only after pretreatment with serotonin precursors and monoamine oxidase inhibitors (LaMotte et al., 1982). In this report serotonin immunoreactive neurons can be localized in the spinal cords of normal, non-human primate fetuses. Spinal cord tissues of fetal non-human primates (Papio papio) (embryonic age 140-147 days) were obtained and fixed by immersion in 3.5% paraformaldehyde and phosphate buffer. Representative segments from different spinal levels were sectioned on a freezing microtome followed by routine incubation in serotonin antiserum (INC) at dilutions ranging from 1:1000 to 1:4000. The sections were then reacted using The Avidin-Biotin method. Control experiments were also performed.

In the normal fetal baboon, serotonin-like neurons were distributed throughout the length of the spinal cord and were located primarily ventral to the region of the central canal in the presumptive Lamina X. The reactive neurons were primarily bipolor (12-15 um) with beaded varicosities appearing to terminate around the central canal dorsally, as well as extending ventrally toward the spinal artery. No 5HT immunoreactive terminals from these intraspinal neurons appeared to innervate the remainder of the spinal cord grey matter. A quantitative estimate revealed that greater numbers of immunoreactive neurons as seen in tissue sections were found in the caudal spinal segments rather than in the cervical spinal segments.

These results indicate that an intrinsic spinal cord serotonin system is normally present during fetal development of non-human primates. In addition, these findings suggest that these spinal cord 5HT immunoreactive neurons may have an important function during fetal development, either in regulating differentiation in the spinal cord grey matter or in modulating certain neuronal cell groups that may have a critical physiological function during fetal growth. (Supported by NS 19379 and by Scope C HD13063 to R.I. Stark).

130.15 CHOLINEACETYLTRANSFERASE IMMUNOHISTOCHEMICAL EVALUATION OF AN EXCITOTOXIN LESION OF NUCLEUS BASALIS. G.R. Stewart, B.K. Hartman, C. Cozzari* M.T. Price and J.W. Olney, Washington Univ Schl Med, Dept Psychiatry, St. Louis MO.

The development of an accurate and sensitive immunostaining procedure for cholineacetyltransferase (ChAT), the biosynthetic enzyme for acteylcholine, has greatly advanced the localization and characterization of cholinergic neurons throughout the brain and spinal cord. The cholinergic neurons within the basal forebrain (BF) are of particular interest because they degenerate in patients with senile dementia Alzheimers type. These cells, located primarily in the ventral globus pallidus, project ipsilaterally upon most cortical regions as well as the amygdala. A lesion placed within the BF of rats and monkeys causes a rapid and profound loss of ChAT from the neocortex. But a recent study reported that cortical ChAT levels return to normal by 12 wk following a lesion produced with the excitotoxin ibotenic acid (Wenk & Olton, Brain Res 293,184,1984). Using ChAT immunohistochemistry (IHC) we are evaluating the effects of an excitotoxic lesion on BF cholinergic cells. In addition, we have observed that the basolateral nucleus of the amygdala (BLA) in normal animals, receives an exceedingly dense and well defined cholinergic innervation from BF cells.

Lesions were produced by injecting the excitotoxin N-methyl-DL-aspartate (NMA, 1.5ul, 100nmol) unilaterally into the ventral globus pallidus. Animals were sacrificed from 3 days to 20 weeks following surgery and the brains processed for ChAT IHC.

At short survival times (ie, less than 2 wk) there was a drastic reduction of ChAT positive cells from the lesioned side of the brain as well as complete loss of ChAT staining from the ipsilateral BLA. In long-term animals (up to 20 wk), the loss of ChAT positive cells persisted indicating the irreversible nature of the excitotoxic lesion. At 20 wk ChAT staining was discernible in the ipsilateral BLA but was quite faint compared to the contralateral BLA.

Our findings suggest that ChAT IHC is a useful adjunctive method for evaluating an excitotoxin lesion of BF cholinergic neurons and that the BF/BLA cholinergic system may prove of special interest as a model for studying CNS cholinergic denervation-reinnervation phenomena. Moreover, our data confirm the usefulness of NMA as an inexpensive but highly effective lesioning agent. (Supported by RSA MH38894 (JWO), MH37967.)

130.16 CO-LOCALIZATION OF IMMUNOREACTIVITY FOR CHOLINE ACETYLTRANSFERASE (CHAT) AND VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) IN BIPOLAR NEURONS IN CEREBRAL CORTEX. F. Eckenstein* and R.W. Baughman. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Immunohistochemical staining in the rat with a well characterized antiserum to choline acetyltransferase (Eckenstein and Thoenen, EMBO J., 1:363, 1982) revealed a population of small, bipolar neurons present in all cortical areas and in all cortical layers, but concentrated in layers 2 and 3 (Eckenstein and Thoenen, Neurosci. Lett., 36:211, 1983). These cells remarkably resemble VIP-positive cells in terms of their morphology, number and distribution. We therefore tested for co-existence of these two antigens by staining serial horizontal 6-micron cryostat sections alternately for ChAT and VIP, with a highly sensitive double PAP procedure. Eighty percent of the ChAT-positive cells were stained in an adjacent section for VIP, indicating a very high degree of co-localization of ChAT and VIP in these bipolar neurons. Since another major source of cortical cholinergic innervation originates from cells in the basal forebrain, we looked for VIP staining in these neurons. Even with colchicine injections, no VIP immunoreactivity was detectable. Thus there appear to be at least two types of cholinergic innervation in cortex, an intrinsic one co-localized with VIP and an extrinsic one not containing VIP. This difference suggests that these two types of ChAT-positive terminals might be distinguished on the basis of the presence or absence of VIP-staining, an approach that we are now pursuing. For complete characterization of the synaptic relationships of the ChAT-positive fibers it is necessary to visualize them at the ultrastructural level. We have achieved satisfactory pre-embedding staining for both ChAT and VIP, and one of the most striking features we have observed is that in addition to contacting neuronal elements, many ChAT-positive and VIP-positive structures are closely associated with blood vessels, including capillaries. Examination of staining patterns in the light microscope also reveals an extensive network of ChAT- and VIP-positive fibers running along blood vessels. This suggests that in addition to influencing neuronal activity the ChAT/VIP neurons may play a role in regulating cortical blood flow.

130.17 SIMULTANEOUS DETECTION OF DOPAMINE, NORADRENALINE AND ACETYLCHOLINE IN BRAIN BY USING SPECIFIC ANTIBODIES. M. Geffard*, A.M. Rock*, M.L Souan*, J. Dulluc*, and M. Le Moal (SPON:Y.Ben-Ari). Inserm U259, Psychobiologie des Comportements Adaptatifs, rue Camille Saint-Saëns, 33077 Bordeaux, France.

Highly specific antibodies were raised against labile neurotransmitter molecules (Geffard et al.,1982,1984) and they were developed and characterized according to the following principles : (i) the original care was taken to preserve structure of the transmitter molecule during the synthesis of immunogenic conjugates. In the case of CA, glutaraldehyde was preferentially used as fixative to link the CA-amino group to the ε-amino group of lysine residue in the BSA molecule. Special care was taken during the coupling reaction to prevent the oxidation of the catechol moiety ii) conditions were developed which favored the development of antibodies to the transmitter-conjugate for example. After a coupling by glutaraldehyde the obtained imines were saturated in order to form an aliphatic chain and to become less antigenic than the catechol ring iii) a rational specificity study was carried out in order to characterize the physicochemically antibody site iv) the immunodetection of each molecule required the use of coupling agents which also serve as fixatives, thus the transmitter molecule is retained in tissues; the antigenicity is increased in accordance with specificity results and the histological structure of the tissue is preserved.

The antibody affinity and specificity of each antiserum were demonstrated in vitro and with immunocytochemistry. The radiolabelled synthesis of a DA and NA analogs were used to quantify the antigen-antibody reaction and characterize the specificity. For anti-DA antibodies, the best displacement of (^{3}H) DA-G-ALM was observed with DA-G-ALM (KA=6,7x10^{7}1.mole^{-1}). The other CA derivatives exhibited a slight immunoreactivity NA-G-ALM (KA=5x10^{5}1.mole^{-1}), L-DOPA-G-ALM (KA=3.8 x10^{4}1.mole^{-1}); OA-G-ALM (KA=5x10^{4}1.mole^{-1}), TA-G-ALM (KA=3.1 x10^{4}1.mole^{-1}). For anti-NA antibodies, the best displacement of (^{3}H) NA-G-ALM was observed with NA-G-ALM itself (KA=3.8x 10^{7}1.mole^{-1}). The most immunoreactive compound was DA-G-ALM (KA=2.4x10^{6}1.mole^{-1}). For anti-acetylcholine antibodies the most immunoreactive compound was the conjugated ACH reproducing the haptenic group in immunogens. Choline and phosphatidylcholine, were not recognized by the anti-Ach antiserum.

These biochemical results correlate well with immunohistochemical results which also will be reported (Geffard et al. 1984 in press).

130.18 GABA IN RAT CEREBRAL AND CEREBELLAR CORTEX:ELECTRON MICROSCOPIC IMMUNOCYTOCHEMISTRY. P.Seguela*, H.Gamrani*°, M.Geffard*, A.Calas*° and M.Le Moal*(SPON:J.Epelbaum). Inserm U259, Psychobiologie des Comportements Adaptatifs, rue Camille Saint-Saëns, 33077 Bordeaux, France.*°Cytologie,33400 Talence, Fr.

In order to develop specific antisera for the GABA-Glutaraldehyde-Lysine complex, one must satisfy the following conditions:1)obtain a high degree of coupling on the protein carriers, 2)reduction of the imines formed during condensation 3)use of several alternative immunizations with different carriers. Radioimmunological tests with a radiolabeled ligand-protein complex were completed prior to the use of these antisera in light (PNAS 84, in press) or electron microscopic immunocytochemistry. For the staining procedure, male Wistar rats were perfused with ice cold solution containing 5 % glutaraldehyde and 1 % sodium metabisulfite in 0.1M sodium cacodylate buffer (pH 7.6). Slices 5mm thick were post-fixed 1h in the same fixative. Fifty um sections were incubated in normal bovine serum for 12h at 4°C(1/4000) Sections were stained with the PAP technique. Sections were immersed for 1h in 1 % osmium tetroxyde, dehydrated in graded alcohols and flat-embedded in Epon 812. Pieces of selected areas were removed from the specimens, thin sections were collected on copper grids, stained with 2 % uranyl acetate solution, and observed with a Philips EM 300 electron microscope. In cerebral cortex, three types of labeled neuronal elements (perikarya, dendrites and axon termials) were distributed within all cortical layers as described with GAD immunocytochemistry (Riback, 78). Morphological features of many immunopositive cell bodies were typical of stellate neurons. In somata and dendrites reaction product was concentrated along the cisternae of endoplasmatic reticulum, the mitochondrial, plasmic and nuclear membranes and at the surface of microtubules. We never observed staining in the Golgi apparatus, which is in contrast to the results reported for GAD immunocytochemistry. In cerebellar cortex electron microscopic results confirmed the immunopositivity of Purkinje, basket, Golgi and stellate cells. Punctate profiles in light microscopy correspond to the labeling of nerve terminals or small dendrites. Glomeruli could be clearly recognized: negative mossy fibers surrounded by unlabeled dendrites which were contacted by immunopositive terminals. The excellent preservation of tissue with glutaraldehyde fixation and high specificity of these antibodies permitted for the first time a comparisation between the GAD and the GABA subcellular localization.

131.1 PLASTICITY OF CENTRAL CATECHOLAMINERGIC NEURONS IN AGED RAT BRAIN: REINNERVATION AND FUNCTIONAL RECOVERY AFTER AXOTOMY. C.J. Phelps and J.R. Sladek, Jr., Department of Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

In a recent study (Phelps and Sladek, Brain Res. Bull. 11:735, 1983), regenerative capacity was observed in catecholaminergic (CA) fibers in the medial forebrain bundle (MFB) after surgical axotomy, in aged, as well as in young adult, rats. Plasticity which persists in aging also was indicated by observed hyperinnervation of magnocellular neurons in intact aged rat brain (Sladek et al., Peptides 1:131, 1980). As a continuation of the former study, regeneration at the lesion site, reinnervation of supraoptic nucleus (SON), and functional manifestations were examined among aged rats subjected to long-term axotomy of fibers which contact and influence SON neurons.

Twenty- and thirty-month-old m.o. F344 male rats were subjected to bilateral knife cuts, positioned just caudal and medial to SON. Lesion sites and SON were examined microscopically at 2, 7, 21 and 60 days. CA fluorescence was induced by aqueous formaldehyde-glutaraldehyde (FAGLU) or aluminum formaldehyde (ALFA) perfusions. Water consumption and urine volume were monitored from four days prior to surgery through postsurgical survival times.

Subtotal denervation in SON and typical axonal degenerative profiles at the lesion site, were evident 2 days postsurgically; axonal transmitter "pile-up" persisted as long as 3 weeks in aged brains. Fine-sized, new fibers appeared at the wound site as early as 7 days postsurgically, persisted even 60 days, and were traceable for significant lengths. SON neurons were consistently rimmed with fluorescent varicosities, including lateral areas which lost fluorescence shortly after lesioning. This apparent reinnervation was not observed at earlier surgical intervals.

Water consumption and urine volume decreased dramatically after surgery in all animals. Water consumption returned to normal levels by 6 days in 20 m.o. rats, and by 9 days in 30 m.o. rats. Urine volume remained depressed until 32 days postsurgically in both age groups. By 42 days after surgery, urine volume was greater than presurgical measurements, and water consumption increased markedly among 20 m.o., but not 30 m.o., animals.

The data indicate morphological reinnervation in aged animals, similar to that observed in young rats (Davis et al., SN Abs. 9:859, 1983) and suggest functional influence of this recovery on vasopressin secretion.

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131.2 **REACTIVE SYNAPTOGENESIS IN THE HIPPOCAMPUS OF AGED AND YOUNG-ADULT RATS.** K.J. Anderson*, S.W. Scheff, and S.T. DeKosky, Depts. of Anatomy and Neurology, Univ. of Kentucky and V.A. Medical Centers, Lexington, KY 40536.

Selective lesions that result in a partial loss of neuronal input appear to signal residual, undamaged inputs to sprout and replace synaptic connections that have been lost. Previous investigations have compared this process of reactive synaptogenesis between young and old animals & have demonstrated that the aged brain has a diminished capacity for reinnervation following massive denervation of a target area. Many factors, such as clearance of degenerative debris and the rate of synaptic loss, may contribute to this reduced plasticity observed in senescence. Studies concerning reactive synaptogenesis in the aged animal have focused on the responses of the hippocampal dentate gyrus. We have chosen to study the lesion-induced plasticity of an adjacent area of the hippocampal formation, area CA1 of regio superior, in young-adult and aged rats.

Young-adult (3-6 months of age, n=20) and aged (24-30 months of age, n=20) Fischer 344 rats were used in this experiment. Rats received a unilateral, intraventricular injection of kainic acid (0.6 µg/µl) which selectively destroyed the CA3-CA4 hippocampal pyramidal neurons. Following a two day interoperative interval, the rats received an ipsilateral transection of the fimbria-fornix. Animals were allowed to survive for 4, 10, 30, or 60 days following surgical removal of the fimbria and were perfused with 1% gluteraldehyde and 4% paraformaldehyde in phosphate buffer. Both hippocampi were removed and processed for electron microscopy. Photographic montages were constructed of area CA1 that extended from the alveus to the hippocampal fissure. The density of synapses, both intact and degenerating, was determined and plotted as a function of age, zone of analysis and time following the lesions.

At this time, our preliminary results indicate that synaptic density is reduced to 40% of control in the hippocampus ipsilateral to the lesions and 60% of control contralaterally. Both aged and young animals exhibit a return of synaptic density to near control levels by 60 days postlesion, however, the senescent animals display a significant reduction in the initial phase of the reactive response. (Supported by NIH grants NS16981, NS00444, BRSG S07R05374 and the V.A. Medical Research Service.)

131.3 INCREASED DURATION OF Ca^{2+}-DEPENDENT K^+ CONDUCTANCE IN HIPPOCAMPAL NEURONS FROM AGED RAT BRAIN. P.W. Landfield and T.A. Pitler*. Dept. of Physiol. & Pharmacol., Bowman Gray School of Med., Winston-Salem, NC 27103

Previous studies in this laboratory are consistent with the view that intracellular Ca^{2+} is elevated in aged hippocampal cells, and that this elevation results in an inactivation or blunting of Ca^{2+}-dependent processes (e.g., frequency potentiation) during repetitive stimulation (Landfield, Soc. Neurosci. Abst., 1981; Landfield et al., 1983, ibid, and in press; Pitler and Landfield, ibid, 1984). However, additional tests of this possibility are necessary.

In the present study, we investigated the after hyperpolarization (AHP) following a single depolarizing current pulse (40 msec, .2-.6 nA), injected through the intracellular pipette. That this hippocampal AHP results from a Ca^{2+}-dependent K^+ conductance is well documented (Hotson and Prince, J. Neurophysiol., 1980; Alger and Nicoll, Science, 1980; Schwartzkroin and Stafstrom, ibid, 1980).

Intracellular recordings were obtained from CA1 cells meeting criteria for good penetrations, in hippocampal slices from young (4-6 mo-old) and healthy aged (26-30 mo-old) Fischer rats.

The maximal AHP within the 200 msec following the onset of a depolarizing current pulse was measured for each cell, and the percent of this maximum remaining at every 100 msec following depolarization onset was calculated. Cell responses were matched for number of spikes triggered.

Following 2 spikes, aged rat cells exhibited a distinctly longer AHP than did cells from young rats. At 600 msec following depolarization onset, 68±16% of the maximum AHP remained for aged cells, and only 23±9% remained for young rat cells. At 1000 msec, 36% of maximum remained in the aged, while only 2% remained in the young. Following 3 spikes, age differences were also pronounced.

Thus, the duration of Ca^{2+}-dependent K^+ conductance is increased in aged rat hippocampal cells. This conductance is thought to directly reflect free intracellular Ca^{2+}, and therefore, these data provide evidence that Ca^{2+} is elevated in brain cells of normally aging mammals. Since high intracellular Ca^{2+} may inactivate Ca^{2+}-dependent processes in hippocampus (Pitler and Landfield, this meeting), or contribute to structural disintegration, the increased Ca^{2+} in aged cells may be relevant to both functional and structural aspects of age-related decline.

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131.4 POSSIBLE ROLE OF DOPAMINE IN THE FUNCTIONAL RECOVERY FROM HEMIPLEGIA IN AGED RATS. R.T. Knight* and S. Brailowsky. Dept. of Neurology, Univ. of California-Davis, V.A.M.C., Martinez, CA. 94553.

Chronic (7 days) application of GABA (100 ug/ul/hr) to the motor cortex of rats trained to walk coordinately on a narrow beam, produced a transitory behavioral syndrome of contralateral hemiplegia. Both young (3-6 months) and aged (24-30 months) animals showed functional recovery 1 to 2 weeks after the end of the drug administration, although aged subjects took longer to reach control conditions. Acute administration of haloperidol (0.1 mg/kg) in the recovered animals provoked a re-emergence of the unilateral deficit in both aged and young rats, with the aged group showing a more prolonged recovery period (3 days) from the neuroleptic administration than the young group (1 day).

These results suggest participation of dopaminergic mechanisms in the recovery process after brain lesions and indicate an increased susceptibility to dopaminergic blockers in aged animals recovering from a cortical insult.

The use of these drugs in brain-lesioned subjets, and particularly in geriatric patients, is considered potentially harmful, at least in the early stages of the recovery process.

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131.5 RECIPROCAL CHANGES IN D-1 AND D-2 DOPAMINE BINDING SITES IN HUMAN CAUDATE NUCLEUS AND PUTAMEN DURING NORMAL AGING. (3H)FLUPHENAZINE AS A DOPAMINE RECEPTOR LIGAND. D.G. Morgan , J.O. Marcusson*, B. Winblad*, and C.E. Finch. Andrus Gerontology Center, Univ. of Southern California, Los Angeles, CA, 90089-0191.

D-1 and D-2 dopamine binding sites were simultaneously measured in membrane preparations from human postmortem brain material. Spiperone displacement of (3H)fluphenazine binding resolved two components with a transition plateau at 10 nM spiperone. Additional characterization studies indicated that the component with high affinity for spiperone was the D-2 dopamine receptor, while the other component corresponded to the D-1 binding site. No adrenergic or serotonergic binding sites were labeled by (3H)fluphenazine in human neostriatum. Nonspecific binding was defined as that remaining in the presence of 100 uM apomorphine.

Saturation analyses were performed on caudate nucleus and putamen membranes from 26 donors ranging from 17-100 years of age. In caudate nucleus, (3H)fluphenazine D-2 Bmax declined 30% between 20 and 80 years (r =-0.62; $p < 0.01$); a similar decline was detected for (3H)spiperone D-2 Bmax (r =-0.68). Both radioligands gave the same range of Bmax values (100-300 fmol/mg prot.; r =0.69). The (3H)fluphenazine D-1 Bmax increased 70% between 20 and 80 years (r =0.47; $p < 0.05$) and the ratio of D-1 Bmax/D-2 Bmax increased from 1.0 to 2.1 (r =0.68).

In putamen, no significant change in the D-2 component was apparent. However, (3H)fluphenazine D-1 Bmax doubled between 20 and 80 years (r =0.63) and the D-1 Bmax/D-2 Bmax also doubled (r =0.55; $p < 0.02$).

No influence of age on Kd was found for either D-1 or D-2 components. Postmortem time was not correlated with Bmax or Kd for any of the binding sites measured.

	Bmax (fmol/mg prot.)		Kd (nM)	
	Caudate	Putamen	Caudate	Putamen
D-1	333 +22	213 +22	4.09 +.33	4.03 +.31
D-2	916 +12	155 +10	.94 +.23	1.41 +.30

We conclude that age alters the relative proportions of postsynaptic dopamine receptor sites in human neostriatum. Therefore, one would expect that the elderly will respond differently to dopaminergic drugs than their younger counterparts.

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131.6 HIGH-AFFINITY AGONIST BINDING TO STRIATAL D-2 DOPAMINE RECEPTORS IN AGED MOUSE. J.A. Severson. Dept. of Psychiatry, USC School of Medicine, Los Angeles, CA 90033.

Direct binding of 3H-agonists and 3H-antagonists to striatal dopamine (DA) receptors declines in an age-correlated manner. For 3H-antagonists, the decline in receptors, termed D-2, appears to begin early in life and is progressive to old age. Striatal 3H-agonist binding declines more rapidly with age than 3H-antagonist binding and most of the decline may occur before midlife. High-affinity agonist binding to the D-2 receptor appears to require the interaction of the D-2 receptor with a guanine nucleotide binding regulatory protein (N). Thus, differences in the rates of receptor loss with age, as measured by the binding of 3H-agonists and 3H-antagonists, may result from the loss of D-2 receptor or N, or both.

3H-Spiperone (3H-SP) binding to D-2 DA receptors in homogenates from male C57BL/6J mice, ages 3, 12 and 24 months, was inhibited by increasing concentrations of DA. In the first experiment, 7 concentrations of DA, 10^{-7} to 10^{-4}M, were used in the absence and presence of 150 uM GMP-pNHp. Inhibition curves were analyzed for IC50 by ALLFIT and the IC50 converted to $_{9}$Ki. In the second experiment, 20 concentrations of DA, 10^{-9} to 10^{-4}M, were used in the absence of guanine nucleotides and the displacement curves were analyzed by LIGAND. All displacement curves in the second experiment were best described by a 2-site model.

No age differences were observed in the degree of shift of displacement curves induced by GMP-pNHp. However, quantitative determination of high-affinity (R_H) and low-affinity (R_L) agonist binding sites indicated a decline in the percent and the density of the D-2 receptors measured as R_H. Scatchard analysis of 3H-SP binding isotherms indicated that the total D-2 receptor density (Bmax) declined also. However, the age changes in R_H occurred before midlife, while the decline in Bmax was progressive between 3 and 24 months. These data suggest age-related declines in the total striatal D-2 dopamine receptor density and the membrane protein that is obligatory for the formation of R_H. Additionally, the apparent loss of N occurs before midlife.

131.7 DECLINE IN FATIGUE RESISTANCE, PTP AND APPEARANCE OF α ADRENERGIC RECEPTORS IN AGING SKELETAL MUSCLE. R.C. Carlsen and D.A. Walsh*. Depts. of Human Physiology and Biological Chemistry, Univ. of Calif. Sch. of Med., Davis, CA 95616.

Muscular strength and endurance decline with age. In part, this may be due to structural changes in skeletal muscle, but there may also be a decline in the tension-generating capacity of muscle fibers. We have investigated age-related changes in contractile capability in a predominantly (90%) type IIA fast-twitch muscle from the rat hind-foot. In addition, we have tested the effect of catecholamines on aging muscle contractile properties. The flexor digitorum brevis (FDB) was stimulated directly in vitro (35°C) using platinum sheet electrodes placed on either side of the muscle. Comparisons were made between young (5-7 months), late middle age (2 years) and old (3 years) muscles from Sprague-Dawley rats. Isometric twitch properties were minimally affected by age. Twitch time course did not change appreciably. Time to peak tension increased by 12% at 2 years and by 22% at 3 years, but this was mirrored by a tendency for half-relaxation time to decrease. Twitch tension was actually highest in 3 year old FDB, but maximum tetanic tension was substantially less than young controls in both 2 year and 3 year FDB. Twitch/tetanus ratio was 0.20 in young FDB, 0.26 in 2 year old and 0.41 in 3 year old muscles. The fatigue index also declined in 3 year old FDB (0.23±.05) but not in 2 year old (0.41±.06). Nonetheless, the integral of the force x time profile over the full 2 minute stimulus period indicated that 2 year old muscles actually had a decreased capacity for work over time. Post-tetanic potentiation (PTP) and the staircase response both declined in aging muscles, to the point that they were not present in 3 year old FDB. PTP after 150 stimuli reached 47% in young FDB, 20% in 2 year old muscle and twitch tension actually fell by 37% in 3 year old FDB. Applied catecholamines (epinephrine, norepinephrine, isoproterenol) did not potentiate twitch responses in either young or 2 year old FDB, but epinephrine and norepinephrine produced a long-lasting (2-10 minutes) contracture in 3 year old FDB. The contracture was also evoked by phenylephrine and blocked by phentolamine indicating the involvement of α adrenergic receptors. Contracture was not affected by preincubating the muscle with curare, but was eliminated by preincubation in 2 mM EGTA. We conclude that aging FDB develops a population of α adrenergic receptors which may produce an increase in Ca^{++} influx and affect muscle protease activity.

(Supported by NIH AM 13613 and NS 15002).

131.8 AGE CHANGES IN NEUROMUSCULAR PHYSIOLOGY OF C57BL MICE N. Anis & N. Robbins, Dept. of Developmental Genetics and Anatomy, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

In order to test the generality of and genetic influence on previous findings of altered synaptic transmission in old CB6F-1 mouse muscles (Banker, Kelly & Robbins, J. Physiol. 339: 355, 1983), we studied similar physiologic parameters of transmission in C57Bl/6J mice, one of the parent strains of the F-1 hybrid. Studies were carried out in soleus and extensor digitorum longus muscles from young (7-10 mo.) or old (24-25 mo.) mice and the findings were similar in both muscles.

Resting membrane potential was 3-4mV lower in old than in young muscles. Miniature endplate potential (m.e.p.p.) frequencies were the same or in some cases much higher in old than young muscles, while amplitudes were about 50% greater. Separate studies showed that the increased m.e.p.p. amplitude was not due to increased input resistance or to a greater number of acetylcholine receptors per endplate.

In low Ca high Mg solutions, e.p.p. amplitude and quantal content were increased 2.5 and 1.5 times, respectively, i.e. the increase of endplate potential (e.p.p.) amplitude in old muscles was greater than that simply due to an increased unit amplitude.

The lack of decrease in m.e.p.p. frequency at old C57BL neuromuscular junctions, in contrast to findings in CBF-1 muscle, is not surprising given the literature showing that this parameter changes variably with age. However, the increased m.e.p.p. amplitude is a new and interesting age change, not previously reported. Finally, there is good agreement between C57 and the F-1 hybrid in the finding of increased quantal content of transmitter release. Thus, the latter may be a more ubiquitous finding at the aging synapse.

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131.9 NATURE OF K^+ ACTIVATED HYPERPOLARIZATION (KAH) IN MOUSE DIAPHRAGM AND ITS POSSIBLE CHANGE BY AGING. Toshihiko Nishimura* and Alexander G. Karczmar. Department of Pharmacology, Loyola University Medical Center, Maywood, Illinois 60153.

This study concerns muscle membrane potential change related to the active ionic transport and its possible change by aging. The diaphragm was dissected from 6 and 24 months old CBF-1 mice (male) and some experiments were carried out with 6 to 10 weeks, 6 and 10 months old CF-1 mice (male). Conventional microelectrode technique was employed to record membrane potential changes. The diaphragm was soaked in K^+ free modified Krebs solution for 90 min. at 22°C. During this period, it was expected that $(Na^+)i$ should increase and $(K^+)i$ should decrease due to inhibition of Na^+-K^+ exchange pump. Application of 10mM K^+ to Na^+ loaded muscles caused KAH. Ouabain (0.3mM) suppressed KAH reversibly. TTX (0.3μM) and TEA (10mM) did not affect KAH. KCl- and K-Glutamate-induced KAH were identical.

In most cells, KAH exhibited triphasic response, as rapid hyperpolarization was followed by depolarization during K^+ application and K^+ washout caused another hyperpolarization. This triphasic response was observed in 90.9% and 76.0% of muscle fibers of 6 and 24 months old CBF-1 mice, respectively. In 6 months old mice rarely and in 24 months old mice more frequently, a monophasic KAH was observed.

Na^+ loaded muscles were depolarized, as resting membrane potentials were 54.6±7.0 (mean ± S.D.) mV and 53.6 ± 4.8 mV in 6 and 24 months old CBF-1 mice, respectively. KAH amplitudes were 16.6 ± 5.3 mV and 14.6 ± 4.9 mV in 6 and 24 months old CBF-1 mice, respectively. A change with age in the triphasic response could not be documented at this time; the incidence of monophasic KAH was increased in 24 months old mice. This indicates that some change of membrane property related to Na^+-K^+ exchange pump may occurr during aging process. This work was supported in part by Potts Foundation Grant. CBF-1 mice were supplied by NIH Institute on Aging.

131.10 GROUP IA-ALPHA MOTONEURON SYNAPSES IN THE AGED CAT. P.A. Boxer*, M.H. Chase, and F.R. Morales, (SPON: J.K. Engelhardt), Depts. of Physiology and Anatomy and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Significant changes in the basic electrophysiological properties of alpha motoneurons have been reported in aged cats (Boxer, et al., Soc. Neurosci. Abstr. 8:480, 1982). The present studies were designed to examine the effects of sensescence on the waveform parameters of compound Group IA monosynaptic EPSPs.

Experiments were performed on age-documented healthy cats: 5 cats were 14 to 15 years of age and 6 adult controls were 2 to 4 years of age. The animals were anesthetized with sodium pentobarbital. The ventral roots L6 to S1 were cut and intracellular recordings were obtained from triceps surae motoneurons with 3M KCl electrodes (action and resting potential amplitudes were greater than 60 mV). Homonymous and heteronymous monosynaptic EPSPs were elicited by stimulation of the triceps surae nerves at twice Group IA threshold at a rate of 0.5 or 1 Hz. EPSPs were averaged and their amplitude, 10-90% rise time, rate-of-rise and half-width were measured. There was no significant difference in the amplitude of the homonymous EPSPs between the two groups of cats (5.1 ± 2.6 mV in the aged cats vs. 4.4 ± 2.1 mV in the adults, $p>0.05$). However, in the aged cats the rise times were significantly prolonged (0.91 ± .24 ms vs. 0.54 ± .14 ms in the adults, $p<0.001$); the rates-of-rise were also slower. The half-widths of the EPSPs were longer in the aged cats (6.4 ± 1.5 ms vs. 5.1 ± 1.6 ms in the adults, $p<0.01$). Qualitatively similar results were observed in the case of heteronymous EPSPs.

The prolongation of the rising phase of the EPSPs as well as the decay phase in the aged cats suggests that the synaptic currents underlying the EPSP are prolonged. These results are unlikely to reflect a loss of synapses in the soma and proximal dendrites, since no difference in the amplitude of the EPSPs was found. However, the data can be explained by postulating a greater asynchrony in the presynaptic impulses at the terminal arborization of the IA axons in the aged cats.

Supported by the NIA (AGO 04307) and the Intra-Science Research Foundation.

131.11 PROGRESSION OF AGE CHANGES IN NEUROMUSCULAR NERVE TERMINAL MORPHOLOGY IN MOUSE HINDLIMB MUSCLES M.A. Fahim and N. Robbins. Gerontol. Ctr., Univ. So. Calif, Los Angeles, CA 90089 and Dept. of Dev. Genetics and Anatomy, Case Western Reserve Schl. of Medicine, Cleveland, OH 44106.

In order to determine whether there was any correlation between physiologic and morphologic progression of nerve terminal age changes, neuromuscular junctions from extensor digitorum longus (EDL) and soleus muscles of CB6F1 mice from 5 to 25 mos of age were examined by quantitative microscopy of zinc iodide-osmium stained preparations. Over this time period, nerve terminal area and total length, normalized to fiber diameter, changed slightly or not at all in EDL, but in soleus there was a steady increase. The percentage of nerve terminals with sprouts was about the same throughout this period in both muscles, but there was over a 100% increase in the number of unconnected synaptic regions. Since both muscles show substantially increased quantal release content with age (Kelly & Robbins, J. Physiol. 343: 375, 1983) it is clear that nerve terminal expansion (e.g. the 20% increase in soleus nerve terminal area) cannot account for the physiologic results. Also, the large increase in number of regions implies definite morphologic remodelling without substantial expansion of total nerve terminal area (especially in EDL). Thus, it appears that only quantitatively different, but qualitatively similar morphologic events occur with aging at EDL and soleus neuromuscular junctions of the CB6F-1 mouse, but they do not account for the changing physiology. Supported by NIA grant AG00795 to N.R.

131.12 AGING OF CHOLINERGIC SYNAPSES IN THE AVIAN IRIS. E. Giacobini, T. Mattio* and E. Mussini*, Dept. Pharmacol., Southern Illinois Univ. Sch. Med., Springfield, IL 62708 USA and C.S. Biol; Fisiopat. Musc., Ist. Patologia Generale, Universita di Padova, 35100 Padova, Italy.

Based on the results of our studies on the ciliary ganglion iris preparation, a hypothesis of aging of the cholinergic synapse has been proposed (Giacobini, E., Adv. Cell. Neurobiol., 3:173, 1983). This hypothesis contemplates age-related changes in carrier-mediated mechanisms of uptake and release of the neurotransmitter and its precursor (choline) leading to "chemical denervation". Morphometric analysis of neuromuscular junctions in the iris showed a significant reduction of the axonal junctional membrane at five years. A 50% decrease in the volume of vesicles per unit volume of the synapse was evident at three years. Experiments were designed to determine the ability of the 3-year iris to undergo depletion-reloading-release of ^3H-acetylcholine (^3H-Ach). The 3-year tissue released significantly less ^3H-ACh than the 4-month tissue as determined by the area under the release curve (peak area). Also, the 3-year tissue showed a lower peak release of ^3H-ACh than the 4-month iris. The time needed for the 3-year tissue to reach its peak release was significantly longer than at 4-month and its rate of release was significantly slower. These neurochemical results correlate well with the morphological data which demonstrates that two important features for neurotransmitter release (vesicular volume and synaptic length) were decreased in the 3-year (or 5-year) old tissue. These results support the hypothesis that age-dependent decline in cholinergic transmission is related to modifications of presynaptic mechanisms of release and uptake of the neurotransmitter and its precursor. [Supported by AFOSR Grants 81-9229 and 83-0051, by grants from the Nowatski Eye Res. Fdn., E.F. Pearson Fdn. and Natl. Res. Council of Italy to the Unit for Muscle Biology (I. Mussini)].

131.13 GREATER FRACTION OF LOW-AFFINITY, SLOW-CHANNEL ACETYLCHOLINE RECEPTORS AT THE NEUROMUSCULAR JUNCTION OF AGED RATS. D.O. Smith and M.R. Chapman*. Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53706.

In the rat diaphragm, there is an age-related increase in the number of nerve terminals per end plate. This is associated with increased vesicular and cytoplasmic ACh release per end plate. Correlated postsynaptic changes were examined in the diaphragm obtained from rats aged 10 (mature adult) and 28 (aged) months. ACh receptor binding kinetics were assayed by incubating minced tissue in monoiodinated ^{125}I-labeled α-bungarotoxin at 37°C. The average ($\pm$s.e.) association rate constants of specific binding, K_{+1}, were 36775 $\pm$2781 and 14390 $\pm$1576 $M^{-1}s^{-1}$ in mature adult and aged rats, respectively ($p<0.001$). Retardation of the initial rate of toxin binding by d-tubocurarine (dTC) was assayed, and the results were found to exhibit nonlinear Hofstee plots, which is consistent with two classes of binding sites. The values of EC_{50} for the two components were similar for both age groups, namely 7uM and 43 nM which represent "low-affinity" and "high-affinity" sites, respectively. However, the percentages of high-affinity sites were 50% and 25% in the 10- and the 28-month animals, respectively ($p<0.05$).

The average open-time of the ACh-activated ion channels was also determined by measuring the time constant of decay of extracellularly recorded miniature end-plate potentials (m.e.p.p.s). Data from each age group exhibited bimodal distributions, with peaks at about 0.6 and 0.9 ms, indicating the presence of two different populations of channels. The percentages of sites exhibiting "fast" (0.6 ms) open-time kinetics were 63% and 31% in the 10- and the 28-month animals, respectively ($p<0.05$). M.e.p.p. frequencies recorded extracellularly and intracellularly from the same fibers were not appreciably different, indicating that the channel open-time measurements were obtained from most --if not all-- of the end-plate region.

Lower binding affinities and slower mean channel open times have been reported for extrajunctional receptors in denervated muscle. Thus, the results of this study may indicate an increased fraction of "extrajunctional-type" ACh receptors at the end plates of aged rats. Supported by NIH grant AG01572.

131.14 THE EFFECTS OF EXERCISE AND STRESS ON AGE-RELATED CHANGES IN END-PLATE ARCHITECTURE. J.L. Rosenheimer, W.W. Spirduso and R.P. Farrar* Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53711 and Dept. of Physical and Health Education, Univ. of Texas, Austin, TX 78712

There is an age-related decrease in terminal branch number at end plates of the extensor digitorum longus (EDL) and soleus muscles of the rat. However, branch number increases with age in the diaphragm. To examine the effects of exercise and/or stress on this phenomenon, the end-plate architecture of these functionally diverse muscles was examined in rats aged 18 to 19 mos and 24 to 25 mos. Data obtained from sedentary controls (C) were compared to those from rats that had been exposed chronically to foot shock (CS) or that had been shocked and exercised on a treadmill (ES) from 3 mos of age. The average number of nerve terminals and the frequency of occurrence of sprouting and degeneration were recorded.

At 18 to 19 mos there was a significant decrease ($p<.0005$) in branch number at the soleus end plates of the CS animals as compared to the C animals; branch number in the ES animals was similar to that of the C group. By 24 to 25 mos this pattern of change was manifest in both hind-limb muscles. The diaphragm also exhibited a significant ($p<.05$) decrease in terminal branch number in the CS animals as compared to C at 24 to 25 mos; this value was further depressed in ES rats.

The number of sprouting nerve terminals increased significantly in all muscles of ES animals as compared to C animals. Degenerating nerve terminals were observed most frequently in the CS animals. However, these trends were seldom significant.

It is concluded that the apparent stress from chronic shocking accelerated the age-related decrease in terminal branch number in the hind limbs. Moreover, it caused branching to decrease in the diaphragm. However, exercise counteracted the effects of stress in the hind-limb muscles, but accentuated these effects in the diaphragm. Supported by NIH grants AG02071 and AG01572 and an NIH training grant to the Neurosciences Training Program at the Univ. of Wisconsin.

131.15 NA,K-ATPASE IN YOUNG AND AGING CEREBRAL CORTEX: HUMAN AND RHESUS MACAQUE BRAIN. S.C. Specht, R. Fiol, J. Sánchez*, S. Castro* and L. Hernández*. Depts. of Pharmacology and Pathology, Univ. Puerto Rico Sch. Med., San Juan, P.R. 00936.

Morphological and functional changes in cerebral cortex suggest that neuronal Na,K-ATPase may decrease importantly during brain aging. Studies in young and aged rat have yielded conflicting results. Hence, this study was undertaken with human and non-human primate (rhesus macaque) brains to determine if recovery of neuronal enzyme is decreased in aged brain and if the relative proportions of the two catalytic subunits α and $\alpha(+)$ change during aging. Human brain tissue was obtained at autopsy 8-12 hours after death frozen rapidly and maintained at -70°C until analyzed. The ages at death were 16,22,28 (young) and 58,62,70 and 78 years (old). Ages of the rhesus macaques were 8 and 27 years. The cortical areas sampled were frontal, motor, parietal, occipital, temporal and hippocampal; optic nerve was also analyzed. Synaptosomes were prepared from cortical grey matter by sucrose density centrifugation and Na,K-ATPase was purified from synaptosomal membranes by selective detergent extraction. The catalytic subunits were separated by SDS-polyacrylamide gel electrophoresis. The apparent molecular weights of α and $\alpha(+)$ were 101 and 97 KD; enzymes from human and rhesus macaque brains were indistinguishable in terms of apparent molecular weight. No age-dependent change in the relative proportion of α and $\alpha(+)$ was noted. The recovery of synaptosomes was 41% lower in old brain (mg protein/g grey matter) and the recovery of purified Na,K-ATPase was 48% lower (g protein/mg synaptosomal protein). Thus, the data support the hypothesis that neuronal Na,K-ATPase declines during aging of the primate brain. (Supported in part by NS-07464 and Biomedical Research Support Grant to the Univ. P.R. Sch. Med. The rhesus macaques were originally from the Caribbean Primate Research Center, Division of Comparative Medicine, Univ. P.R. Sch. Med., Medical Sciences Campus.)

131.16 ABSENCE OF AGE-DIFFERENCES IN PROTEIN SYNTHESIS IN RAT BRAIN AS MEASURED WITH A CELL-FREE SYSTEM. J.W. Cosgrove and S.I. Rapoport. Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health, 10/6C103, Bethesda, MD 20205.

Previous studies (reviewed in Makrides, S.C., Biol. Rev., 58: 343, 1983) using cell-free protein synthesis systems have suggested that protein synthesis in the mammalian brain declines as a function of age. We examined brain protein synthesis in relation to age using systems derived from the postmitochondrial supernatant of the whole brain of male Fischer-344 rats, at 3, 12, 24, 30, and 34 months of age. The optimum conditions for amino acid incorporation in the in vitro protein synthesis system are 200mM potassium ion and 5mM magnesium ion. The conditions which are optimal for amino acid incorporation do not change as a function of age. Amino acid incorporation in each of these systems is dependent on addition of an energy source and an energy regenerating system (creatine phosphate and creatine phosphokinase). We used the drug aurintricarboxylic acid (ATA), a specific inhibitor of initiation of protein synthesis, to determine whether these cell-free systems derived from rat brain have the capacity to reinitiate protein synthesis in vitro. Amino acid incorporation in the cell-free protein synthesis system derived from the brain at each age is sensitive to the drug ATA at a concentration of 1×10^{-4}M. This suggests that the cell-free system derived from animals at different ages has the capacity to initiate protein synthesis in vitro. Measurement of protein synthesis capacity in these cell-free systems which can reinitiate protein synthesis in vitro, indicated that there is no relation between brain protein synthesis and age. Our results suggest that previous studies of an age related reduction in brain protein synthesis, which employed non initiating cell-free protein synthesis systems, must be interpreted with caution. Furthermore, the age-invariance of protein synthesis, despite reported age changes in cell number and morphology in the rat brain, suggests that compensatory mechanisms operate to maintain resting synthetic activity in the healthy brain.

131.17 CALMODULIN COMPARTMENTATION AND AGING IN THE MOUSE BRAIN. P.C. May, H.H. Osterburg* and C.E. Finch. Andrus Gerontology Ctr., USC, Los Angeles, CA 90089.

Calmodulin (CaM) may be an important regulator of dopaminergic transmission within the basal ganglia. Several studies suggest distribution of CaM between cytosol and membranes influences striatal dopaminergic activity. This study examined whether possible age-related alterations in CaM or tubulin levels or distribution contribute to the basal ganglia deficits which occur with age. A procedure was developed for extraction and RIA analysis of mouse brain CaM and tubulin from three subcellular fractions:1, soluble; 2, EGTA-releasable, membrane bound; and 3, detergent-extractable, membrane bound. The amount of CaM and tubulin in each compartment was evaluated in three age groups of male C57BL/6J mice : young (3-4 mo.), middle-aged (9-10 mo) and old (29-31 mo). In striatum, small (10-15%) but significant ($p<.05$, Neuman-Keuls analysis) age-correlated decreases were detected in all three fractions; interestingly, the changes occurred in different age groups. Soluble CaM levels which averaged 1050±30 ng/mg protein for young and middle-aged mice decreased to 940±30 ng/mg protein in the oldest age group. Decreases in detergent-extractable membrane bound CaM occurred primarily between young and middle-aged mice (290±9 and 253±13 ng/mg protein, respectively) while CaM in the EGTA-releasable compartment declined progressively from 67±2 ng/mg protein in the youngest to 57±2 ng/mg protein in the oldest age group. Cortical levels of CaM were less altered with age and only the EGTA-releasable fraction decreased. In addition, soluble CaM was assayed by measuring the CaM-dependent activation of cyclic nucleotide phosphodiesterase(PDE). In general, RIA and PDE activation assays gave equivalent results, suggesting no alteration in biological activity of CaM via post-translational modification or other mechanisms occurs with age. Soluble and membrane bound tubulin did not change significantly with age in either brain region. These results suggest that decreases in CaM may contribute to the age-related decline in mammalian basal ganglia function.

These studies were supported by NIA grants to C.E.F.(AG 00117, AG 03230). P.C.M. was supported by NIA training grant 2T32 AG 00037-06.

AGING III

132.1 CHANGES IN RESPONSIVENESS OF CEREBRAL CIRCULATION AS A FUNCTION OF AGE. T. Kent*, R. Yang*, H. Croskell*, and S. Preskorn. Psychopharm. Lab, Kansas Univ. Sch. of Med., Kansas City, KS 66103.

A triple-label radioisotope method was used to measure the following parameters of cerebral microcirculation in 8 month and 28 month old Sprague-Dawley male rats: cerebral blood flow (CBF), the single-transit brain extraction of ^{3}H-water (Ew), cerebral blood volume (CBV), and an estimate of bolus transit time through the brain (BTT). The permeability-surface area product of water (PS) was also calculated from the measurements of CBF and Ew. This technique represents a modification of a dual-label method employing ^{3}H-water and ^{14}C-butanol to measure Ew and CBF (Brain Res. 249:23-30, 1982). The modification involved using external gamma tagged EDTA administered via the femoral vein to measure the total transit time (TTT) necessary for the bolus to travel from the vein and through the brain. This interval determined the optimum time for animal sacrifice to measure CBF and Ew following intravenous administration of ^{3}H-water and ^{14}C-butanol. The EDTA radioisotope was also used to measure regional CBV following animal sacrifice.

The rats were anesthetized using metofane and placed on a respirator so that arterial CO_2 content ($PaCO_2$) could be manipulated. CO_2-induced changes in the above parameters were compared between groups to assess for age-related effects. TTT was longer in the 28 month old animals than in the 8 month old animals across $PaCO_2$ range (22 to 75 mmHg). Forebrain CBF was higher in the 28 month old than in the 8 month old animals. There was no significant difference between the groups in PS, CBV, and Ew measurements. Hence this data was pooled to examine the relationship between PS and CBV. A linear correlation was found (table 1). The ln(1-Ew) vs -1/CBF was plotted for the pooled data. A linear correlation was found with the slope being PS. Forebrain PS values obtained in this manner were found to be higher in the 28 month old than the 8 month old animals.

Table I Correlation of BV vs PS

Region	RT	CT	DI	CB	MP
r^2	.26	.41	.60	.44	.03
p	.02	.01	.001	.01	N.S.

RT=rostral telencephalon, CT=caudal telencephalon, DI=diencephalon, CB=cerebellum, MP=medulla-pons. Supported by grants: MH00272 (RSDA), NS 17252, and PMFA (fellowship). Animals were supplied by NIA.

132.2 AGE-EFFECTS IN BRAINSTEM AUDITORY EVOKED POTENTIALS (BSAEP) IN RATS. G.V. Simpson*, O. Prospero-Garcia*, D. Scabini*, S. Brailowsky and R.T. Knight*. (SPON: E.W. Yund). Dept. of Neurology, Univ. of Calif.-Davis, V.A.M.C., Martinez, CA. 94553.

A technique of conducting free-field BSAEP audiometry in unanesthetized, unrestrained, chronically implanted young (7 months) and aged (25 months) rats is described. This method was used to determine click BSAEP thresholds in these animals. The threshold for the aged group (44.7 db SPL, SD 4.7) was increased ($p < 0.001$) by 18 dB relative to the young group (26.7 dB SPL, SD 3.5).

Following threshold determination, BSAEPs were recorded to clicks at 35 dB above individual threshold. Latencies of positive waves I and IV of the BSAEP did not differ between the young and aged groups. Latency of the prominent negative wave (No) following wave IV was increased in the aged group (4.75 msec, SD 0.23 in the young group vs. 5.94 msec, SD 0.23 in the aged group; $p < 0.001$). The latency of the No wave was measured at 15, 25 and 35 dB above threshold in the aged group. A linear decrease in latency with increasing intensity was found.

These data indicate a significant non-recruiting hearing loss in aged rats. The prolonged latency of the No component of the BSAEP in the aged group further suggests slowed conduction in brainstem auditory pathways.

Supported by the Medical Research Service of the Veterans Administration and by a grant from the N.I.A. (AG 02484).

132.3 REDUCED CALCIUM UPTAKE BY RAT BRAIN MITOCHONDRIA AND SYNAPTOSOMES IN RESPONSE TO AGING. R. Farrar*, L.J. Chandler*, E.M. Barr, W.W. Spirduso, and S.W. Leslie. (SPON: A. Baylor). Dept. of Physical Education and Division of Pharmacology, College of Pharmacy, The University of Texas at Austin, Austin, Texas 78712.

Synaptosomes were isolated from cerebral cortex of 3, 18, and 24 month old male, Fisher 344 rats and $^{45}Ca^{++}$ uptake was measured at 1, 3, 5, 15 and 30 second time periods following 65mM KCl depolarization. Identical experiments were performed in which 5mM KCl was added to examine age-related changes in resting $^{45}Ca^{++}$ accumula- tion by synaptosomes. Both "fast-" and "slow-phase" voltage- dependent $^{45}Ca^{++}$ uptake were significantly reduced in synaptosomes from 18 to 24 versus 3 month old rats. No age-related change in resting (5mM KCl) $^{45}Ca^{++}$ accumula- tion was observed. ATP-dependent and respiration-linked $^{45}Ca^{++}$ uptake was examined in mitochondria isolated from whole brains of 3 and 28 month old male, hooded Long Evan rats. Both ATP-dependent and glutamate-malate- ADP stimulated $^{45}Ca^{++}$ uptake by mitochondria were markedly reduced in response to aging. Respiratory control ratios were the same for 3 and 28 month old mitochondria, suggesting that the decrement in $^{45}Ca^{++}$ was not caused by an age related decline in respiratory activity of mitochondria. The results of this study show that both voltage-dependent calcium entry into presynaptic nerve terminals and calcium uptake by mitochondria in brain decline with advanced aging. Age-related changes in cytosolic calcium levels could underly, at least in part, cellular decrements in brain observed with aging. (Supported in part by NIAAA grant AA05809, RSDA AA00044 to Leslie and NIH AG02071 to Spirduso and Farrar).

132.4 EFFECTS OF EXERCISE ON REACTIVE CAPACITY AND ^{3}H-SPIPERONE BINDING IN YOUNG, MIDDLE AGED, AND OLD F344 RATS. W.W. Spirduso, P.E. Gilliam, R.P. Farrar, M. Ardies, and R.E. Wilcox[a]. Dept. Physical and Health Education and Dept. Pharmacology[a], U. of Texas, Austin, Texas, 78712. F344 males (N=144) 6 mos of age were shaped 7 days on a reactive capacity (RC) task, in which the animal released a lever as quickly as possible to avoid being shocked. A conditioned stimulus (CS; buzzer) preceded an unconditioned stimulus (shock) by CS-UCS intervals of 1000, 500, 300, and 200 msec. Following initial RC shaping, the rats were divided into 2 groups (N=72). One group ran on a motor driven treadmill 20 M/min, 0.0 grade, 30 min/day, 5 day/week. Animals failing to maintain this pace were carried back to an electric grid and the rear of the belt and received a mild shock. The remaining animals constituted a pair-fed sedentary group which was also "yoked" to the running group in terms of daily shocks received. Average amounts of shock delivered were one shock every 6 min for 30 min. One control group, receiving neither running nor shock, was tested at 6, 12, 18, and 24 mos of age.

RC tests consisting of 7-day 50 trial sessions were repeated every 6 mos for available animals. At 12, 18, and 24 mos, a control-shock group and an exercise group were sacrificed and tissue from the striatum of each rat was assayed by ^{3}H-spiperone binding. RC was not significantly different at 6 and 12 mos of age, but RC of control-shock animals was significantly faster than that of runners at 18 and 24 mos. The difference between the running and control-shock groups was increased at each age, and the between-group differences in ^{3}H-spiperone binding were inversely related to age. As reported in Sprague-Dawley rats (Gilliam et al., 1984), moderate running at a young age increases ^{3}H-Spiperone binding. However, at older ages this same intensity in F344 rats, which represents a higher percent of maximum work capacity, was accompanied by decrements in both RC and ^{3}H-Spiperone binding. It is hypothesized that the stimulating effect of systematic but escapable shock experienced by the control-shock groups, interacts with an age-related increase in exercise-induced stress to produce the behavioral and binding changes seen with age.

Sponsored by Grants to W.W. Spirduso and R.P. Farrar., AG02021, R.E. Wilcox and W. Riffee, MH 33442.

132.5 AGE EFFECTS AND CIRCADIAN PERIODICITY IN LOCAL CEREBRAL GLUCOSE UTILIZATION IN OVARIECTOMIZED FEMALE RATS. P.M. Wise[1], I.R. Cohen-Becker*[1], N.G. Weiland*[1], R.C. Walovitch[2], and E.D. London[2]. [1]Dept. of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201 and [2]NIDA Addiction Research Center, Baltimore, MD 21224.

Previous reports have demonstrated age-related declines in local cerebral glucose utilization (LCGU), an index of cerebral function, in male Sprague - Dawley and Fischer - 344 rats (Smith, C.B. et al., Brain 103:351, 1980; London, E.D. et al., J. Neurochem. 37:217,1981). Cyclic reproductive function is dependent upon normal circadian periodicity and declines with age. Therefore, we studied the effects of age on daily circadian rhythms of LCGU, with particular emphasis on the hypothalamic areas involved in LHRH release.

Studies were performed in young (3-4 mo) and old (18-21 mo) female Sprague-Dawley rats, which were ovariectomized 9 days before further studies. On the day before LCGU measurement, the external jugular vein was cannulated to the level of the right atrium, and the cannula was externalized through the nape of the neck. LCGU was measured by the autoradiographic [^{14}C] 2-deoxy-D-glucose ([^{14}C DG]) method (Sokoloff, L. et al., J. Neurochem. 28:897, 1977) in undisturbed, unrestrained rats, using standard values for the lumped constant and rate constants for the uptake and phosphorylation of [^{14}C DG]. Studies were performed at 1400h and 2200h (lights on at 0400h and off at 1800h).

Assuming that age per se does not alter the value of the lumped constant for the calculation of LCGU, age-associated declines were observed in brain regions including cortical areas, the corpus striatum, amygdala, and hippocampus. The following hypothalamic nuclei also showed LCGU decrements: medial preoptic, supraoptic, suprachiasmatic, paraventricular, and arcuate. Circadian periodicity in LCGU was observed in the suprachiasmatic nucleus and pineal gland of young and old animals.

Although these preliminary findings indicate that circadian periodicity of LCGU is not absent in the aged brain, decrements in LCGU in specific hypothalamic nuclei important to LHRH release may contribute to declining reproductive function. (Supported in part by NIH AG-00168 and AG-02224 to PMW).

132.6 LATERAL DIFFUSION OF MEMBRANE LIPIDS CHANGES WITH AGING IN MOUSE DORSAL ROOT HANGLION NEURONS. H.Horie,Y.Kawasaki* & T.Takenaka. Dept. of Physiol. Sch. of Med. Yokohama City Univ., Yokohama, 232 Japan and Mitsubishi-Kasei Institute of Life Sciences, Tokyo, 194 Japan.

The difference of effects of fibronectin on cell survival and differentiation between newborn and adult DRG neurons suggests that the membrane structure and function of DRG neurons might change with aging(Horie,H. & Kim,S.U.,Brain Res.,294,178,1984). It is thought that the membrane fluidity of DRG neurons might also change with aging. We measured the membrane fluidity of DRG neurons by Fluorescence Photobleaching Recovery(FPR) method.

Dorsal root ganglia from 1-3-day-old mice(N-DRG) were dissociated in 0.25% trypsin and those from 45-60-day-old mice(A-DRG) were dissociated in 0.25% collagenase. The cells were seeded onto polylysine coated coverslips(PL) or polylysine and fibronectin double coated coverslips(PL-FN). These cells were incubated for 10 min at 37°C in Ham's F12 medium containing the fluorescent analog of fatty acid,5-(octadecylthiocalbamoylamino) fluorescein,F18. F18 labels specifically living cell surfaces. The lateral motion of the labeled cell surface component was measured by the FPR method. The fraction of recovery,f, was defined as : f=(recovered fluorescence intensity)/(reduced fluorescence intensity by bleaching). In N-DRG neurons seeded on PL-FN in F12 containing 10% fetal calf serum(FCS) the lateral diffusion coefficient of F18 at 30°C was $0.21 \times 10^{-8} cm^2/sec$ after 20 hr in culture and $0.21 \times 10^{-8} cm^2/sec$ after 44 hr in culture. The mobile fraction,f,was about 0.90 after 20 hr in culture and 0.88 after 44 hr in culture. In A-DRG neurons seeded on PL-FN in containing 10% FCS, the lateral diffusion coefficient of F18 at 30°C was $0.12 \times 10^{-8} cm^2/sec$ after 20 hr in culture and $0.13 \times 10^{-8} cm^2/sec$ after 44 hr in culture. The mobile fraction was 0.84 after 20 hr in culture and 0.77 after 44 hr in culture. When both the neurons were cultured in F12 alone medium, the values of the lateral diffusion coefficient of F18 were same as those cultured in 10% FCS containing medium and the values of the mobile fraction of N-DRG and A-DRG neurons seeded on PL-FN coverslips were larger than those seeded on PL coverslips

These results suggest that the lateral diffusion of membrane lipid of DRG neurons decreases with aging and it might relate to the change of the capability of differentiation with aging. Fibronectin improved the mobile fraction of newborn and adult neurons and it might depend on improvement of cell survival by fibronectin.

132.7 AGE RELATED CHANGES IN ORBITOFRONTAL POLYSENSORY CORTEX IN THE ADULT BABOON (PAPIO PAPIO). L.A. Benevento, G.D. Pappas and Liu Hepei*[1]. Department of Anatomy, University of Illinois College of Medicine, Chicago, IL 60612.

We studied the postmaturational development of the adult baboon (2 to 30+ years old) orbitofrontal association cortex. Orbitofrontal cortex receives convergent polysensory inputs and is implicated in neuronal mechanisms underlying cognition and attention (Benevento, L.A., et al. Exper. Neurol. 57:849, 1977)-functions which alter dramatically with age. We observed that the small to medium pyramidal cells of layers II, III, V, and VI, which participate in the integration of both inhibition and excitation resulting from different modalities, do not show obvious morphological age-related changes. Thus, we undertook a morphometric study of these neurons and the surrounding neuropil in order to determine possible morphological correlates of aging.

While there is no significant overall decrease in the number of small and medium pyramidal cells in either young or old adults, all neurons show a decrease in synapses with age. Although the percentage of the surface of the perikarya covered with synapses is the same in young and old brains, the cell bodies do become 36% smaller, so that there is a 36% decrease in the number of axosomatic synapses. In the neuropil the number of synapses per unit area decreases 10%. This 10% decrease in synapses occurs even though there is 38% more membrane per unit area of neuropil in older animals due to the much finer caliber of neuronal and glial processes. Apparently, the development of finer neuronal aborization of dendrites and, perhaps axons, continues postnatally through old age.

Observations which have implications for metabolic changes with aging were made in the cytoplasm and nucleus. For example, in the young, the number of nuclear pores varies about 10%. In the old, there is an overall 66% decrease in the number of pores, while the area of the nuclear envelope has decreased only 36%. This means that the nuclear-cytoplasmic pathway for macromolecules is probably curtailed. This, in turn, may decrease the ability of the neuron to maintain its dynamic synthetic functions with age.

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132.8 RELATIVE STABILITY OF PIRIFORM CORTEX IN AGING RATS CONTRASTS WITH CHANGES IN OLFACTORY BULB AND OLFACTORY EPITHELIUM. C.A. Curcio, N.A. McNelly,* and J.W. Hinds. Dept. Anatomy, Boston U. Sch. Med., Boston MA 02118

A series of studies from this laboratory has documented growth and atrophy of the olfactory bulb (OB) and olfactory epithelium (OE) of the Sprague-Dawley rat from maturity to senescence. Major events occurring in these structures include changes in the volume of mitral cells (MC) and changes in the number of septal olfactory receptors. These effects are large, consist of a growth phase followed by atrophy, and are temporally related such that events in the OE precede those in the MC. A transneuronal degeneration hypothesis of aging would predict that loss or atrophy would be similarly transmitted to the next synaptic station in the olfactory pathway; therefore MC terminal fields in the piriform cortex (PCx) were studied in aging rats.

Two animals each at 3, 12, 18, 24, 27, 30, and 33 mo of age (50% survival=30 mo) were perfused with mixed aldehydes. Alternating 50 and 100 µm Vibratome sections were cut through the PCx. The 50 µm sections were Nissl-stained and used for determining the volume of a defined part of layers Ia and Ib and the lateral olfactory tract (LOT). The 100 µm sections were processed for electron microscopy (EM). From EM montages spanning the width of layer Ia and the extent of layer II, the following parameters were measured: numerical (Nv) and surface (Sv) density of synapses (originating primarily from OB) and volume fraction (Vv) of neuropil components in layer Ia, and Nv and size of layer II neurons. Data were collected from coded tissue and were analyzed with ANOVA and regression methods.

No significant age effects were found for laminar volumes, although LOT volume showed an upward trend. Both Nv and Sv of synaptic apposition zones were stable. A modest (18%) but significant decline in Vv of dendrites and spines in Ia was mirrored by an increase in Vv of glial processes; no change in Vv of axons and terminals was observed. Neither nuclear volume, soma volume, nor Nv of layer II neurons changed with age, although the incidence of nuclei containing filamentous lattices increased four-fold.

Thus, contacts made in the PCx by MC axons remain relatively stable in senescence, despite the marked volumetric changes in the MC somata. Age-related dendritic regression in layer II neurons may be attributable to a factor other than deafferentation. However, functional deafferentation due to reduced input to MC remains a possibility.

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132.9 REORGANIZATION OF ULTRASTRUCTURE IN AGING RAT HIPPOCAMPUS: A STEREOLOGICAL ANALYSIS. M.D. Applegate*, G. Campbell* and P.W. Landfield (SPON: M. Levitt). Dept. of Physiol. & Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103.

Prior studies on synaptic density in aged mammalian brain have yielded conflicting results, even in the same brain structures. However, not all quantitative studies have corrected for synaptic size (which affects density measures) and only a few have analyzed the volume fractions of other major elements in the neuropil. To further clarify these issues, we conducted an extensive stereological analysis of a strictly-defined region in stratum radiatum of field CA1 of the hippocampus.

Young-mature (6-7 mo-old) and aged (27-29 mo-old) rats were intracardially perfused, and transverse sections of hippocampus were embedded in Epon according to standard procedures. The blocks were very carefully trimmed to permit cutting of ultrathin and semithin sections from a similar restricted region of field CA1 in each animal. Micrographs were printed at a final magnification of 32,000x and represented 850 μm^2 of stratum radiatum per animal.

Stereological analyses were conducted blind using the point counting method. Points were classified as axon terminal, spine, dendritic shaft, glial or axonal areas, with several subdivisions of these categories. Synaptic densities were counted and their lengths measured separately for use in numerical density correction formulae.

The results show a decline in synaptic density, and a decrease in total presynaptic terminal volume. There were few if any changes in dendritic shaft or axonal area. The decrease in terminal volume was countered by a concomitant increase in glial volume.

To date, therefore, the primary ultrastructural aging changes found in hippocampal neuropil are presynaptic. These alterations could be due either to synaptic degeneration or to an increase in overall size of the hippocampus. However, increases in CA1 dimensions are inconsistent in our semithin studies, or in volumetric studies (cf, West and Coleman, Soc. Neurosci. Abstr., 1983). In addition, we have noted astrocytic profiles containing what appear to be degenerating terminal elements. Thus, synaptic degeneration may contribute to synaptic density decline in aged rat hippocampus.

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132.10 BEHAVIORAL ALTERATIONS IN AGED CATS. R.L. Lloyd* and M.S. Levine. (SPON: S.S. Soltysik). Mental Retardation Research Center, UCLA Los Angeles, CA 90024.

These experiments were designed to assess some of the behavioral changes that occur in aged cats. Three areas were examined: motor activity, reactivity to auditory stimuli and spatial reversal learning. Three groups of cats of differing age were tested: 1-3 yrs (N=17) 5-9 yrs (N=9) and greater than 10 yrs (N=15). Motor activity was assessed in an open field gridded into squares by a series of photocell beams. Significant decreases in motor activity occurred in cats over 5 yrs of age compared to animals 1-3 yrs of age. Average activity scores (counts /3 min) decreased from 61±7 (S.E) in 1-3 yr-old cats to 29±6 in 5-9 yr-old cats and 36±7 in cats over 10 years of age. There were no significant differences between 5-9 yr and cats greater than 10 yrs. Reactivity to auditory stimuli was assessed by determining the responses to taped cat vocalizations. Each animal received two sessions spaced 24 hrs apart. Reactivity to each vocalization was rated on a 6 point scale. All cats displayed similar patterns of initial reactivity. However, with repeated stimulus presentations cats over 10 yrs of age showed significantly less habituation than the other cats. During the second session all cats over 5 yrs of age displayed significantly less habituation than cats 1-3 yrs old. Spatial reversal learning was assessed in a walk through T maze. Initially cats were given 50 trials of free choice in which responses to either side of the maze were reinforced to determine the position preference of the animal. On subsequent trials animals were reinforced only for responses to the side opposite their position preference until criterion performance was reached. The reinforced side was then reversed. This procedure continued for 5 reversals. Cats 1-3 and 5-9 yrs of age had considerable difficulty performing reversals and made many errors (84±8 mean±S.E and 64±9 respectively). In contrast, cats over 10 yrs of age readily reversed and made significantly fewer errors (39±4).

The results of these experiments indicate that marked behavioral changes occur in aged cats. Hypoactivity appears to begin before 5 yrs of age and continues to occur after 10 yrs. Hyperreactivity begins between 5-9 yrs of age and is most apparent after 10 yrs of age. Lack of perseveration during spatial reversals appears to occur after 10 yrs of age.

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132.11 NEUROPHYSIOLOGICAL AND MORPHOLOGICAL ALTERATIONS IN CAUDATE NEURONS IN AGED CATS. M.S. Levine, J.S. Schneider, R.L. Lloyd*, C.D. Hull, A.M. Adinolfi, J.P. Villablanca*, M.K. Boylan* and N.A. Buchwald. Mental Retardation Research Center, UCLA Los Angeles, CA 90024.

These studies were designed to assess some of the morphological and neurophysiological alterations that occur in caudate (Cd) nucleus neurons in aged cats in order to understand how information processing in this nucleus is affected during aging. Extracellular unit recordings were performed in awake chronically prepared animals. The ability of Cd neurons to encode spatial somatosensory information was assessed. To date, recordings have been obtained from a population of 203 cells in the head of the Cd nucleus of two cats over 10 yrs of age and compared to similar data obtained from cats 1-3 yrs of age (2 cats, 130 units). Neurons in the aged cats were less responsive to somatosensory stimulation than those in 1-3 yr old cats. Only 13 neurons (6%) responded to sensory stimulation of the face. In 1-3 yr old cats about 25% of the cells respond to such stimulation. In the aged cats "receptive fields" were moderate (front muzzle or vibrissa field area) or large (entire face) in size while in 1-3 yr old cats most receptive fields were smaller and tended to be restricted to the front part of the face. Thus, Cd neurons in aged cats demonstrate a marked decrement in the ability to encode somatosensory information. In a series of morphological experiments computer assistance was used to reconstruct and quantify dendrites of medium-sized Cd spiny neurons from silver impregnated material in cats 1-18 yrs of age (1-3 yr-75 cells, 8 yr-15 cells, 13 yr-30 cells, 14-15 yr-30 cells and 18 yr-30 cells). Marked age-related morphological changes occurred. Total dendritic length and average dendritic length/neuron increased from 1-3 yrs (4202±154 mean±S.E and 836±38 respectively) to 8 yrs of age (5156±218 and 920±56 respectively) and then decreased by 18 yrs (2458± 185 and 563±47 respectively). Radius of the dendritic field was maximum at 1-3 yrs (206±5) and then decreased monotonically until 18 yrs of age (140±6). Number of dendrites/neurons and number of branches/dendrite remained unchanged. There was a marked loss in dendritic spines on distal segments. Spine density decreased from .95±.04 spine/u in 1-3 yr old cats to .57±.05 spine/u in 18 yr old animals. These neurophysiological and morphological results indicate that marked changes in the ability of Cd neurons to process information occurs during aging in the cat.

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132.12 AGE RELATED NEUROPATHOLOGICAL CHANGES IN HUMAN SYMPATHETIC GANGLIA. A. Hervonen,[1], P. Helen*[1], J.E. Johnson, Jr.[2], H. Alho*[3], and S.I. Rapoport,[1]. Lab. of Neurosciences, NIA, Bethesda, MD 20205[1], Section on Exper. Morphol., Gerontol. Res. Ctr, Baltimore, MD 21224 [2] and Dept. of Biomedical Sci. Univ. of Tampere, SF 33101 Tampere, Finland.

The age related accumulation of signs of cellular degeneration has been well described in CNS neurons. Little attention has been focused however on the corresponding features in the autonomic nervous system, which is essential in maintenance of homeostasis of the aging organism. Age related changes in autonomic function have widespread consequences for remote parts of the body. The homogenous population of sympathetic postganglionic neurons is a useful model of neuronal aging.

Sympathetic ganglia from 28 patients (ages 16 to 92 years) without neurological disease were obtained from sympathectomies. Postmortem material from patients with Alzheimer disease was also studied. The cervical and thoracic sympathetic ganglia were treated for Thioflavine and Bielchowsky staining, for fluorescence histochemistry of catecholamines and age pigments and for electron microscopy.

The major age related changes in patients without neurological disorders were:(1) loss of cytoplasmic catecholamine fluorescence,(2) increase of autofluorescent pigment in the perikaryon,(3) shift of autofluorescence emission towards higher wavelengths,(4) hypertrophy of dendritic processes, and (5) neuroaxonal dystrophy. Electron microscopy demonstrated that the aged neuron contained bundles of neurofilaments, mitochondrial accumulations and a variety of inclusion bodies. Neuritic plaques or classical neurofibrillary tangles were not found.

Two patients with pathologically verified Alzheimer disease (age 75 and 87 years) showed some features not found in the same age group without the disease. First, cytoskeletal abnormalities resembling neurofibrillary tangles were found, and secondly, Lewy-bodies were frequent.

The degenerative changes were more variable at more advanced ages between individuals and within one sample. More material from patients with Alzheimer disease is needed to determine whether the changes are disease or age related. Autonomic neurons may demonstrate neuropathology in a primary neurodegenerative disease. The expression of the underlying disease can be different in central and peripheral neurons.

132.13 AGE—RELATED HEARING LOSS AFFECTS DENDRITIC SPINE DENSITY IN MOUSE NEOCORTEX: A GOLGI STUDY. M. D. McGinn, K. R. Henry and R. G. Coss*
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The neuronal organization of the brain has been found to alter during ageing. Neurons become varicose, lose dendritic branches and dendritic spines, and become swollen and distorted. While most of these effects may be intrinsic, our results suggest that in mice, at least, some neuronal effects of ageing are associated with reduced peripheral sensory acuity.

For Golgi study, 4 aged (460 days old) male CBA mice with good bilateral hearing were compared with 5 CBA mice of the same age and sex which had an approximately equal bilateral hearing loss. Four young adult (100 days old) CBA mice with normal hearing were also included. Thresholds in the young mice did not differ from the older CBA's with good hearing. In the old mice with hearing loss, the mean auditory thresholds were elevated at least 25 dB at 2 kHz and up to 60 dB at higher frequencies.

Apical dendrites of layer V pyramidal cells, within the auditory neocortex, which were well impregnated by the rapid Golgi method were selected for analysis. Forty micrometer long segments of these dendrites which lay within layer IV were drawn at high magnification with a camera lucida. Dendrite diameter and spine density were determined directly from these drawings. Some cortical neurons in older mice showed dendritic varicosities, loss of dendrites, and swollen and distorted somas. In this respect, there was little difference between the older animals with and without hearing loss. Dendritic spine density was reduced in both groups of older mice. In this case, however, mice with loss of hearing were more severely affected. Neurons were then divided into two classes, large diameter (>2.0 um) and small diameter (<2.0 um) apical dendrites, and spine density was averaged in each class for each animal. A two-way analysis of variance revealed a significant interaction between shaft diameter class and treatment condition ($F_{2,12}$=3.905, p=0.049). A comparison of simple main effects showed that the locus of the interaction was the group of old mice with good hearing. In these animals, spine density increased slightly on large diameter dendrites while spine density on small diameter dendrites dropped sharply ($F_{1,12}$=15.75, p=0.002). In the older mice with hearing loss spine density was reduced on both large and small dendrites. These results suggest that a diminution in sensory acuity may exacerbate intrinsic effects of ageing upon cortical neuronal morphology.

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132.14 DEFICIENT RETENTION OF APPETITIVE AND AVERSIVE LEARNING IN NEW ZEALAND BLACK MICE. C. M. Harris, M. J. Forster, K. C. Retz, N. Frantz*, and H. Lal. Dept. of Pharmacology, Texas Coll. of Osteopathic Med., Fort Worth, TX 76107.

Increasing serum titers of brain reactive antibodies (BRA) represent a correlate of aging in both humans and animals. Moreover, New Zealand Black (NZB) mice exhibit a much more rapid development of serum BRA titers than do C57BL/6J mice. Furthermore, the presence of high BRA titers and impaired avoidance acquisition in young NZB mice, aged C57 mice, and in young C57 mice following receipt of bone marrow cells from aged C57 mice suggests that BRA may gain access to CNS tissue and interfere with learning and/or memory processes by an immunological mechanism (Nandy et al., Life Sci., 33:1499, 1983; Spencer & Lal, Neuroscience Abs., 9:96, 1983; Nandy et al, Neuroscience Abs., 1984, submitted). In order to evaluate the extent to which the BRA-associated behavioral impairments represent deficient memory processing, retention performance of NZB/BlNJ and C57BL/6J mice was investigated in the present study. When tested on a one-way, directional active avoidance task, NZB mice aged 2-3 or 6-7 mo showed slower acquisition (to a criterion of 8 of 10 trials with avoidance) than like-aged C57 mice, although both strains showed equivalent criterion run performances. On 48-hr retention tests, performance of C57 mice deteriorated with age, NZB mice showing deficient retention relative to C57 mice within both age groups tested. Retention performance of young (2-3 mo) NZB mice was roughly equivalent to that of older (6-7 mo) C57 mice. To test the generality of retention deficits in 2-3-mo-old NZB mice, the two mouse strains were also tested for acquisition and retention of choice point discrimination in a water-reinforced T-maze task. Whereas both strains showed equivalent numbers of trials to the acquisition criterion (9 of 10 correct choices) on this task, NZB mice took fewer trials to 24-hr reversal acquisition than did C57 mice. The present studies demonstrate age-related retention deficits in C57 mice as well as "precocial" retention deficits in young NZB mice. The present findings also suggest that acquisition deficits of NZB mice may depend upon the essential nature and/or difficulty of specific task requirements. By contrast, retention failure in NZB mice may well prove common to a variety of learning situations. (Supported by National Institute of Aging Grant 1 RO-3 AG3623.)

132.15 INTRAHIPPOCAMPAL SEPTAL GRAFTS AMELIORATE LEARNING IMPAIRMENTS IN AGED RATS. F.H. Gage, A. Björklund, U. Stenevi*, S.B. Dunnett* and P.A.T. Kelly*. Department of Histology, University of Lund, S-223 62 Lund, Sweden.

Aging can result in naturally occurring brain damage, with anatomical, biochemical and behavioral changes which appear to be substantial yet selective. Recent advances in the localization and characterization of the age-related neurodegenerative processes and of the related Alzheimer's and Parkinson's deseases, have given rise to animal models which allow the investigation of basic etiologies and potential long term therapies for impairments associated with aging.

In this presentation we will focus on the functional deficits in learning and memory and the potential for grafts rich in cholinergic neurons to effect these behavioral impairments. Specifically, grafts of embryonic tissue, rich in cholinergic neurons, were implanted as a dissociated cell suspension into the depth of the hippocampal formation in aged rats with severe impairments in spatial learning abilities. 2 1/2 - 3 months after transplantation, the grafted rats, but not the non-grafted controls, were significantly improved in their performance in a spatial learning test. This was due, at least in part, to an improved ability of the grafted rats to use spatial cues in the task. In all animals the grafts had produced an extensive acetylcholine esterase-positive terminal network in the surrounding host hippocampal formation. Thus, we propose that the action of cholinergic neurons in the graft onto elements in the host hippocampal circuitry is a necessary, but perhaps not sufficient prerequisite for the observed functional recovery.

Intracerebral CNS grafting is a useful experimental tool for the investigation of recovery from brain damage. The basic findings of the present experiments suggest that grafts can provide the necessary requirements for functional recovery in the animals with age-related impairments.

132.16 AGE DIFFERENCES IN PERFORMANCE OF RATS AND MICE IN A 14-UNIT T-MAZE. D. Ingram*, E. Spangler*, J. Freeman* and W. Richards* (SPON: M. Heft). Gerontology Research Center, NIA, NIH, Baltimore City Hospitals, Baltimore, MD 21224

In a series of studies, Goodrick (cf. J. Gerontol., 27: 353, 1972) demonstrated the effects of aging on performance of rats in a 14-unit T-maze. The task involved food deprivation over several weeks. We have altered this protocol to assess age differences in performance of rats and mice in an automated, 14-unit T-maze involving footshock escape/avoidance. Male Wistar rats and C57BL/6J mice were given preliminary training in one-way active avoidance (US=0.6 and 1.0 mA for mice and rats, respectively) in a straight runway (1 m long). The criterion was 8/10 successful avoidances (CS-US interval=10 sec) across two consecutive 10-trial daily sessions. The day after meeting this criterion, each animal was provided the first of two 10-trial sessions in the 14-unit T-maze with the second session 24-hr later. The animal was required to traverse each quadrant of the maze within 10 sec to avoid a footshock (0.6 and 1.0 mA for mice and rats, respectively). Movement of the animal through the maze is detected by a series of infrared photocells, and these data are stored in a microprocessor. All age groups demonstrated learning in this task; however, the rate of acquisition was slower and the asymptotic level of performance was higher as a function of age in both rats and mice. The mean number of errors per trial for rats 6, 12, 18, and 24 mo of age was 5.4, 7.7, 7.4, and 11.2, respectively. This age difference was significant according to a one-way ANOVA, $F(3,20)= 6.53$, $p < .01$. The mean number of errors per trial for mice 6, 12, 18, 24, and 30 mo of age was 4.7, 5.1, 6.2, 6.5, 7.4, respectively, which was also a significant age effect, $F(4,33)= 2.80$, $p < .05$. Preliminary analysis of learning strategies revealed perseverative responses at certain maze locations in all age groups but which persisted longer in older groups. Results of additional studies indicated that intact vision was not important to maze performance as different age groups could learn in the dark. Still other findings suggest that retention can be demonstrated over several weeks, but preliminary analyses suggest the existence of age differences in long-term retention. Thus, our modified complex maze protocol appears to produce reliable age differences in the performance of rats and mice.

132.17 AN AGE-RELATED DECLINE IN FRONT-PAW SHOCK INDUCED ANALGESIA. R. J. Hamm* and J. S. Knisely* (SPON: R. M. Fay). Dept. of Psychology, Virginia Commonwealth U., Richmond, VA 23284.

The purpose of the present study was to examine the relationship between the age-related decline in neurochemical indexes of the opioid receptor system and the function of the endogenous opioid pain-inhibitory system activated by front-paw shock. Five-seven mo old (N=12), 15-17 mo old (N=13) and 22-24 mo old (N=11) rats were exposed to 90 sec of scrambled electric shock delivered to their front paws. Because the 15-17 mo old and the 22-24 mo old rats had approximately a 0.1 mA higher shock threshold than the 5-7 mo old rats, the 5-7 mo old rats were exposed to a 1.6 mA shock intensity to induce analgesia while the 15-17 and 22-24 mo old rats were exposed to a 1.7 mA shock. After shock termination, tail-flick latencies were measured at 0,1,2,4,6,8,10,12 and 14 min. Post-shock tail-flick latencies were converted into percent maximum possible effect. A 3(Age) x 9(Time) ANOVA of %MPE yielded a significant main effect of Age ($p<0.0001$) and Time ($p<0.0002$).

To assess opioid involvement, two weeks after the above procedure naloxone (10 mg/kg) was administered i.p. to rats in each age group. Fifteen min after this injection, rats were exposed to front-paw shock as decribed above. Separate 2(Naloxone-Saline) x 9(Time) ANOVA were calculated for each age group. These analyses revealed that naloxone significantly attenuated the analgesia produced from front-paw shock in the 5-7 mo old ($p<0.02$) and 15-17 mo old rats ($p<0.01$). Because front-paw shock did not produce an appreciable level of analgesia in the 22-24 mo old rats, the effect of naloxone was not significant in that age group.

These results clearly show that the endogenous pain modulatory system activated by front-paw shock declines in function with increasing age. In addition, the present results confirm that front-paw shock produces a form of analgesia that is mediated by the function of an endogenous opioid system. This research also suggests that there is a parallel between the age-related decline in neurochemical indexes of the opioid receptor system and the function of these receptors in producing analgesia in response to aversive stimulation.

132.18 ENRICHMENT FOR ONE HOUR A MONTH RESULTED IN ACCELERATED GROWTH IN ADULT GERBILS. MaryLou Cheal, Kathleen Foley*, and Robert Kastenbaum*. Department of Psychology and Adult Development and Aging Program, Arizona State University, Tempe, AZ 85287.

Brief periods of enrichment during youth and adulthood were predicted to have long-term effects on subsequent ontogeny. The underlying hypothesis derives from lifespan habituation theory and represents an attempt to determine if exposure to challenging enriching stimuli would prevent the loss of the ability to adapt to change in later life.

In a lifespan longitudinal study, simple behavioral and somatic measurements were recorded monthly from 31 male and 31 female gerbils. Following measurements, the gerbils were placed in a large interesting environment for one hour. At one month of age, the gerbils' small size and immature homeothermic mechanisms necessitated a laboratory-housed enrichment environment. Starting at two months of age, enrichment was in an outdoor desert setting that provided multiple stimuli with natural meaning for the animals.

The brief amount of enrichment (one hour per month) resulted in acceleration of the development of two systems: the ventral gland and the hind limb. The growth of the ventral gland was significantly accelerated in gerbils with enriched experience in comparison to controls (Age X Enrichment Effect: $F(4, 232) = 3.65$, $p < .01$). Because the ventral gland is known to be dependent on gonadal hormones, enrichment may be accelerating the age of sexual maturation.

Additionally, in male gerbils enrichment resulted in longer hind limbs from four to six months of age (Age X Sex X Enrichment Effect: $F(5, 290) = 4.86$, $p < .001$). The development of the hind limb paralleled development of locomotion, rearing, and jumping.

Whether this minimal exposure to enrichment will have lasting effects will be determined in future months. Data through twelve months of age will be presented.

133.1 LOCALIZATION OF SUBSTANCE P, SEROTONIN AND METHIONINE ENKEPHALIN IN SEXUALLY DIMORPHIC NUCLEI OF THE RAT LUMBAR SPINAL CORD. P.E. Micevych, A. Coquelin, and A.P. Arnold. Departments of Anatomy and Psychology, and Brain Research Institute, University of California, Los Angeles CA 90024.

The fifth and sixth lumbar segments of the male rat spinal cord contain two sexually dimorphic nuclei, the spinal nucleus of the bulbocavernosus (SNB; Breedlove and Arnold, 1980) and the dorsolateral nucleus (DLN, Jordan et al., 1982). These nuclei contain neurons which accumulate androgens and innervate muscles of the male perineum. Substance P (SP), serotonin (5-HT), and methionine enkephalin (M-ENK) have been observed innervating ventral horn motor neurons. We used antisera directed against SP, 5-HT, and M-ENK to examine the immunohistochemical distribution of these substances in adult male Sprague-Dawley rats. We compared their distribution in the SNB and DLN to the staining patterns observed in lumbar motor nuclei not thought to be sexually dimorphic, the retrodorsolateral nucleus (RDLN) and the ventral motor pool (VMP). Bound primary antibodies in free-floating 30um vibratome sections were visualized using either the indirect immunofluorescence or avidin-biotin peroxidase techniques. Both SP- and 5-HT-like immunoreactive (LI) fibers/terminals are in close apposition to perikarya and dendrites in all four nuclei. These two substances are also found along fibers coursing between the SNB and DLN. The SP-LI and 5-HT-LI fibers send collaterals towards the VMP. Within the DLN, 5-HT-LI fibers were preferentially distributed in the medial aspect, while SP-LI fibers course through the entire nucleus. Moderate levels of M-ENK-LI puncta were distributed around perikarya in the SNB and were concentrated along fibers coursing between the DLN and SNB. A very dense accumulation of M-ENK-LI was restricted to the medial aspect of the DLN. No M-ENK-LI was observed in the lateral DLN. M-ENK-LI was not found in either the VMP or RDLN. Thus, M-ENK-LI appears to be associated with sexually dimorphic nuclei in the rat lumbar ventral horn. Spinal transection at mid-thoracic levels profoundly depleted SP- and 5-HT-LI elements, indicating a supraspinal origin for the ventral horn SP- and 5-HT-LI fibers and/or terminals. The M-ENK-LI was not altered by transection, implying that the M-ENK-LI fibers/terminals may originate in local spinal interneurons.
Supported by the UCLA Medical School and USPHS grant HD15021.

133.2 ONTOGENY OF SEXUAL DIMORPHISM IN A RAT SPINAL NUCLEUS: II. FORMATION AND LOSS OF EARLY PROJECTIONS. D.R. Sengelaub and A.P. Arnold. Dept. Psychology, UCLA, Los Angeles CA 90024.

The medially located spinal nucleus of the bulbocavernosus (SNB) contains three to four times as many motoneurons in adult male rats compared to females. This large dimorphism in cell number is produced perinatally, principally by a differential cell loss. To determine the role the early projections of the SNB might play in the creation of the sexual dimorphism in cell number, horseradish peroxidase (HRP) was used to label SNB motoneurons retrogradely during the period in which the dimorphism in cell number emerges.

Injections into the SNB target muscles of either .05ul of 2% choleratoxin-HRP or a .25mm3 pledget of 20% HRP in polyacrylamide gel were made at embryonic (E) days 18, 20, 22, and postnatal days 1, 4, and 10. Spinal cords were processed with TMB and examined for the number and location of retrogradely labeled cells. At E18 and E20, the numbers of cells labeled in the SNB of males and females are equal, indicating that there are substantial initial projections of SNB motoneurons in both males and females. From E18 to E22 (the day before birth) the number of labeled SNB cells triples in males but only doubles in females. This sex difference in labeled cells increases postnatally and by postnatal day 10 injections of this size label approximately 80% of the motoneurons in the male SNB but fail to label any motoneurons in the female SNB.

SNB cell number increases from E18 to E20, and during this time labeled cells can be seen outside the SNB, in both the dorsolateral motoneuron column and midway between the two motor columns. The frequency of these ectopic cells is equivalent in males and females, and decreases over this period with few or none labeled by the day of birth. The morphology of these cells, their axon trajectories, and their coincidence in time with the increase in SNB motoneuron number, suggest that these labeled cells reflect a migration of cells medially into the SNB from the lateral column in both males and females.

These results indicate that the formation of early projections of the SNB in males and females is comparable and that the differential death of motoneurons and resultant dimorphism in cell number are not the result of a failure of female SNB motoneurons to send their axons to the periphery.
(Supported by USPHS grant HD15021).

133.3 ONTOGENY OF SEXUAL DIMORPHISM IN A RAT SPINAL NUCLEUS: I. HORMONAL CONTROL OF NEURON NUMBER. E. J. Nordeen, K. W. Nordeen*, D. R. Sengelaub, and A. P. Arnold, Dept. of Psychology, UCLA, Los Angeles, CA 90024.

In adult rats, the spinal nucleus of the bulbocavernosus (SNB) contains many more motoneurons in males than in females. Females given androgens during a 'critical' perinatal period have an increased number of SNB motoneurons as adults. Previous research shows that androgens do not regulate SNB neuron number by altering neurogenesis. Thus, we examined SNB ontogeny in males, females and androgenized females to determine if sex differences in SNB neuron number arise through androgenic regulation of motoneuron death and/or migration.

Between embryonic days (E)16-22 timed pregnant females (Sprague-Dawley) received either 1 mg/day of testosterone propionate (TP) in oil or were left untreated (E23 = day of birth = postnatal day (PN)1) . Pups born to TP-treated dams were cross-fostered to other lactating females and injected with 2 mg of TP on PN1, 3 and 5. Male, female and androgenized female (TP-female) pups were perfused at E18, E20, E22, PN4 or PN10 and lumbar spinal cords were embedded in paraffin, sectioned and stained with cresyl violet. All SNB motoneurons were counted and corrections were made for split nucleoli. Spinal cords were examined for the presence of degenerating cells within the SNB region.

In all groups, the SNB contains very few (60-80) recognizable motoneurons at E18, although motoneurons are obvious in other spinal nuclei. Between E18-E22 there is a pronounced increase in SNB motoneurons that is more dramatic in males and TP-females than in normal females. By E22, males and TP-females have significantly more SNB motoneurons than normal females (287 and 272 vs. 216). Between E22 and PN10, females lose twice as many SNB motoneurons as either males or TP-females. By PN10, males and females have approximately adult numbers of SNB motoneurons (219 vs. 55) and TP-females do not differ significantly from males (201). Degenerating cells can be seen in the SNB of all groups during the development of sexually dimorphic cell number, but are not present at PN10. At PN4, during the maximal decline in SNB cell number, females have more degenerating cells in this region than either males or TP-females.

These results suggest that sex differences in SNB neuron number arise principally through differential cell death during the early postnatal period. Perinatal androgen treatment increases SNB neuron number in females by attenuating this cell death. The sexually dimorphic increase in SNB motoneurons that occurs during the late prenatal period suggests that androgens may also influence prenatal cell death and/or motoneuron migration.
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133.4 THE ANDROGENIC INDUCTION OF SPINAL SEXUAL DIMORPHISM IS INDEPENDENT OF SUPRASPINAL AFFERENTS. Renata B. Fishman and S. Marc Breedlove, Department of Psychology, University of California, Berkeley, CA 94720.

The spinal nucleus of the bulbocavernosus (SNB) and its target muscles, the bulbocavernosus (BC) and levator ani (LA) are present in adult male, but not female, rats. These muscles are present in females at birth, but disappear shortly thereafter. Treatment of neonatal females with testosterone propionate (TP) masculinizes the SNB in terms of cell number, cell size, nuclei size, and maintenance of BC/LA musculature. It is not yet known where androgen exerts its primary effect to accomplish these morphological changes. Possible sites of hormone action include SNB motoneurons, BC/LA muscles, or supraspinal regions innervating SNB cells. The purpose of the present study was to determine whether or not such supraspinal afferents are necessary for the testosterone-induced masculinization of SNB system structures in females.

Sprague-Dawley female rats aged 0-12 hours received either a mid-thoracic spinal transection or sham operation. Either TP or sesame oil vehicle (.05ml) was injected s.c. immediately following surgery and again on the third day of life. At 30 days, rats were sacrificed and their spinal cords and perineal muscles were removed. There was no indication of regeneration of supraspinal afferents to the lumbar spinal cord. Spinal cords were transversely sectioned at 50 µm and stained with thionin. SNB cell size, nuclei size, cell number, and BC/LA muscle weights were measured.

Neonatal TP significantly masculinized SNB cell number, soma size, and nuclei size in both transected and sham operated controls. (Two-way ANOVAs, $p<.05$). However, there were no significant effects of transection on any of these indices ($p>.20$). The perineal muscles were present only in TP-treated females, but there was no significant effect of transection upon the weight of BC/LA muscles ($p>.20$).

	NUMBER SNB CELLS		SNB SOMA SIZE (μm^2)	
	TP	OIL	TP	OIL
Transected -	84.0 ±18.6	48.4 ±5.4	782.5±33.2	619.3±38.1
Sham -	80.1 ±4.7	43.3 ±7.4	747.3±37.4	593.3±37.2

These results demonstrate that supraspinal afferents are not necessary for the androgen-induced masculinization of the SNB system, suggesting that the site of hormone action may be either the SNB motoneurons and/or the muscles themselves.

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133.5 ULTRASTRUCTURAL CHARACTERISTICS OF CELLS OF THE MOTOR NUCLEUS OF V DURING DEVELOPMENT IN NORMAL AND HYPOTHYROID RATS. Y. Narayanan* and C.H. Narayanan. Dept. of Anat., LSU Sch. of Med., New Orleans, LA. 70119.

Previous work from our laboratory has shown that induced thyroid deficiency by the administration of 0.5% propylthiouracil to pregnant rats greatly impairs suckling behavior in the newborn. In prenatal stages, the frequency of mouth opening and closing which represent basic components of suckling behavior is greatly reduced in treated fetuses. Since two-neuron reflex arcs formed by the mesencephalic nucleus of V and the motor nuelus of V are involved in mouth opening/closing, we have examined the ultrastructural charcteristics of the motor nucleus of V of normal and hypothyroid rats in fetal and neonatal stages. Midbrain and rostral hindbrain regions were dissected out from selected cases of control and experimental animals, and the region of the pons close to the point of entrance of the trigeminal fibers marking the location of the motor nuelus of V was processed for electron microscopy.

Differences in cellular morphology between control and treated group of animals are recognizable as early as 20 days of gestation age. Neurons of control animals clearly show beginnings of NISSL organization, increase in cytoplasm, cytoplasmic organelles, neurofilaments and microtubules, while the neurons of the experimental group lack any such cytoplasmic organization. At three weeks after birth, the motor neurons in control animals are well differentiated, with well organized NISSL bodies surrounded by areas rich in microtubules, neurofilaments, mitochondria and well developed Golgi. The nuclues is vesicular, the karyoplasm consisting of a cottony matrix material with chromatic and perichromatinic particles suspended in it. The nucleolus is large, dense, spheroid and appears vacuolated with well differentiated pars granulosa and pars fibrosa. The motoneurons of the experimental group show a poor organization of NISSL, and a drastic reduction in cytoplasmic organelles, microtubules and neurofilaments. The cristae of mitochondria appear dilated and the Golgi complex is poorly represented. The nucleolus showed a clumped appearance, which seemed to be a constant feature of the experimental at all ages. The overall configuration, fine structural features of neurons of the motor nucleus of V are consistent with the view that thyroid hormones play an important role in selected aspects of neuronal differentiation. Supported by the National Institutes of Health-NICHD. HD12064.

133.6 ESTROGEN MASCULINIZES THE PATTERN OF ANDROGEN ACCUMULATION IN THE BRAIN OF A SONGBIRD. K.W. Nordeen*, E.J. Nordeen, and A.P. Arnold. Dept. of Psychology, UCLA, Los Angeles, CA 90024.

In zebra finches, sex differences in vocal ability parallel sex differences in the morphology and hormone accumulation of several song related brain nuclei. If females (who do not normally sing) are given estrogen shortly after hatching, the morphology of song control areas is masculinized. Androgens given to these females in adulthood promote further growth in song regions and induce male-typical vocalizations. In the present study, we determined whether neonatal estrogen also masculinizes the pattern of androgen accumulation in two of these brain regions, hyperstriatum ventrale pars caudale (HVc) and magnocellular nucleus of the anterior neostriatum (MAN).

Newly hatched female zebra finches were implanted subcutaneously with silastic ropes containing either 25 ug of estradiol (E2) or cholesterol (Ch). At 3-6 months of age, 4 E2-treated and 4 Ch-treated birds were gonadectomized. The following day each bird was injected with 20 ng/gm body weight of tritiated dihydrotestosterone in 95% ethanol. The animals were decapitated 1 1/2 hours later, and the brains were processed for autoradiography. Sections were exposed for 4, 6 or 8 weeks and stained with thionin. At least 150 cells/animal were analysed for each nucleus. The number of grains over a cell was counted and compared with the expected number of grains calculated from the cell's area and the background grain density. The Poisson criterion was used to discriminate labeled from unlabeled cells.

The percentage of labeled cells in MAN and HVc was significantly greater in E2-females than in controls. Cells also tended to be more densely labeled in E2-females than in Ch-females. In MAN, 49% of the cells were labeled in E2-females whereas 18% were labeled in Ch-females. In both groups large cells were more likely to be labeled than small cells. In HVc, 36% of the cells were labeled in E2-females and 11% in Ch-females. In this nucleus, there was no relationship between cell size and androgen accumulation.

Estrogen influences the development of cells that accumulate androgens in adulthood Estrogens either increase the survival of androgen accumulating cells or regulate androgen accumulation within these cells. In either case, this estrogenic regulation probably contributes to sex differences in the neuroanatomical and behavioral response to androgens in adulthood.
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133.7 NEUROGENESIS IN THE MATE-CALLING CIRCUIT OF XENOPUS LAEVIS FROGS: A ^{3}H-THYMIDINE STUDY. D.L. Gorlick and D.B. Kelley. Dept. of Biological Sciences, Columbia University, New York, NY 10027.

Mate-calling by male Xenopus laevis is controlled by a circuit of six brain nuclei: VS(ventral striatum), APOA(anterior preoptic area), VT(ventral thalamus), DTAM(dorsal tegmental area of the medulla), Ri(nuclei of the inferior reticular formation), N.IX-X(motor nucleus of cranial nerves 9 and 10). Two of these areas (DTAM and N.IX-X) are known to be sexually dimorphic in frogs. The developmental origins of sex differences in neuron cell number are not known. As a first step, we used thymidine autoradiography to investigate the birthdates of neurons in the calling circuit. Individual X. laevis were injected with a single pulse of ^{3}H-thymidine (1 μCi/g) at stages 11 (gastrula) through 64 (just prior to metamorphosis). Animals were sacrificed at metamorphosis (stage 66) and brains processed for autoradiography. Three classes of birthdates were observed: 1) Early; Cells in VS were all born during a brief period from stage 11 through stage 25, 2) Prolonged; Cells in VT, Ri and N.IX-X were born over a protracted period from stage 11 through stage 50 - the number of labelled cells peaked in VT at stages 43-44 and in N.IX-X at stages 26-28, 3) Late; DTAM and APOA were born during short periods near the end of development - DTAM between stages 49 and 56 and APOA from stage 49 through stage 64.

Neurons in the calling circuit concentrate steroid hormones in adults and juveniles. The sexual dimorphism of the larynx, to which N.IX-X cells project, is determined by androgen secretion in the male. Hormones may also play a role in the development of sex dimorphism in the central nervous system, possibly by influencing cell proliferation or death of neurons that effect vocal behavior. Our results show that there are differences in the times of origin of calling circuit areas. Results of this study are being used to guide our investigations into the mechanisms by which sex differences in the brain develop.

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133.8 SEXUAL DIFFERENTIATION OF THE GERBIL BRAIN MAY INVOLVE SELECTIVE CELL DEATH IN FEMALES. C. Ulibarri* and P. Yahr. Dept. Psychobiology, University of California, Irvine, CA 92717.

Gerbils have a sexually dimorphic area (SDA) between the preoptic area and the anterior hypothalamus. In males, the SDA is hook-shaped and contains a dense cell group, the pars compacta (SDApc). In females, the SDA is ovoid and lacks an SDApc. This research focuses on the normal development of the SDA and the role that gonadal steroids play in that development.

Brains of male and female gerbils of various ages were sectioned and thionin-stained. At birth (Day 1), both sexes have an SDApc, but the rest of the SDA is not visible. By Day 10, the female SDApc has disappeared, but the male SDApc has enlarged. The rest of the SDA is visible but ovoid in both sexes. By Day 15, the male SDA is hook-shaped. The female SDA remains ovoid. This dimorphism becomes more pronounced as the animals mature. Postnatal regression of the SDApc in females may be due to selective cell death. The testosterone (T) secreted by the developing male may promote the survival of the SDApc cells.

To test this, males were castrated on Day 1 or 2 and females were injected with 50 or 100 µg T propionate (TP) on Day 2. Controls received sham surgeries or oil injections. As adults, neonatally castrated males received ovarian transplants and neonatally androgenized females received implants of T. Animals were tested for scent marking behavior before and after adult hormonal manipulations. Brains were prepared as above. Both doses of TP masculinized marking behavior and prevented regression of the SDApc: 17 of 29 neonatally androgenized females had an SDApc. Males castrated on Day 2 scent marked at female levels, but 8 of 10 still had an SDApc. Their prior exposure to T apparently allowed SDApc development to proceed.

These studies suggest that sex differences in the SDApc develop through hormonal control of cell death and survival.

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133.9 TESTOSTERONE INCREASES DENDRITIC GROWTH OF NORMAL AND INTRAPARENCHYMALLY-TRANSPLANTED MEDIAL PREOPTIC NEURONS. R. P. Hammer, Jr. and G. W. Arendash. LNP,NIMH, Bethesda, MD 20205 and Dept. Biol., Univ. S. Florida, Tampa, FL 33620

We examined the effect of postnatal testosterone treatment on dendritic growth of medial preoptic area (MPOA) neurons in female rats, as well as MPOA neuron transplants from newborn male rats into newborn female rats. Rats given such transplants exhibit enhanced sexual behavior as adults (Arendash and Gorski, Science 217: 1276). After transplant surgery, recipients were given daily subcutaneous injections of testosterone propionate (200µg) or oil vehicle (5µl) for 5 days. When animals were 30 days of age, brains were removed and prepared for Golgi-Cox analysis. Sections were thionin counterstained to accurately identify intraparenchymal transplants which were well integrated into MPOA tissue with axons and dendrites traversing the host-transplant interface. Neurons from intact MPOA, intraparenchymal MPOA transplants, intraventricular MPOA transplants, and intact caudate were selected in 15 testosterone- and 17 oil-treated animals. For each neuron,the number of primary dendrites and the number of dendritic terminations were counted and the average soma diameter and the greatest extent of the dendritic array from the soma were measured.

Independent of testosterone treatment, the number of dendritic terminations, a measure of dendritic branching, was greater ($p<0.01$) in intraventricular MPOA transplant neurons than in intraparenchymal transplant or intact MPOA neurons. Testestorone increased ($p<0.05$) dendritic extent in intact MPOA neurons and in MPOA intraparenchymal transplant neurons, but not in intraventricular transplant MPOA or spiny caudate neurons. Testosterone increased ($p<0.01$) soma size only in intact MPOA neurons, but did not alter the number of primary dendrites in neurons from any region or transplant location.

Testosterone may cause increased dendritic growth by acting at MPOA steroid hormone receptors, thereby promoting increased protein synthesis and membrane production. Thus, caudate tissue, which contains few steroid hormone receptors did not show testosterone-dependent dendritic proliferation. The adult complement of primary dendrites in MPOA neurons is already present at birth (Hammer and Jacobson, Int. J. Devel. Neurosci. 2: 77), so neither testosterone treatment nor transplantation altered this parameter. The location of the transplant, however, significantly affected parameters of dendritic growth and branching.Such differential measures may illustrate trophic effects of the milieu into which dendrites grow.

133.10 ESTROGEN AND INSULIN STIMULATION OF NEURITIC GROWTH IN VITRO. C.D. Toran-Allerand, K.H. Pfenninger and L. Ellis. Ctr. Reprod. Sci. and Depts. of Neurol. and of Anat. and Cell Biol., Columbia Univ., New York, N.Y. 10032.

Exposure of cultures of the murine hypothalamus, preoptic area (POA) and cerebral cortex to estradiol elicits a striking enhancement of neuritic growth in estrogen receptor-containing explant regions (Toran-Allerand, C.D., Progr. Br. Res., 61, 1984, in press). We document here the morphological responses of estradiol-treated explants of the hypothalamus, POA, olfactory bulb and cerebral cortex to insulin. Explants of the E-17 murine hypothalamus/POA and fronto-cingulate cortex and of the E-18 rat fronto-cingulate cortex and olfactory bulb were placed on ammoniated collagen (Maximow assemblies) or polylysine (petri dishes) substrata, respectively, and exposed to the serum-containing media standard for our respective laboratories. Some media were also supplemented with insulin (10-50 ug/ml) and/or estradiol (50 ng/ml). Exposure of both types of cultures to estradiol plus insulin elicited a dramatic enhancement of neuritic growth, localized to estrogen receptor-containing regions. As early as 72 hrs in vitro, responsive outgrowth was characterized either by a dense, luxuriant growth of thick, radiating fascicles of exceptionally long neurites (collagen) or a dense halo of very fine radial neuritic outgrowth (polylysine) similar to that elicited by NGF in peripheral neurons. The dramatic growth response to estradiol/insulin far exceeds that seen when either hormone is added alone. The supra-physiological concentrations of insulin required suggest that it activates the receptor for a closely related molecule such as one of the insulin-like growth factors (IGF). Insulin and IGF have been shown to promote neuritic outgrowth in vitro. The sites and mechanisms of action (direct or indirect) of estrogen/insulin stimulation are not known at present. However, the dramatic acceleration and enhancement of the response to estrogen by high insulin levels suggests that estrogen's effect on the developing CNS may be additive to or, more likely, synergistic with that of an endogenous insulin-like factor whose receptors are particularly localized to brain regions containing estrogen receptors. Other hormone/growth factor synergisms have been reported for insulin/NGF (Rescio-Pinto et al., PNAS, 1984, in press) and T_3-dexamethasone/EGF (Hoath et al., Life Sci., 32: 2709, 1983). Supported by: NIH, NIMH, the Whitehall Found. and the Natl. Spinal Cord Injury Found.

133.11 STRUCTURAL AND FUNCTIONAL IMPAIRMENT OF ADRENERGIC INPUT TO INTRAOCULAR CEREBELLAR GRAFTS BY THYROID HORMONE DEFICIENCY. Michael Hall*, Åke Seiger, Ann-Charlotte Granholm*, and Michael R. Palmer. Dept. of Histology, Karolinska Institute, S104 01 Stockholm, SWEDEN and Dept of Pharmacology, Univ. of Colo. Med. School, Denver, CO 80262.

Embryonic cerebella were transplanted to the anterior eye chamber of normal and thyroidectomized adult rats. The cerebellar grafts received adrenergic innervation from the sympathetic ground plexus of the host iris. The density of adrenergic fibers innervating the grafts was reduced to half in the thyroidectomized group, without any effect on the fluorescence intensity or the morphology of individual nerve fibers. The reduction in adrenergic ingrowth was entirely prevented in grafts raised in thyroidectomized recipients which were substituted daily with l-thyroxine (100 ug/kg, s.c.). Electrophysiologically, there were no differences in spontaneous firing rates of neurons recorded extracellularly from cerebellar grafts in the two groups. However, both locally applied norepinephrine and isoproterenol were an order of magnitude less potent for causing inhibitions of spontaneous neuronal activity from transplants in thyroidectomized recipients as compared to controls. These sensitivity differences were obvious both when the catecholamines were applied by micro-pressure ejection as well as when they were superfused over the transplants. The thyroidectomy-induced catecholamine sensitivity changes could be prevented by chronically substituting animals with l-thyroxine, but returned upon cessation of this hormone treatment. It was concluded that thyroid hormone deficiency alters both the development and function of the adrenergic innervations of intraocular cerebellar grafts. (Dr. Seiger is a member of the European Neuroscience Association. This work was supported by Swedish Medical Research Council grants 14X-06555, 25P-6326, Karolinska Institutes Forskningsfonder, Magnus Bergvalls Stiftelse, the "Expressen" Prenatal Research Foundation and USPHS grants AA05915 and HD07072.)

133.12 DEVELOPMENT OF HYPOTHALAMIC AND NIGRAL DOPAMINERGIC SYSTEMS IN VIVO AND IN CULTURE. W.J. Friedman, C.F. Dreyfus, B.S. McEwen and I.B. Black. Rockefeller Univ. and Cornell Univ. Med. Coll., N.Y., NY 10021.

Development of dopaminergic neurons in the embryonic mouse substantia nigra and basomedial hyothalamus was studied by monitoring tyrosine hydroxylase (TH). Catalytic activity and immunocytochemical reactivity was initially detected in both systems on embryonic day 13 (E13) in vivo. TH activity increased approximately 8-fold in both areas between E13 and E16, and maintained plateau values through early postnatal life. To begin characterizing underlying molecular mechanisms, E15 explants from these two regions were grown in culture, in the presence of human placental serum, a heretofore requisite medium component. However, to begin defining the developmental role of extracellular factors, we substituted rat serum, which is easily manipulated, for the human serum. In fact, hypothalamus and nigra exhibited normal morphologic and biochemical ontogeny in rat serum. To examine the potential role of steroid hormones in development, serum was obtained from adrenalectomized and ovariectomized rats. Virtual absence of corticosterone, estrogens and androgens was confirmed by radioimmunoassay. Hypothalamic and nigral TH and morphology developed normally in the steroid-depleted medium, suggesting that physiologic levels of hormones were not necessary for normal brain dopaminergic ontogeny in vitro. We are presently employing this system to define effects of endogenous and exogenous agents on brain dopaminergic ontogeny.

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133.13 SEXUALLY DIMORPHIC DEVELOPMENT OF CHOLINERGIC ENZYMES IN RAT HIPPOCAMPUS. R. Loy. Departments of Anatomy and Center for Brain Research, University of Rochester, Rochester, NY 14642.

Removal of the septal afferent to the hippocampus elicits a sexually dimorphic ingrowth of sympathetic, noradrenergic axons into devervated regions. This suggested that the septo-hippocampal system may develop differentially in response to early gonadal hormone exposure. Loy and Milner (Birth Defects: Orig. Art. Ser., 19:417, 1983) found that acetylcholinesterase (AChE) staining of hippocampal interneurons matures earlier in female than in male rats. The present biochemical studies confirm and quantify this finding, and extend the effect to the synthetic enzyme, choline acetyltransferase (ChAT).

Sprague-Dawley (Charles River) rat pups were decapitated, the brains removed and the dentate gyrus (DG) dissected free of Ammon's horn (AH). Septal samples included medial and diagonal band nuclei. Frozen tissues were weighed, homogenized in water, and triplicate 2 ul samples assayed radioenzymatically for AChE and ChAT activities. On PND-1, 3, 5 and 18 the combined wet weights of DG and AH are greater in females than in males at all ages. On PND-90 (adults) only the DG weights remain greater in females. Specific activity of AChE is also higher in females on PND-1 and 5 in the DG, but not in AH. This sex difference is no longer apparent at older ages. In the septum, however, adult levels of AChE are 16% lower in females but are not different between the sexes at other ages tested. By contrast, ChAT levels in the DG are higher in females on PND-18, but not at younger ages; this sex difference is maintained in adults, where the specific activity in male DG is only 81% of females. There is no sex difference in ChAT activity in AH at any age. ChAT activity is also sexually dimorphic in the septum, with female levels 10-20% higher at PND-3, 5, 7, and 18, but not in adults.

As in the histochemical studies previously reported, AChE in DG is higher in females the first week after birth but not in adults. ChAT activity is higher in female DG during late development and in the adults. It is not clear from these data if the sex dimorphism in cholinergic enzyme activity occurs within the hippocampus or as a result of steroid action on the septal cell bodies. However, the findings that there is no sex differences in specific activity of either enzyme in AH, and that there is a sex difference in wet weight of DG, but not AH, favor a local regulation of cholinergic activity in the target cells of the dentate gyrus.

Supported by PHS Grant NS-20288. These experiments were performed with the assistance of R. A. Sheldon.

133.14 ONTOGENY OF IMMUNOREACTIVE-LUTEINIZING HORMONE RELEASING HORMONE (IR-LHRH) CONTAINING CENTERS IN THE BRAIN AND THEIR RELATIONSHIP TO SEXUAL MATURATION. L. Halpern-Sebold*, M. P. Schreibman and H. Margolis-Kazan*. Biology Department, Brooklyn College, Brooklyn, N. Y. 11210

The ontogeny of ir-LHRH centers in the brain was studied in sibling platyfish (Xiphophorus maculatus)"genetically programmed" to reach puberty at two ages (early maturers (E), 10 wks; late maturers (L), 26 wks) using immunocytochemistry, cytology and morphometry.

The results indicate a sequential development of 3 ir-LHRH areas in the brain that is directly related to stage of sexual maturation and not age. The nucleus olfactoretinalis (NOR) is first to contain ir-LHRH, followed by the nucleus preopticus periventricularis (NPP) and then the nucleus lateralis tuberis (NLT). This anterior to posterior sequence of development ("cascade effect") is similar in both genotypes except in L, ir-LHRH is never found in NLT perikarya, and specific steps occur in older animals and take longer to complete. The delay creates significant differences in cytometric, cytological, and immunocytochemical characteristics of the 3 regions in fish of the same age but different genotype.

Ir-LHRH perikarya first appear in the NOR at 5 wks (stage 1 gonopodial development) in E and at 11 wks (still stage 1) in L at a maximum number which remains constant into adulthood. Dimensions of the NOR and its perikarya (both genotypes) increase from stage 1 to 2 (initiation of sexual maturation) when ir-fibers appear between the NOR and the NLT and NPP. NPP ir-perikarya appear at stage 2 and are maximum at stage 6 (E have 50% more than L). In both genotypes NLT measurements increase to stage 2 and then decrease to stage 6. Ir-NLT fibers appear in stage 2 (E and L). NLT ir-perikarya soon appear in E but are never seen in L. L also have fewer ir-gonadotrops.

Our results also demonstrate a positive correlation between the number of ir-LHRH neurons and the number of pituitary gonadotrops in E and L from birth to adulthood. Supported by NIA (AGO-1938) and PSC-CUNY.

133.15 IMMUNOHISTOCHEMICAL LOCALIZATION OF HYPOTHALAMIC LHRH IN THE HOMOZYGOUS ATHYMIC (nu/nu) AND HETEROZYGOUS (nu/+) MOUSE. D. Bloom* and P. Micevych (SPON: E. Decima). Dept. of Psychology and Dept. of Anatomy, and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Congenitally athymic or neonatally thymectomized mice exhibit disturbances in reproductive functioning that include reduced levels of serum LH and FSH. Since exogenous GnRH can stimulate LH secretion in these animals, the reproductive disturbances may be the result of a defect in the hypothalamic LHRH system. The presence of the thymus during a critical perinatal period seems to be required for the development of a normal brain and neuroendocrine system. To investigate the possibility that the LHRH system has developed an abnormal distribution pattern in the athymic mouse, the LHRH-like immunohistochemistry (LHRH-LI) of this animal was compared to that of its heterozygous thymic littermate. Untreated and colchicine treated animals were perfused with 4% paraformaldehyde. Thirty-micron sections were incubated with antiserum raised against LHRH (R. Elde), and visualized by ABC immunocytochemistry. Heterozygous mice, whose endocrine functions are normal, have an LHRH-LI distribution similar to that reported for the rat: Fusiform perikarya are highly concentrated in the diagonal band of Broca, with an orientation that parallels the fiber tracts, and in the preoptic periventricular area, where the orientation has a dorsal-ventral axis. Fewer perikarya are seen in the septum, medial preoptic area, retrochiasmatic area, lateral hypothalamic area, bed n. of stria terminalis, and anterior hypothalamic area. A high density of LHRH-LI fibers is observed in the organum vasculosum, subfornical organ, and median eminence. Moderate concentrations are seen in the arcuate n., diagonal band of Broca, septum, medial habenula, stria medularis, medial preoptic n., nucleus and tract of stria terminalis, preoptic periventricular n., preoptic suprachiasmatic n., median preoptic n., areas dorsal to the optic chiasm, suprachiasmatic n., and fasciculus retroflexus. Fibers were highly associated with periventricular areas (III ventricle) and appeared to extend between the ependymal cells of the ventricular walls. The cell bodies and fibers of the LHRH-LI system in the athymic mouse appeared similar in all respects to those of the heterozygous animal. No significant differences could be observed in cell number, distribution, orientation, or gross cytological characteristics that could explain the reported endocrine disorders. Distribution of LHRH-LI in regions associated with the pituitary appeared normal.

133.16 NEUROTRANSMITTER MEASUREMENTS IN MICRODISSECTED HYPOTHALAMIC NUCLEI OF THE DEVELOPING RAT BRAIN. J. Kranzler, N. MacLusky*, C. Leranth*, C. Hurlburt*, and F. Naftolin*, Departments of Obstetrics and Gynecology and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

Numerous studies have attempted to clarify the biochemical changes which underlie the sex differences in physiology and behavior observed in the adult rat. Although sex differences in neurotransmitter levels, steroid receptors and protein distributions have been observed in adulthood, the mechanisms underlying the development of these differences remain unknown. Sexual differentiation is thought to result from the action of gonadal steroids on the brain during perinatal and early postnatal life. We have therefore adapted Palkovit's technique (Brain Res. 59:449, 1973) to the developing rat brain. Brains were mounted on chucks using a Gelfoam support, and frozen in dry ice. Alternate thin (20-40μm) and thick (180-280μm) frozen sections were cut at -20°C. The thick sections were kept frozen at -60Cwhile the thin sections were stained with cresyl violet for visualization of the landmarks required for orientation in the hypothalamus. Orientation in the anterior-posterior direction was documented by viewing the stained thin sections under a stereomicroscope. The positions of specific anatomic structures were confirmed using the atlases of Heller et al. (J. Neurosci Methods 1:41,-1979) and Sherwood and Timiras (U.C. Press, 1970). The medial preoptic area (MPOA), arcuate-median eminence (A-ME) and ventromedial nucleus (VMN) were then removed from the corresponding thick frozen sections using a needle with a 500 μm diameter. This technique has been applied to the measurement of neurotransmitter levels by high performance liquid chromatography (HPLC) in 12-day old rats, pooling tissue from two animals/sample and using a modification of the HPLC procedure of Anderson et al (J. Chromatog. 223:315,1981). RESULTS: Norepinephrine: MPOA 148 ± 20.5, A-ME 45.0 ± 5.2, VMN 32.5 ± 7.2; Dopamine MPOA 33.7 ± 9.1, A-ME 13.1 ± 2.8; VMN 5.1 ± 1.5; 5 HT: MPOA 16.1 ± 1.7, A-ME 16.2 ± 2.5, VMN 11.8 ± 0.9; 5-HIAA: MPOA 15.7 ± 1.3, AME 12.1 ± 1.7, VMN 9.4 ± 1.6. (Means ± SEM, N=7-9; in ng/mg protein). This method appears to offer the necessary sensitivity and anatomic resolution to evaluate the effects of gonadal steroids on neurotransmitter levels in discrete cell groups of the developing rat brain. (CL is a Mellon Fdn. fellow. Supported by HD13587 and GM 07205).

133.17 SYMPATHETIC MATURATION: ORGANIZATIONAL AND POSTORGANIZATIONAL EFFECTS OF TESTOSTERONE. J.E.Melvin and R.W.Hamill. Monroe Community Hospital/Univ Roch Med Ctr, Roch,NY 14603.

Previous studies indicate that hormonal factors regulate the biochemical maturation of sympathetic ganglia: tyrosine hydroxylase (T-OH),an index of noradrenergic ontogeny, fails to mature normally following castration at 10-11 days of age. The present studies extend these observations by examining the organizational and postorganizational effects of the hormone testosterone on the biochemical development of cholinergic and noradrenergic ganglion elements.

To examine the postorganizational role of testosterone, choline acetyltransferase (CAT) activity, a biochemical marker of presynaptic cholinergic maturation, T-OH and ganglion protein were examined in hypogastric ganglia from male Sprague-Dawley rats 1,2,4,6,8,10, and 12 weeks following castration on day 10. T-OH activity failed to develop normally by 1 postoperative week and never exceeded values observed at the time of castration. In contrast, both CAT activity and protein continued to develop following castration, but at diminished rates; both were significantly reduced at 1 and were 40% of control at 12 postoperative weeks. To investigate the organizational effects of testosterone, rats were subjected either to castration or sham operation within 12 hours of birth; sham operated and castrated controls received vehicle immediately following surgery; a third group received testosterone decanoate (20 mg/kg body weight); a fourth group received vehicle following castration but testosterone injections were initiated two weeks later. Testosterone treatment on day one fully restored T-OH and CAT activities; whereas delayed treatment only partially ameliorated the biochemial developmental deficits:

	Control	Veh Rx'd	Day 1 T-Rx'd	Day 15 T-Rx'd
T-OH(pmoles/g)	2288±326	73±32	2607±130	622±282
CAT(nmoles/g)	4.78±47	1.03±12	4.29±0.07	2.08±0.42

Postorganizational results suggest that testosterone is necessary for any further development of postsynpatic T-OH activity, while presynaptic CAT activity and total ganglion protein continue to develop at parallel diminished rates, possibly implying that the number of preganglionic synapses is related to total ganglion protein, an indication of neuron number and/or size. Our organizational studies indicate that a critical time exists during the first 2 weeks of life when testosterone must be present for either neuronal survival and/or a normal response to testosterone during the postorganizational phase of development.

133.18 EFFECTS OF DIHYDROTESTOSTERONE ON DEVELOPING SYMPATHETIC NEURONS AND SYNAPSES. L.L. Wright and A.J. Smolen. Boston University School of Medicine, Boston, MA 02115; and Medical College of Pennsylvania, Philadelphia, PA 19129.

Adult male rats have 20-30% more neurons in the superior cervical ganglion (SCG) than do adult females. This difference is not present at birth, indicating that the gender difference arises postnatally. Neonatal rats treated with testosterone propionate (TP) or 17-beta-estradiol (E) during the first two postnatal weeks have more neurons and synapses in their SCGs at 15 days of age than do vehicle treated littermates (Wright & Smolen 83a,b). To determine whether a non-aromatizable androgen would have a similar effect, dihydrotestosterone (80 ug/g) was suspended in oil and injected subcutaneously into male rats on alternate days from the day of birth until day 14. The animals were sacrificed and the SCGs removed on postnatal day 15, and the ganglia were processed for electron microscopy.

Numbers of neurons were counted from semithin Epon sections spaced 200 microns apart throughout the ganglia, and were corrected using the Abercrombie (46) method. There was no difference in the number of neurons in the vehicle control animals (27287, se = 1271) and DHT treated animals (25787, se = 1023). This is in contrast to the effects of treatment with TP (38508, se 1765) or E (54097, se 5731).

Synpases were counted from 30 grid squares from each ganglion, and the numbers extrapolated to determine synapses per ganglion. There was also no difference in the number of synapses between control (1.54×10^6, se = .35) and DHT treated animals (2.1×10^6, se = .31), while with the same course of treatment, TP or E increased the numbers of synapses significantly (Wright & Smolen, 83a,b).

Thus, the non-aromatizable androgen, DHT does not produce the same effects that TP or E does in the developing SCG, suggesting that the actions of testosterone may be via aromatization to estradiol.

This work has been supported in part by a grant from the Dysautonomia Foundation.

133.19 EFFECTS OF THYROID HORMONES ON DEVELOPMENT OF THE SYMPATHO-ADRENOMEDULLARY AXIS IN THE RAT. C. Lau, M. Franklin*, L. McCarthy*, A. Pylypiw* and L.L. Ross. Dept. of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

It is well-established that thyroid hormone plays a key role in development of the nervous system. In the rat, previous studies have shown that while functional connections between the splanchnic nerve and the adrenal medulla are not established until about 1 week postnatally, neonatal administration of triiodothyronine (T_3) accelerates the maturation of functional neurotransmission in the adrenal medulla. The present study aims to elucidate the underlying mechanisms with which T_3 may exert its effects. Neonatal rats were given T_3 (0.1mg/kg, s.c.) daily beginning 1 day after birth. Preganglionic innervation of the adrenal medulla was examined by retrograde axonal transport of horseradish peroxidase (HRP) using the tetramethylbenzidine method. At 8 days of age, 3% HRP conjugated with wheatgerm agglutinin was injected into the left adrenal gland and the pups were sacrificed 48 hours later. In both control and T_3-treated rats, HRP-labeled neurons were found in the ipsilateral intermediolateral (IML) cell column of the T1 through T13 segments of the spinal cord with the majority of the labeling located in the T6 to T10 segments. In control rats, a total of 1078±110 labeled cells were observed. In the T_3-treated pups there was a 60% increase of labeled neurons. To evaluate the effects of thyroid hormones on the cholinergic synaptic development in the adrenal medulla, activities of the marker enzyme choline acetyltransferase (CAT) were measured. In control rats, CAT activity was 0.9 nmol/gland/30 min at 2 days of age. This undergoes a rapid 4-fold increase in the ensuing 2 weeks. Hyperthyroid neonates exhibited a small but significantly persistent pattern of elevated CAT activity. These results suggest that the premature onset of neurotransmission in the sympatho-adrenomedullary axis of hyperthyroid rat is due in part to preganglionic hyperinnervation leading to precocious synaptogenesis. Concomitant with the premature onset of splanchnic-adrenal function in the hyperthyroid pups, deficits of adrenal catecholamine (CA) stores and CA biosynthetic capabilities were found. Studies in progress explore the basis of these adrenomedullary deficiencies and its relationship, if any, to the precocious neurotransmission.

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133.20 HORMONES AND GROWTH FACTORS INDUCE THE SYNTHESIS OF GLIAL FIBRILLARY ACIDIC PROTEIN IN RAT BRAIN ASTROCYTES R.S. Morrison, J. De Vellis, L.F. Eng and R.A. Bradshaw*. Dept. of Biological Chemistry, California College of Med., Univ. of California, Irvine, CA 92717.

Glial fibrillary acidic protein (GFAP) is the major constituent of glial filaments, and is restricted within the CNS to astrocytes. As with other classes of intermediate filament proteins, the regulation of GFAP expression is poorly understood. Utilizing highly purified cultures of astrocytes and a chemically defined (CD) medium, we have demonstrated that the expression of GFAP is subject to regulation by hormones and growth factors. The concentration of GFAP/mg protein was induced 2-4 fold in the presence of hydrocortisone, putrescine, prostaglandin F2α (PGF2α), and pituitary fibroblast growth factor (FGF). Augmentation of the levels of GFAP continued for up to 3 weeks after conversion to CD medium and paralleled the morphological maturation of astrocytes. The accumulation of GFAP resulted from an increase in its specific rate of synthesis. Conversion of astrocytes from serum-supplemented (SS) to CD medium did not alter its rate of degradation. GFAP appeared quite stable under both sets of conditions, exhibiting a half-life of approximately 7.5 days. The data demonstrate that astrocytes exhibit plasticity with respect to expression of GFAP, which may have implications for gliosis.

134.1 DIFFERENCES BETWEEN ON AND OFF GANGLION CELLS IN DARK-ADAPTED GOLDFISH RETINA. J. D. Nussdorf, M. Falzett and M. K. Powers. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

We recorded action potentials from single axons in the optic tract of awake, self-respiring goldfish under conditions designed to maximize sensitivity to light, and to resemble conditions of ongoing psychophysical tests in our lab. Stimuli were large diffuse monochromatic flashes one sec in duration, presented on a tangent screen to fully dark adapted fish.

We found that OFF neurons (N = 36) were generally more sensitive than ON neurons (N = 55). At 520 nm (near peak absorption of the rod photopigment), OFF cells were 0.65 log unit more sensitive than ON cells. Moreover, on average, OFF cells were within 0.5 log unit of psychophysical absolute threshold, and 35% of these cells equalled or exceeded psychophysical sensitivity. The difference in sensitivity between ON and OFF cells increased at longer wavelengths. At 650 nm OFF neurons (N = 10) were 1.13 log units more sensitive than ON neurons (N = 11). Even though ON cells were generally less sensitive than OFF cells, their spectral sensitivity matched that of the rod photopigment, while OFF cells did not. Instead, the OFF cells matched psychophysical spectral sensitivity, which is known to be mediated by both rods and long wavelength cones.

We conclude that at absolute threshold (1) OFF cells tend to be more sensitive than ON cells, (2) ON cells are rod-dominated, while OFF cells receive contributions from rods and cones, and (3) the OFF channel may mediate psychophysical absolute threshold. Finally, psychophysical methods have demonstrated that goldfish continue to discriminate wavelengths near absolute threshold. We speculate that the neurophysiological differences reported here between ON and OFF channels may be responsible for such wavelength discriminations.

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134.2 IMMUNOCYTOCHEMICAL LOCALIZATION OF A SYNAPTIC VESICLE-ASSOCIATED ANTIGEN IN THE DEVELOPING RAT RETINA. P.V. Sarthy and W. Bacon*. Department of Ophthalmology, Univ. of Washington, Seattle, Washington 98195.

In order to examine the appearance of synaptic vesicles and to correlate it with the formation of the synaptic layers, we have determined the staining pattern of a murine monoclonal antibody (Serum 48) to a synaptic vesicle-associated protein (Matthew et al., J. Cell Biol., 91:257, 1981) in developing rat retina. The antigen was detected by the indirect immunofluorescence technique using 20 μm cryostat sections of paraformaldehyde (4%)-fixed retinas. In the adult retina, the antiserum stained both the outer plexiform (OPL) and the inner plexiform layers (IPL). The nuclear layers and the nerve fiber layer (NFL) were devoid of any staining. In order to establish the association of the antigen with synapses, we examined localization of the antigen in a 45-day-old RCS-rdy rat retina in which the OPL is lost due to degeneration of the photoreceptors. We found reduced staining in the outer retina although staining in the IPL was unaffected. In prenatal and early postnatal (P) retinas, the antiserum stained two bands which corresponded to the respective locations of the NFL and IPL. Further, staining in the NFL increased until P-4 and began to decline subsequently, and by P-8 little staining was left in this layer. In contrast, in the IPL the intensity of staining increased gradually and leveled off by P-10/12. In the outer retina, a band of fluorescence corresponding to the OPL was first observed at P-5 and increased in intensity up to P-9/10. As expected from the developmental pattern of the retina, all bands appeared initially in the central retina and subsequently in the peripheral retina.

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134.3 PEPTIDERGIC GANGLION CELLS IN THE TURTLE RETINA W.D. Eldred, T. Isayama*, A. Reiner & R.E. Carraway*. Dept. Biology, Boston Univ., Boston, MA 02215; Dept. Anatomy & Cell Biology, Univ. Michigan, Ann Arbor, MI 48109; Dept. Physiology, Univ. Massachusetts Med. Sch., Worchester, MA 01605.

In recent years, many different neuropeptides have been localized within amacrine cells in the vertebrate retina, but not within ganglion cells. We have found numerous ganglion cells, of several specific types, in the turtle retina which apparently contain the neuropeptide LANT-6. These labeled neurons had axons, cell bodies in the ganglion cell layer, and dendritic arborizations in the inner plexiform layer which were either monostratified, bistratified, or diffuse.

The positive identification of these neurons as ganglion cells was approached in two ways. In the first set of experiments horseradish peroxidase was applied to the optic tectum in order to retrogradely label the cell bodies of retinal ganglion cells projecting to the tectum. These same preparations were than labeled with antisera directed against LANT-6 and processed using DAB-cobalt for the retrograde label, and DAB alone for the immunocytochemistry. The co-occurence of retrograde HRP granules and immunocytochemical labeling indicated that many, but not all, of the retrogradely labeled ganglion cells contained LANT-6-like immunoreactivity (LLI). In the second set of experiments several turtles were monocularly enucleated and the brain was examined for subsequent changes in LLI in the retinal recipient areas. All retinal recipient areas of turtle brain contain observable levels of LLI fiber staining. Following enucleation and at least one month survival, LLI was either eliminated from retinal recipient regions (suprachiasmatic region, neuropil of dorsal lateral and ventral lateral geniculate nuclei, area pretectalis, the lentiform region, and the nucleus of the basal optic root) or markedly reduced (retinal recipient superficial tectum). The results of the enucleation studies are consistent with the presence of LLI within a large percentage of ganglion cells. The localization of LLI within many retinal ganglion cells and their target areas within the brain, suggests that LANT-6 may play a significant role in visual processing. This work supported by EY04785 to WDE, NS19620 to AR and AM28565 to REC.

134.4 SUBSTANCE P AND LEUCINE-ENKEPHALIN IN GANGLION CELL AXONS IN THE ANURAN RETINA. R.O. Kuljis and H.J. Karten. Departments of Neurology, Psychiatry and Neurobiology, S.U.N.Y. at Stony Brook, N.Y. 11794.

Fifteen adult specimens of Rana pipiens were anesthetized with MS 222 and subjected to a single linear scratch in the retina with a fine needle. One to 27 days after surgery, substance P(SP)- and leucine-enkephalin(LENK)-like immunoreactivities were analyzed in the retina, optic nerve and optic tectum by indirect fluorescence and peroxidase-antiperoxidase methods. In 6 animals horseradish peroxidase (HRP) was applied to the stumps of the transected optic nerve 1-3 days prior to the retinal scratch. The retinae of the latter animals were double stained for SP and HRP with fluorescein and rhodamine.

SP- and LENK-containing fibers were demonstrable in increasing numbers with longer postoperative survival. These peptide-containing fibers were located in the optic nerve fiber layer (ONFL) in the vicinity of the lesion, only in its peripheral border (i.e. the side of the lesion closer to the ora serrata), and often ending in a dilated expansion reminiscent of dystrophic axons and/or growth cones. Only occasional labeled somata were seen in the ganglion cell layer, as in normal retinae. A portion of the HRP-containing axons in the ONFL were also SP-positive in those retinae labeled prior to scratching. Almost no peptide-containig optic nerve axons were observed in the portion of the retina central to the lesion (i.e. closer to the optic nerve head) or in the optic nerve, as in normal animals. In the optic tectum, modifications in the pattern of peptide-like immunoreactivity were identical to those following retinal deafferentation (Kuljis & Karten, JCN 217,'83), in those portions of the tectum deafferented by the retinal scratch. The remainder of the tectum displayed the normal pattern of peptide-like immunoreactivity.

Our observations indicate that peptide-like immunoreactivity is demonstrable in retinal ganglion cell axons in the ONFL of the retina after trauma. In contrast, peptides are not convincingly demonstrable immunocytochemically in ganglion cells or their processes in normal or in colchicine-treated retinae. These findings corroborate previous observations suggesting the existence of various types of peptidergic retinal ganglion cells (Kuljis et al., JCN 225,'84). The possibility of posttraumatic expression of new peptide phenotypes cannot be entirely ruled out at present.

134.5 TOPOGRAPHY OF THE RETINAL PROJECTION TO THE NUCLEUS LATERALIS GENICULATUS OF GOLDFISH. A.D. Springer and A.S. Mednick. Department of Anatomy, New York Medical College, Valhalla, N.Y. 10595.

In addition to the optic tectum, the goldfish retina projects to at least twelve other targets. Of these, nucleus lateralis geniculatus (NLG) is unique in that its borders can be readily identified. Two approaches were used to determine the topography of the retino-NLG projection: a) cobaltous-lysine was applied to peripheral cuts at different positions in the retina and b) half of the retina was ablated and the surviving retinal ganglion cell axons were labeled with cobaltous-lysine. The retinal fibers that innervate NLG originate from all retinal quadrants and occupy the middle part of the main optic tract, overlapping the optic tract position of fibers from temporal retina. Both experimental approaches revealed that dorsal retina projects to the lateral part of NLG and that ventral retina projects to the medial part of NLG. Peripheral temporal retina projects to the rostral pole of NLG. However, peripheral nasal retina does not project to the caudal pole of NLG. Examination of parasagittal sections through NLG in which the retinal projections were labeled with cobaltous-lysine or [^{3}H]proline indicated that NLG is a folded structure. It has a dorsal surface containing fibers from temporal retina, as indicated by nasal retinal ablations. The caudal genu contains fibers from central retina as indicated by peripheral retinal ablations. The ventral surface contains fibers from nasal retina as indicated by temporal retinal ablations. Peripheral nasal fibers project to the rostral pole of the ventral surface and peripheral temporal fibers project to the rostral pole of the dorsal surface of NLG. Furthermore, the dorsal surface extends more rostrally than the ventral surface. In addition, after arborizing in NLG, most retino-NLG axons rejoin the optic tracts and continue to innervate the most superficial retino-recipient tectal lamina. Temporal retinal ganglion cell axons, after arborzing in NLG, enter the superficial retino-recipient tectal lamina directly without rejoining the optic tracts. Supported by Grant EY03552.

134.6 DIFFERENT PROJECTIONS OF DORSAL AND VENTRAL RETINAL GANGLION CELL AXONS TO THE SUPRACHIASMATIC NUCLEUS AND OTHER NON-TECTAL RETINO-RECIPIENT TARGETS IN GOLDFISH. A.S. Mednick and A.D. Springer. Department of Anatomy, New York Medical College, Valhalla, N.Y. 10595

Retinal ganglion cell axons project to various targets in the goldfish brain, including the optic tectum. We examined the retinal projections to 9 non-tectal targets: suprachiasmatic nucleus (SCN), nuclei opticus pretectalis dorsalis and ventralis (NOPrD, NOPrV), accessory optic nucleus (AON), nucleus corticalis (NC), nucleus opticus commissurae posterior (NOCP), nucleus opticus dorsolateralis (NODL), nucleus opticus ventrolateralis (NOVL), and the tuberal region (TR) of the hypothalamus. The retinal projections were examined by applying cobaltous-lysine to small peripheral retinal lesions made in specific quadrants of the retina or by ablating part of the retina and applying cobaltous-lysine to the remaining optic axons.

The SCN appears to receive a projection only from ventral retina. The thalamic nuclei (NODL, NOVL, and NOCP) receive fibers from all quadrants of the retina. NOPrD, which is located near the dorsal optic tract, appears to receive fibers primarily from ventral retina, with dorsal retina contributing a small projection. NOPrV, which is located near the ventral optic tract, appears to receive projections primarily from dorsal retina, with ventral retina providing a small projection. The AON mainly receives fibers from dorsonasal retina, with dorsotemporal retina contributing a small projection. The NC appears to receive fibers exclusively from dorsonasal retina, and the TR appears to receive fibers from dorsal retina. Thus, some targets appear to receive optic fibers only from ventral retina (SCN), while others receive fibers from dorsal retina (TR, AON, NC). Other targets receive fibers from all retinal quadrants (NODL, NOCP, NOVL) or predominantly from one-half of the retina (NOPrD, NOPrV). The reasons for the selective innervation of some retinal targets by axons from specific retinal areas are unclear. Supported by Grant EY03552.

134.7 RETINOFUGAL ORGANIZATION IN TWO GYMNOTIDS, EIGENMANNIA VIRESCENS AND STERNARCHUS. E. Sas*and L. Maler. Dept. Anatomy, Health Sci., Univ. of Ottawa, Ottawa Canada K1H 8M5

The excellent development of the electrosensory system in these Gymnotid Teleosts, prompted us to investigate the following questions: a) to what degree does the wide use of electroreception for detection of objects and communication, affect the visual representation in these fish? b) are the retinal projections similar in these Gymnotids living in bottom layer waters? c) do they differ from those of diurnal teleosts? To elucidate these points we placed electrophoretic injections of HRP, WGA conjugated HRP,or ^{3}H-leucine into the posterior chamber of the eye; or exposed the cut end of the optic nerve to cobalt chloride (Lázár et al., '83). The optic nerves cross completely at the optic chiasma in both Gymnotids; unlike those of the weakly electric Mormyrids. The retinal afferents terminate in a layered fashion in stratum opticum and stratum fibrosum et griseum superficiale of the optic tectum, along its whole rostro-caudal extent, and only a meagre projection to rostromedial stratum album centrale.

In Eigenmannia the width of the main retinotectal projection is similar to that of Mormyrids; slightly denser and wider than in Sternarchus but narrower and less segregated in sublayers than in goldfish. The tight retinotectal projection in Sternarchus becomes drastically reduced in the caudal fifth of the optic tectum, thus it may pose a problem when attempting to record visual responses from this area. This reduced visual representation perhaps is correlated to the smaller, less protruding eyes of this fish which appear covered by a fine membrane. The main retinofugal target areas (pretectal area, optic tectum, thalamus, and hypothalamic optic N.) were found to be fairly similar to those reported in other teleosts. The particularities found in Gymnotids will be discussed. We may conclude that the visual system in these nocturnal Gymnotid fish, although not as impressively, represented as the electrosensory system, it may play a critical role during jamming of the electrosensory system, (Bastian, '82).

134.8 MORPHOLOGY OF RETINOGENICULATE TERMINALS IN THE TURTLE, PSEUDEMYS SCRIPTA. A. Sjostrom* and P.S. Ulinski. Dept. Anatomy and Comm. on Neurobiology, Univ. Chicago, Chicago, IL 60637.

The dorsal lateral geniculate complex in turtles receives a bilateral, topographic retinal input. Several morphologically and physiologically distinct classes of retinal ganglion cells exist in turtles (e.g. Kolb, 1982, Phil. Trans. R. Soc. Lond. B, 298:355; Marchiafava, 1983, Vis. Res., 23:325), but nothing is known about the morphology of their axon terminals within the geniculate. This study examined the morphology of individual retinogeniculate terminals using serial section reconstructions of axons filled by HRP injections in the optic tract.

We successfully filled a large number of retinogeniculate terminals and prepared detailed drawings of 87 terminals which we classified into three types based on the size and number of varicosities in the terminal, and (if a terminal formed a spatially restricted arbor) the volume of the arbor. Type I retinogeniculate terminals are spatially restricted, large-volume arbors having a low density of large varicosities (1.4 x 1.4 μm in size). The mean $\pm$ S.E.M. number of varicosities per arbor was 47.0 $\pm$ 5.0 μm. Arbors had volumes of 14.0 x 10^3 $\pm$ 3.5 μm^3. The density of varicosities in the arbor was 6.4 x 10^{-3} $\pm$ 1.0 vari-cosities/μm^3. Type II retinogeniculate terminals consist of restricted, small-volume arbors having a high density of small varicosities (0.5 x 0.5 μm in size). The number of varicosities per arbor was 74.0 $\pm$ 5.0. The volume was 6.6 x 10^3 $\pm$ 1.1 μm^3. The density of varicosities in the arbor was 17.4 x 10^{-3} $\pm$ 1.9 varicosities/μm^3. The volume of the arbors and the number and density of varicosities asso-ciated with the two types of terminals differed statis-tically at 0.03 probability level or better with a Mann-Whitney U test. Type III retinogeniculate terminals, in contrast to Types I and II, do not form spatially re-stricted arbors. Rather, they consist of sparsely branched axons that parallel the optic tract and contain scattered en passant varicosities 0.9 - 1.3 μm in length.

These findings raise the possibility that different classes of retinal ganglion cells differ in their mode of termination within the geniculate complex, but the precise relation between the three types of retinogeniculate terminals and the classes of ganglion cells remains to be determined. Supported by PHS Grant NS 12518.

134.9 RETINAL GANGLION CELLS LABELED AFTER INJECTION OF HRP INTO THE DORSAL LATERAL GENICULATE NUCLEUS. E. Kicliter and N. Lugo-García. Department of Anatomy and Laboratory of Neurobiology, University of Puerto Rico School of Medicine, San Juan, PR 00901.

Ground squirrels have three functional types of optic nerve fibers (Michael, J. Neurophysiol. 31: 249-282, 1968; Gur and Purple, Vision Res. 18: 1-14, 1978). Of these, only the contrast-sensitive and opponent color types project to the dorsal lateral geniculate; directionally selective units terminate preferentially in the superior colliculus (Michael, J. Neurophysiol. 35: 815-832, 1972; 36: 536-550, 1973). We were interested in determining whether the ganglion cells which terminate in the dorsal lateral geniculate have specific sizes of somata, comprising only part of the entire size range of ganglion cell somata. If so, then perhaps specific functional types might be associated with morphological types based on soma size.

In order to answer this question we made iontophoretic injections of horseradish peroxidase (HRP) into either the optic tract or dorsal lateral geniculate of thirteen-lined ground squirrels (Sigma type VI HRP, 10% in 0.01 M NaCl, discontinuous anodal current). After three day survival periods the retinas of these animals were reacted according to the Hanker-Yates method (Histochem. J. 9: 789-792, 1977).

After injections of the optic tract labeled ganglion cell somata ranged in diameter from 4-20 μm. These cells were often distributed as doublets or triplets in the retina. After lateral geniculate injections the majority of labeled cells ranged from 10-16 μm in diameter and were more regularly spaced within the retinal region of labeled cells. The finding that not all sizes of ganglion cells project to the dorsal lateral geniculate suggests that functional categories of ganglion cells in ground squirrels may be associated with specific sizes of somata.

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134.10 GANGLION CELL DISTRIBUTION IN RED SQUIRREL RETINA. A. Flores and E. Kicliter. Dept. Biology, Catholic Univ., Ponce, PR 00731 and Lab. Neurobiology and Dept. Anatomy, Univ. of Puerto Rico Sch. of Medicine, San Juan, PR 00901.

Distribution of ganglion cells in the retina of the red squirrel (Tamiasciurus hudsonicus) was studied in Nissl-stained wholemounted preparations. Ganglion cells were identified by cytologic criteria and were counted and measured from camera lucida drawings. Maps of the retinas showing isodensity contours were prepared. The overall distribution of ganglion cells was characterized by a horizontally-oriented visual streak below the linear, horizontally-oriented optic nerve head and a preponderance of ganglion cells in the inferior retina. Ganglion cell density ranged from 6000 cells/mm^2 in the visual streak to 1000 cells/mm^2 in the retinal periphery. No area centralis within the visual streak was discerned.

Frequency/cell size histograms were made of the retinas of six animals and distribution according to soma size was studied in various regions of the retinas. Ganglion cell soma diameter ranged from 8-25 μm. Average ganglion cell soma size increased with eccentricity from the visual streak. Medium-sized cells (11-14 μm diameter) were present throughout the retina and, together with small cells (8-11 μm diameter), were abundant in the visual streak. Although large cells (soma diameters greater than 14 μm) were more numerous toward the periphery of the retina, a considerable number were found in the visual streak.

The results indicate that the highest density region in the inferior retina, corresponding to the superior visual field, is most important for vision in this species. In accordance with findings in other species, the small and medium-sized ganglion cells are most numerous in retinal regions associated with acute vision.

Some intriguing comparisons can be made between the organization of ganglion cells in red squirrel retina with that in the 13-lined ground squirrel, which inhabits a very different type of visual environment. 1) Both species have visual streaks without areae centralis. 2) in both species average ganglion cell size increases with eccentricity from the visual streak. 3) Red squirrels have fewer ganglion cells. 4) Red squirrels, unlike ground squirrels, have a population of large ganglion cells located throughout the retina, including the visual streak. The latter two comparisons, indicating differences, may be related to adaptations to different visual environments.

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134.11 CHANGES IN THE SUPERIOR COLLICULUS EVOKED POTENTIAL IN 2,5-HEXANEDIONE NEUROTOXICITY. R.D.Wilson*, D.Impelman* and D.A. Fox (SPON:M.S.Amoss). USDA,College Station, TX and College of Optometry, University of Houston, Houston, TX 77004.

Recent reports have indicated that the retinotectal pathway is affected in 2,5-hexanedione neuropathy in rats(Neuropath. Appl. Neurobiol.,8:289, 1982). Morphological changes in pre- and postsynaptic elements of the superior colliculus (SC) were preceded by a reduction in local glucose utilization. We have investigated synaptic function of the optic tract(OT) fibers as inputs to the SC.

Simultaneous recordings were made from the SC and OT while stimulating from the optic chiasm(OX). In control rats, two postsynaptic SC responses were differentiated from presynaptic SC inputs and from each other by their functional characteristics. Postsynaptic components were characterized by peak latency, train responses, antidromic response, recovery cycle and chronaxie. A 100 Hz stimulus train reduced the second postsynaptic component by 75% or more, while the presynaptic components were unaffected. Antidromic response latencies matched presynaptic latencies in the orthodromic SC response. The recovery cycles of the first and second postsynaptic components were characterized by their absolute and relative refractory periods. The absolute refractory periods were .5 ms and .7 - .9 ms, respectively; relative refractory periods were 2.75 - 3.0 ms and 3.1 - 5.5 ms, respectively. The peak delay between the first and second pre- and postsynaptic components was 1.23 - 1.43 ms and 1.10 - 1.67 ms, respectively.

Recordings were made when rats were in advanced stages of 2,5-hexanedione neurotoxicity. The rats had severe posterior paresis with slight forelimb involvement. Response amplitudes from the SC were decreased and peak latencies prolonged. A 55 Hz train reduced the second postsynaptic component to 20%, compared to 40% in controls. Postsynaptic inhibition, which is characteristic of SC recovery cycles, was absent. Absolute refractory periods were increased, with the postsynaptic absolute refractory period being more affected than the presynaptic. These changes in functional characteristics of SC postsynaptic responses suggests that either the distal ends of OT fibers or the SC neurons below the stratum griseum superficialis are affected by 2,5-hexanedione. Supported by (NIEHS grant) ES 03183 (DAF).

134.12 FUNCTIONAL CHANGES IN THE RETINOTECTAL TRACT OF THE HOODED RAT IN 2,5-HEXANEDIONE DISTAL AXONOPATHY. D. IMPELMAN*, R. WILSON* AND D.A. FOX(SPON. B. Brooks) Coll. of Opt., U. Houston, Houston, TX 77004

Toxicity effects on large and small diameter fiber populations were evaluated in depth recordings of t_1(high) and t_2(middle) conduction group responses in hexane-treated rats following the onset of hindlimb paralysis. A preferential large diameter loss occurs in the peripheral axonopathy which is characterized by neurofilament accumulations, paranodal demyelination and swelling of axonal terminals similar to changes in the SC brachium. LE hooded female rats were given 2,5-hexanedione in drinking water using the treatment regimen of Jones & Cavanagh(Neuropath. & Appl. Neurobiol.8:289). Toxicity effects were not correlated with dehydration or weight loss in control experiments. The response properties of smaller diameter t_2 fibers were more affected than t_1 fibers at all stages of toxicity studied. Response amplitudes were reduced for both groups with more dispersion in the t_2 response. T_2 amplitude intensity functions were depressed below t_1 which suggests a selective small diameter fiber loss. Peak conduction velocities (CV), absolute refractory periods(ARPs) and relative refractory periods(RRPs) for t_1 and t_2 in control and hexane animals are tabulated below.

	t_1		t_2	
	CONTROL	HEXANE	CONTROL	HEXANE
CV(m/s)	12.1±0.54	8.5±0.2	5.8±0.16	3.7±0.14
ARP(ms)	0.42-o.56	0.62-0.72	0.71-0.82	0.94-1.43
RRP(ms)	1.1-2.4	0.96-1.62	8.7-13.8	3.7-4.3

Supernormal periods are characteristic of amplitude recovery functions for both conduction groups but are not correlated with chronaxy changes. The hyperexcitability of optic tract fibers is also observed in higher photic following frequency of OT responses in simultaneous recordings of OX/OT responses to 10-16Hz photic trains and suggests there is ephaptic transmission between axons. The data show a differential effect on smaller diameter fibers in the retinotectal distal axonopathy not predicted by morphological changes in distal tract axons. Supported by NIEHS Grant ES 03183.

134.13 CELLS OF ORIGIN OF THE RETINOCOLLICULAR PROJECTION IN THE CAT. R. Hartwich-Young, B.A. Hagadorn* and J.T. Weber. Department of Anatomy, Tulane University Medical School, New Orleans, LA 70112.

The cells of origin of the retinotectal projection to different collicular laminae were investigated by placing injections of wheat germ agglutinin-horseradish peroxidase (WGA-HRP) within individual layers of the cat superior colliculus (SC). The retinae were removed and subsequently processed for tetramethylbenzidine (TMB) reaction product (Mesulam, '78). Following the placement of large injections which include all SC laminae, both retinae contain retrogradely labeled ganglion cells. In the ipsilateral retina, labeled cells are found within the area centralis (AC) and extend into the temporal periphery. Contralateral labeled retinal ganglion cells are confined to the nasal retina and are concentrated within the AC and along the nasal visual streak. Following an injection restricted to the superficial gray lamina (SGS), retrogradely labeled ganglion cells are primarily located in and around the AC of the contralateral retina. Injections that include both the optic layer (SO) and the intermediate gray layer (SGI) result in the labeling of ganglion cells in the contralateral peripheral nasal retina and are most numerous along the visual streak. An injection limited to the SGI labels a substantial number of retinal ganglion cells within the visual streak area, with a smaller number located in the far nasal periphery. Morphometric evaluation of the cell body areas of labeled versus unlabeled retinal ganglion cells reveals that the distribution of labeled cells is similar to that of the unlabeled cells; i.e., small, medium and large sized retinal ganglion cells are labeled following injections within SGS, SO and SGI. Thus, based upon quantitative and qualitative data, it may be assumed that all three types of retinal ganglion cells (alpha, beta and gamma cells) project to retinorecipient laminae of the cat SC. These data are not in agreement with traditional views that only alpha and gamma cells project to the SC and that the deep SC does not receive a direct retinal projection. On the contrary, our results confirm previous findings that beta cells as well as alpha and gamma cells project to the cat SC (Wassle and Illing, '80) and corroborate data demonstrating a direct retinal projection to the cat SGI (Berson and McIlwain, '82; Beckstead and Frankfurter, '83).

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SYNAPSE ELIMINATION, COMPETITION AND NEURONAL DEATH: RETINA AND BRAIN

135.1 EFFECTS OF INTRAOCULAR TTX UPON THE DEVELOPMENT OF THE RAT VISUAL CORTEX. M.A. Matthews and R.V. Riccio. Dept. Anat. LSU Med. Ctr., New Orleans, LA. 70119

The postnatal development of the rat primary visual cortex was investigated under conditions of tetrodotoxin (TTX)-induced impulse blockade of the optic nerve. Each animal was injected into the right eye with 1X10-4M TTX every 2 days to insure a continuous suppression of impulse activity. Efficiency of TTX was monitored by loss of the pupillary light reflex. Planimetric measurements of cortical thickness at 5-21 days postnatal (dpn) revealed no significant differences between experimental (contralateral) and control (ipsilateral) samples. However, a Golgi analysis at similar ages showed a 27% average reduction in spines along apical dendrites of Layer V pyramidal cells. This alteration was found along the entire shaft but the most significant reductions occurred at a distance of 150-425 μm from the soma (within Layers III and IV). Both large (3.0-4.0 μm) and medium (2.0-2.75 μm) diameter apical dendrites showed a reduced spine population but the larger cells exhibited the most severe stunting,which also included a reduction in the length of remaining spines. A small but significant reduction in spines was found on oblique dendrites distributed within Layer IV.

Populations of axodendritic synapses/100μm2 were determined in animals aged 5,9,13 and 21 dpn and subdivided into Type I (symmetric) or Type II (asymmetric) contacts. TTX reduced the number of Type I synapses by 11% at 9 dpn and the principal effect was found in Layer IV and upper Layer V. However, by 21 dpn, significant reductions (8%) also occurred in Layers I and III, and affected both Type I and Type II contacts. Concomitant reductions in the number of vesicles/bouton were observed in the remaining synapses, ranging from 13% in Layers I,V and VI to 30% in Layer IV for Type II contacts, and from 37% in Layers V and VI to 26% in Layers I to IV for Type I contacts. The length of the post-synaptic density was not affected.

It was concluded that intraocular TTX applied during early stages of development may cause a substantial change in the density and distribution of thalamo-cortical projections and possibly intracortical circuitry.

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135.2 INTRAOCULAR TTX CAUSES SYNAPTIC CHANGES IN THE RAT DORSAL LATERAL GENICULATE NUCLEUS DURING POSTNATAL DEVELOPMENT. R.V. Riccio and M.A. Matthews. Dept. Anat., LSU Med. Ctr., New Orleans, LA. 70119

The postnatal development of the rat dorsal lateral geniculate nucleus (dLGn) was investigated in the absence of optic impulses. Rats aged 5-21 days postnatal (dpn), were intraocularly injected in the right eye with 0.7 μl 1X10-4M tetrodotoxin (TTX) every 2 days to induce a chronic elimination of optic activity. Effectiveness of TTX was monitored by loss of the pupillary light reflex. At 5,9,13 and 21 dpn, the contralateral dLGn was removed and processed for quantitative electron microscopy. After similar treatment, the dLGn was removed at 21 dpn and also processed by the rapid Golgi method. Control tissues included the ipsilateral nucleus and the dLGn from normal non-injected animals of the same age.

TTX significantly reduced the number of synaptic glomeruli/100 μm2 area of neuropil during development. No immediate effect was seen at 5 dpn. However, from 9-21 dpn the decrease averaged 40% less than controls and 45% less than normal nuclei. Maximum reductions were evident at 13 dpn. The mean number of spinous protrusions within each complex was also reduced, exhibiting an average decrease of 28% from both control and normal nuclei. Maximum reductions (45% and 35%, respectively) were seen at 13 dpn. Other experiments showed that these reductions were not due to transneuronal degeneration or a loss of optic axons. By 21 dpn, TTX reduced the size of the dLGn by 20%; yet the number of neurons increased by 17%, suggesting a reduction in the amount of neuropil. Sholl (1953) analysis of Golgi-impregnated Class A and Class B neurons indicated that TTX had no effect on the number or pattern of dendritic branching.

These data suggest that the elimination of optic nerve impulse activity causes a retardation in synaptogenesis without inducing transneuronal degeneration. Since our previous studies have found a minimal relationship between impulse activity and the axonal transport of glycoproteins in developing optic nerves, the synaptic changes in the dLGn are probably due principally to the loss of optic impulses and the minor reduction in constituents of the pre-synaptic membrane.

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135.3 DEAFFERENTATION OF THE SUPERIOR COLLICULUS RESULTS IN DECREASED SYNAPTIC DENSITY. Kenneth C. Wikler and Barbara L. Finlay, Department of Psychology, Cornell University, Ithaca, NY 14853.

Neuronal competition for limited synaptic space has been indirectly supported by demonstrations of the relationship between target availability and cell survival. However, it has not been established that synaptic density is conserved under various conditions of innervation. Experimental hyperinnervation of the optic tectum has produced conflicting findings of both an increase (Marotte, L.R., Neurosci., 4, 1981) and no change in synaptic density (Murray, et al., J. Comp. Neurol., 209, 1982; Norden, J.J. and Constantine-Paton, M. Neurosci. Abs., 8, 1982). To test if synaptic density is regulated by postsynaptic tissue or is determined by the magnitude of afferent innervation, we studied the effect of unilateral neonatal enucleation on synaptic density in the superior colliculus of the golden hamster using electron microscopy.

Neonatal hamsters were unilaterally enucleated within twelve hours after birth and allowed to survive for at least five months. Tissues were processed for electron microscopy and embedded in plastic. Semi-thin sections were stained to localize the borders of the stratum griseum superficiale, blocks were retrimmed, and thin sections were collected. A total of over 11,000 square microns of area were sampled from the right and left sides of the superior colliculus. The percentage of total area occupied by neuropil was assessed by making a point-estimate analysis of all electron micrographs. Synapses were counted if they possessed a synaptic thickening and cleft and at least one synaptic vesicle. Synaptic density was compared for the sides ipsilateral and contralateral to the enucleation. A thirty percent decrease in synaptic density in the deafferented side of the superior colliculus was observed (mean ± SEM number of synapses per 1000 square microns of neuropil = 117.61 ± 7.68 for the deafferented blocks and 167.77 ± 10.27 for the control blocks, $p<0.01$).

Our results indicate that decreased retinal innervation does result in a decrease in synaptic density in the superior colliculus. Synaptic density can be reduced by reductions in afference; however, it is not yet clear that target tissues can accommodate increased afferent innervation by increasing synaptic density. To test this possibility, we are currently examining the effects of neonatal partial tectal lesions on synaptic density in the superior colliculus.

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135.4 CELL GENERATION AND DEATH IN THE HAMSTER RETINA: CHANGES IN THE SPATIAL DISTRIBUTION OF CELLS FROM THE LATE POSTNATAL PERIOD TO ADULTHOOD. B.L. Finlay, D.R. Sengelaub, and R.P. Dolan, Department of Psychology, Cornell University, Ithaca NY 14853 and Department of Psychology, University of California, Los Angeles, CA 90024.

In a prior study of the creation of the visual streak in the hamster retina, we examined the spatial pattern of cell degeneration by generation day over the period of maximal cell death in the developing hamster retinal ganglion cell layer. We found that the retinal ganglion layer is generated from embryonic day 10 (E10) to postnatal day 3 (P3) in two waves (E10-12 and E14-P1). The earliest generated cohorts (E10, 11) show a striking elevation in amount of labeled degenerating debris in the retinal periphery while later cohorts show a more uniform distribution of degenerating debris. In order to map directly the consequences of this pattern of early cell degeneration for the formation of the visual streak, we now report the changes in the spatial distribution of cells over the late postnatal period to adulthood.

Pregnant females were injected with ^{3}H thymidine (5 μCi/gm body weight) on embryonic days 10, 11, 12, and 14 (E15=day of birth). Animals were killed on either postnatal day 6 (the peak both in cell number and degeneration rate in the retinal ganglion cell layer), P10 (just after the period of marked cell degeneration) or at adulthood. After autoradiographic processing, the spatial distribution of cells in each cohort was determined from counts of labeled cells made from horizontal retinal sections.

All cell cohorts reach their adult spatial distribution in a pronounced superior-temporal to inferior-nasal progression. The two waves of cell generation correspond to the formation of different cell types, the first consisting of cells with soma size and Nissl inclusions characteristic of ganglion cells, and the second with soma size and nuclear structures characteristic of "displaced" amacrine cells. The E10 and E11 cohorts lose substantially more cells in the retinal periphery, while for the E12 and 14 cohorts, cell loss is uniform across the retinal surface. The spatial pattern of cell loss in each cohort confirms in detail our prior report of the distribution of labeled degenerating debris.

Differential growth of the retina, a mechanism suggested for the creation of differential retinal cell densities, would alter the spatial distribution of all cohorts equally. Since the spatial distribution of each cohort changes differently over the same time period, differential growth alone cannot account for the overall change in local cell density. Since differential cell generation does not occur, cell degeneration must produce the visual streak. Supported by NIH grants NS00783 and NS19245.

135.5 AN AUTORADIOGRAPHIC ANALYSIS OF THE ROLE OF CELL DEATH IN REGULATION OF NEOCORTICAL CELL NUMBER. M.H. Kane*, D.R. Sengelaub, and B.L. Finlay, Department of Psychology, Cornell University, Ithaca, NY 14853; Department of Psychology University of California, Los Angeles, CA 90024.

In the vertebrate spinal cord, anterior horn neurons are produced in approximately equal numbers along the length of the cord and are sculpted into their adult brachial, thoracic and lumbar specializations by differential cell death (Hamburger, Am. J. Anat., 102, 1958). The neocortex of rodents exhibits a possibly analogous inhomogeneity in neuron numbers. The medial neocortex, including the cingulate areas 29a, b and the presubicular area 27, has approximately half the number of cells per "unit column" of cortical depth than do the visual cortical areas 17, 18a and b of the lateral cortical convexity. Large numbers of degenerating cells can be seen in the last-generated external laminae of the medial cortex, but not the lateral cortex, during early development (Finlay & Slattery, Science, 219, 1983). We hypothesized that the initial numbers of cells generated across the cortical surface are uniform, and that differential cell death produces the observed differences in number of neurons in an "unit column" between cortical areas. To test this hypothesis, we have compared the relative number of neurons labeled in medial and lateral neocortex by a single injection of tritiated thymidine in the period of neocortical histogenesis before and after the period of cell death.

Pregnant hamsters were injected with ^{3}H thymidine on either embryonic day 12, when cortical layer 6 is generated, or on embryonic day 14, when a band of cells spanning cortical layers 2, 3 and 4 is generated. The number of labeled cells for both medial and lateral cortical areas was assessed on postnatal days 4-6 (after the termination of migration to the cortical plate, but prior to maximal degeneration) and at adulthood.

In the neonates, for both injection days E12 and 14, equivalent numbers of labeled cells were found in the medial and lateral neocortex (E12, medial cortex, X=47 cells/mm; lateral cortex, X=63 cells/mm; E14, medial cortex, X=78.5 cells/mm; lateral cortex, X=48 cells/mm). For the E12 injection (layer 6), this pattern persisted to adulthood (medial cortex, X=41.5 cells/mm; lateral cortex X=34 cells/mm). For the late injection labeling the external layers, at adulthood only half as many cells were found in the medial cortex (X=21 cells/mm) as in the lateral cortex (X=46 cells/mm). These results support the hypothesis that cells are generated in uniform numbers across the cortical surface and differential cell loss produces some regional cortical differences.

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135.6 RETINAL IMPULSE BLOCKADE BY TTX TRANSITORILY REDUCES CELL DEATH IN THE HAMSTER RETINAL GANGLION CELL LAYER. J.I. Raabe and B.L. Finlay, Department of Psychology, Cornell University, Ithaca, NY 14853.

Synaptic activity is known to be important in the formation of orderly patterns of connectivity in the central nervous system, such as the ocular dominance columns of the cat visual cortex (Stryker, Neurosci. Abs., 1983) and refined retinotopic maps (Schmidt & Edwards, Brain Res., 269, 1983; Meyer, Dev. Brain Res., 6, 1983). A functional neuromuscular synaptic interaction is important in the occurrence of normal motoneuron death in the peripheral nervous system. Neuromuscular blockade prevents naturally occurring cell death (Pittman & Oppenheim, J. Comp. Neurol., 187, 1979). The importance of synaptic activity in the initiation of cell death in the CNS is not known. This study examines the role of synaptic activity in the naturally occurring cell death in the retinal ganglion cell layer of the golden hamster.

A 0.2 μl injection of a 0.02% solution of TTX was administered through the cornea to neonatal hamsters on postnatal day 4 (n=3), just prior to peak degeneration in the retinal ganglion cell layer. A control citrate buffer solution was administered to the remaining eye. Animals survived 40 hours postsurgery, exposing the eye to TTX during the period of peak degeneration, and were sacrificed on postnatal day 6. Retinas were reconstructed to localize the site of injection, and assessed for damage. Only those retinas without obvious punctures to the ganglion cell layer, or possible discontinuities were selected for analysis. The number of degenerating cells, expressed as a fraction of the number of live cells, were compared for the two eyes for three equally spaced horizontal sections. The rates of degeneration were markedly reduced in the eyes injected with TTX as compared to the citrate buffer injected eyes ($\bar{X}$=4.5±.6 vs. 10.8±3.8), which did not differ from normal ($\bar{X}$=11.5±1.0). A separate analysis of the temporal region of the retina, representing the binocular zone and hence overlapping projections of control and experimental eyes, suggests that the rates of degeneration in the citrate buffer eyes are above normal ($\bar{X}$=12.1±6.0 vs. 9.0±1.3). Degeneration in the temporal region of the TTX-injected eyes remained similarly depressed ($\bar{X}$=4.0±0.5 vs. 9.0±1.3) as for the whole eye rates.

These results suggest that either presynaptic spike activity, a functional synaptic interaction, or both, are important in the initiation of normally occurring cell death in the central nervous system.

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135.7 DENDRITIC AND TERMINAL COMPETITION CONCUR FOR THE REGULATION OF DEVELOPMENTAL NEURONAL DEATH IN THE RETINA. R. Linden* & C.A. Serfaty*(SPON L.H. Pinto) Instituto de Biofísica da UFRJ, Rio de Janeiro, Brasil

Expanded uncrossed retinal projections following neonatal enucleation in rodents result from reduced cell death attributed to diminished competition among axons for terminal space. Similarly expanded projections follow removal of neighboring ganglion cells after contralateral optic tract lesions, and have been attributed to reduced dendritic competition. We studied the ganglion cells with ipsilateral axons in whole-mounted retinae of adult rats that had been given either uniocular enucleation or contralateral optic tract lesions, or both operations simultaneously at birth. Cells were labelled with HRP injected along the optic tract. Unoperated rats similarly treated were used as controls.

Numbers of uncrossed-projecting cells were increased over the whole retina. Enucleation produced its main effect upon temporal retina, while optic tract lesions had their major effect on central retina. Results of the double lesion appeared to be a composite of both effects. Notwithstanding these changes, a sharp gradient in the density of ipsilaterally-projecting cells was maintained between the temporal crescent and nasal retina, similar to that of normal rats.

Enucleation led to a decrease in the median of the cell-body size distribution, as compared to the controls. Optic tract lesions, on the contrary, increased the median, but only in nasal retina. The double lesion led to a decreased median in the temporal crescent, and an increased median in nasal retina, combining both effects. In both control and enucleated rats, large alpha-like cells with ipsilateral projections were lacking in nasal retina, while such cells were frequent in all animals given optic tract lesions.

The results suggest that: (a) topographically-dependent dendritic and terminal competition concur for the regulation of cell death when both conditions are altered simultaneously; (b) large alpha-like cells are particularly sensitive to dendritic competition, while smaller cells are particularly sensitive to terminal competition. Interactions of these processes may be related to the generation of differential distributions of ganglion cell types in normally developing retinae. They do not, however, appear to be responsible for the generation of a naso-temporal division.

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135.8 EVIDENCE FOR DENDRITIC COMPETITION AND COMPENSATION UNDER CONDITIONS OF REDUCED RETINAL GANGLION CELL DEATH. K. McColl*, M. Murray and T.J. Cunningham. Dept. of Anatomy, The Medical College of PA, Philadelphia, PA 19129.

When competition at the target of a developing neuron population is reduced, there also is a reduction in naturally occurring neuron death in that population. Although this phenomenon has been documented in experimental studies of several regions of the developing nervous system, very little is known about the specific changes individual neurons undergo in the face of such population excesses. If one eye is removed from a newborn rat there is reduced central competition among the ganglion cells of the remaining eye. As a result, naturally occurring ganglion cell death is reduced, especially in temporal retina. The purpose of this study was to investigate the effect of ganglion cell crowding on the ganglion cell somata and dendrites. We examined alpha cells in the retinae of normal adult rats and littermates which had one eye removed on the day of birth. The cells were stained with a modification of the neurofibrillar method used by Peichl and Boycott (1981) to stain selectively the cell bodies and dendritic trees of this ganglion cell population. We determined the density and shape of fully stained cells in the temporal retina of the two groups. In normal rats, the alpha cells in temporal retina have large, highly branched dendritic trees which are usually distributed symmetrically around the cell body. In rats with one eye removed at birth, there is an increased density of cells in temporal retina. The average size of the cell body is increased but the dendrites are usually shorter and thicker. Some cells have abnormally elongated dendrites on the side of the cell body that extends into regions of the retina where the density of neighboring alpha cells is reduced. The results are consistent with the idea that ganglion cell dendrites also compete for space in the developing retina. The fact that the crowded cells appear truncated rather than grossly shrunken suggests that they attempt to maintain normal cytoplasmic volume in the cell body and dendrites. The changes could also reflect either increased target contact of the cells, their requirement for an appropriate complement of afferent inputs, or both.

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135.9 USE OF THE GOLGI AND NISSL STAIN TO EVALUATE A CHRONIC NEURODEGENERATION OF CORTICALLY TRANSPLANTED FETAL RAT STRIATUM. A.W. Deckel*, R.G. Robinson and D.B. Newman (SPON: G.M. McKhann). Dept. of Psychiatry, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD., 21205, and the Dept. of Anatomy, Uniformed Services University of the Health Sciences, Bethesda, MD., 20814.

It has previously been demonstrated that the neocortex serves as a suitable substrate to facilitate growth of intraparenchymal fetal brain grafts from a variety of regions of the neuraxis. It is generally assumed that all regions of the fetal brain will survive when placed in any region of the recipient brain, providing that the structures necessary for vital functioning are not disturbed. This experiment demonstrates an exception to this rule, finding that the neocortex is not capable of supporting long-term growth of fetal striatal tissue. Adult female Sprague-Dawley rats were transplanted intraparenchymally either with day 18 fetal cortical or fetal striatal tissue. One group of animals (n=8) received bilateral cortical transplants into the intact cortex, and were sacrificed at weekly intervals for the 2 months following transplantation. The second group (n=8) first received intrastriatal kainic acid lesions, followed 1 week later by bilateral grafts of day 18 striatum deposited simultaneously into both the striatum and neocortex. These animals were sacrificed at either 3 weeks or 4 months post-transplantation, and examined histologically either with 30um cresyl violet, or 120um Adams Golgi, staining. The fetal cortical intraparenchymal transplants survived robustly when placed in the recipient cortex, and histologically appeared similar to that of the host cortex. Similarly, fetal striatum transplanted intrastriatally survived well both at 2 weeks and 4 months, with normal dendritic and cell soma morphology. However, when the fetal striatum passed accross the corpus callosum and into the neocortex, a degeneration was observed that was characterized by a chromatolysis and neuronal cell death of transplanted cells that began in the middle regions of the transplant. This process appeared to be gradual, as evidenced by normal development of 2 of the 4 intracortical fetal striatal transplants at 3 weeks post-transplantation, but of a complete or near complete degeneration of the 12 transplants sacrificed at 4 months. We conclude that the neocortex appears toxic to chronically implanted fetal striatum, but not to fetal neocortex.

135.10 TIME COURSE OF RETROGRADE DEGENERATION OF THE CELLS OF ORIGIN OF THE SEPTOHIPPOCAMPAL PATHWAY AFTER FIMBRIA-FORNIX TRANSECTIONS. S. Grady*, T. Reeves, & O. Steward, Neurosurg. Dept., Univ. Virginia Med. Sch., Charlottesville, VA 22908.

Axotomy of peripheral nerves results in a host of retrograde neuronal reactions, including cell death. The effects of axotomy in the CNS have not been extensively explored since a suitable model system has not been developed. The septohippocampal pathway might serve as a useful model because 1) the fiber system can be interrupted selectively, 2) the cholinergic cells-of-origin can be visualized with AChE histochemistry, and 3) the projection of the fibers appears to be exclusively unilateral, allowing within-animal controls. The fimbria-fornix was transected unilaterally in 36 adult male rats which survived for 6, 10, 14, 28 or 30 days. Retrograde cell reactions in the medial septal nucleus (MSN) were assessed in 20 rats by counting nucleoli-containing cellular profiles in thionin-stained horizontal sections (6 μm thickness). Sixteen animals were prepared for acetylcholinesterase (AChE) histochemistry using diisopropylfluorophosphate to suppress neuropil AChE. This procedure results in enhanced visualization of neuronal cell bodies which synthesize AChE. AChE-containing cells in the MSN were counted in 40 μm horizontal sections.

Counts of neurons in thionin-stained sections revealed substantial cell loss following axotomy. There were 31% fewer cells in the axotomized MSN at 6 days post-lesion, and 39%, 45% and 67% fewer cells at 10, 14 and 28 days post-lesion, respectively. In animals processed for AChE histochemistry, there was no difference in the number of AChE-stained neurons at 6 days post-lesion. However, there was a 23% decrease in labeled cells on the axotomized side at 10 days, 25% at 14 days, and 19% at 30 days. The present findings suggest a retrograde cell loss in the MSN after fimbria-fornix transection. The cell loss was not detected in AChE-stained material until after 6 days, whereas the decreases appeared earlier and were more extensive in the cell counts from thionin-stained material. Retrograde cellular atrophy may contribute to the discrepancy, causing lower counting rates in thionin-stained material relative to AChE-stained sections. We propose that the septohippocampal pathway offers a useful model system for evaluating the cellular effects of axotomy in the CNS.

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135.11 ELIMINATION OF TOPOGRAPHICAL TARGETING ERRORS IN THE RETINOCOLLICULAR PROJECTION BY GANGLION CELL DEATH. D.D.M. O'Leary, J.W. Fawcett*and W. M. Cowan, The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

To determine to what extent the axons of retinal ganglion cells (RGC's) make targeting errors during the development of the retinocollicular (RC) projection and to assess the possible role of RGC death in the correction of such errors, we have made localized injections (0.01-0.02 µl) of the fluorescent dye, fast blue (FB), into the caudal part of the superior colliculus (SC) in a series of newborn and 12 day old (P12) albino rats. Two or 3 days after the injections the animals were perfused with buffered formalin and the spread of the FB in the colliculus examined from sections through the midbrain. In retinal wholemounts of animals injected on P12 (after the phase of "naturally occurring" RGC death) and killed on P15, there was a focus of heavy RGC labeling in the contralateral peripheral nasal retina (as predicted from the known topography of the RC projection) and a small number of labeled RGC's scattered over the temporal retina. In animals injected on P0 and killed on P2, on the other hand, much larger numbers of labeled RGC's were seen all over the retina, though the highest density was still in the nasal peripheral retina. This implies that early in development large numbers of RGC's project aberrantly to the wrong part of the contralateral SC, and that the great majority of the fibers that make these targeting errors are eliminated during the first 2 weeks postnatally. Direct evidence that these fibers are eliminated by RGC death rather than collateral withdrawal has come from long-term dye-labeling experiments in which FB was injected into the caudal SC on P0 and the animals killed on P12. These animals have a retinal labeling pattern similar to those injected on P12; there is a focus of correctly projecting labeled cells in nasal retina, with a few aberrantly projecting cells scattered elsewhere. We have estimated the relative numbers of correctly and erroneously projecting cells by measuring the density of labeled cells in the heavily labeled nasal retina, and also in the mirror image region of the temporal retina, and calculating their ratios. The density of labeled RGC's in the nasal retina of the animals killed on P2 was only 6.5 times higher than in the temporal retina, but in the animals injected on P0 and killed on P12, the corresponding ratio was 40:1. This implies that the initial, poorly organized RC projection is sharpened by the preferential death of RGC's which make targeting errors in the SC. It follows from this that "naturally occurring" death of RGC's does not simply represent a quantitative matching of the pre- and post-synaptic populations, but also involves a process of error elimination.

135.12 EVIDENCE FOR MINIMAL NEURONAL LOSS DURING THE POSTNATAL DEVELOPMENT OF THE HIPPOCAMPAL REGIO SUPERIOR IN THE RAT. K. Turlejski*, B.B. Stanfield and W.M. Cowan. The Salk Institute, La Jolla, California 92037.

The normal loss of a substantial proportion of an initially generated neuronal population is a feature of the development of many parts of the nervous system. This phenomenon has been best documented for neural centers which contain rather few cells since in these it is relatively easy to accurately determine the total number of neurons present at successive stages in development. In order to establish to what, if any, extent naturally-occuring cell death is involved in the postnatal development of cerebral cortical structures we have initiated a study of the numbers of neurons present in the regio superior of the hippocampus in the rat during early postnatal life. This field was chosen not only because it is relatively small compared to other cortical fields but also because its borders are easily identified even at very young ages.

Sprague-Dawley rats of various ages were anesthetized and then perfused with 10% neutral buffered formalin. The brains were removed and routinely processed for paraffin sectioning at ten microns. A one-in-five series of sections was mounted and stained with thionin. The total volume of the pyramidal cell layer of the regio superior was determined from areal measurements made from serial tracings on a digitizing tablet and a computer, and sample neuronal density counts were made throughout the field. From the measurements made thus far the mean volume of the regio superior in animals killed on either the day of birth (P0) or on P2 is about 0.245 mm^3, while by P50 the volume has increased to a mean of 1.02 mm^3. The mean corrected neuron density in the regio superior of the P0/P2 animals is 1.74×10^6 neurons/mm^3, but in the P50 animals it is reduced to only 0.374×10^6 neurons/mm^3. Combining these measurements yields a mean total number of 4.24×10^5 neurons in the regio superior at P0/P2, and of 3.83×10^5 neurons in the P50 animals. This difference (which amounts to only 10%) is not statistically significant. Thus, if there is any loss of neurons from the regio superior during postnatal development, it is substantially less than that found in most regions of the nervous system that have been studied so far, where half or more of the initial population of neurons dies. It remains to be seen if other regions of the cerebral cortex, or even other fields of the hippocampal formation, show comparable minimal neuronal loss during development.

135.13 REDUCTION OF OPTIC NERVE AXONS DURING THE POSTNATAL DEVELOPMENT OF THE ALBINO RAT. D. Crespo*, D.D.M. O'Leary and W.M. Cowan (SPON: C. Asanuma). The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

We have estimated the numbers of optic nerve fibers in a series of albino rats from the day of birth (P0) through postnatal day (P)28. The animals were perfused transcardially with saline followed by a fixative containing 1.5% paraformaldehyde, 3.0% glutaraldehyde, 0.6% acrolein and 0.6% DMSO in a 0.1 M cacodylate buffer (pH 7.3). The optic nerves were then dissected out and were postfixed in the same solution, after which they were rinsed in buffer, osmicated, and embedded in Spurr's resin. Semi-thin 1 µm, and ultrathin, sections were cut from about the midpoint of each nerve. The thin sections were mounted on formvar coated slot grids and were examined and photomicrographed on a Zeiss 109 electron microscope. Montages of the whole nerve section were prepared at a magnification of 1900X and analyzed on a digital drawing tablet. The total cross-sectional area of the optic nerve was determined, and the area occupied by large glial processes, cell bodies and blood vessels subtracted. High power photomicrographs (X9000 to X25,000 depending on the age of the animal) were taken of each nerve from areas relatively free of glial processes and covered about 12% of the total cross-sectional area. From these the mean density of nerve fibers was determined, and the number of optic axons in each nerve was calculated.

At birth, the mean number of optic nerve axons was 273,744 ± 20,973 (S.D.). By P3 this number had decreased to about 203,000 fibers, and declined further to 167,665 ± 5903 by P7 During the second postnatal week the number of optic nerve fibers had stabilized at just over 100,000 (103,090 ± 2741 at P14 and 105,809 ± 7610 at P28). This represents a loss of approximately 60% from the number found at birth. In animals from which one eye had been enucleated at birth the number of axons in the remaining optic nerve was found at P28 to be 109,049 ± 3978; this is not significantly different from the number seen in normal P28 rats.

Our findings indicate that the number of optic axons in the albino rat undergoes a 60% reduction during the first two postnatal weeks, and that this reduction is not affected by early removal of the opposite eye.

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136.1 RESTORATION OF GROWTH RELATED-ORDER OF REGENERATED OPTIC AXON FASCICLES IN GOLDFISH. C.A.O. STUERMER, MPI Entwicklungsbiologie, D7400 Tuebingen, FRG.

We asked whether regenerating axons in goldfish tectum reestablish a similar growth-related fascicle order in Stratum opticum (SO) as recently discovered for normal fish (J.Neurosci '84). Normal individual fascicles in dorsal (ventral) hemitectum are composed of axons derived from half-annular regions in ventral (dorsal) hemiretina. Short rostral fascicles derive from central, more peripheral fascicles from more peripheral half-annuli. From each fascicle, axons depart in order, the most temporal axons first, the most nasal ones last.

Regenerated axons were found to reform fascicles in SO, but less orderly.

To test the fascicle order, HRP was applied to the cut ends of regenerated fascicles at various sites in the dorsal tectum (N=22). Two days later the whole-mounted contralateral retina was reacted to show retrogradely labeled ganglion cells.

In 15 retinae most labeled ganglion cells were arranged in a partial annulus or arc in the ventral hemiretina, centered on the optic disc. The arc ranged between 1/6 (i.e. normal) to 1/3 (wider than normal) of radial width. Randomly scattered cells outside the arc were found in addition, mostly with rostral application sites.

When fascicles were labeled in the proximal half of their tectal trajectory, the arc extended roughly 120^0 through ventro-temporal and -nasal retina. Short rostral fascicles gave arcs close to the optic disc. More peripheral fascicles gave more peripheral arcs. Thus, the majority of regenerated axons that originally clustered together tend to regroup again and course in similar tectal positions as normally. The presence of scattered cells indicates that some aberrant axons previously not associated with the fascicle had joined it during regeneration.

When fascicles were labeled in the distal half of their tectal trajectory, the arc was 90^0 or less and always confined to the ventro-nasal retinal quadrant. Scattered cells were rare.

These results indicate that regenerating axons, associated into the fascicle, exit in normal order: the most temporal axons first, the most nasal axons last. Thus regenerated optic axons in fascicles in SO seem to follow similar pathway rules as normal axons, however with less precision.

136.2 OMMATIDIA ARE ADDED ANTERIORLY TO LIMULUS LATERAL EYES DURING POSTEMBRYONIC GROWTH. J.J. Marler* and R.B. Barlow, Jr. Marine Biological Laboratory, Woods Hole, MA, 02543.

The lateral eye of Limulus polyphemus is well-suited to studies concerning the establishment of retinotectal connections, given that a retinotopic map has been described for this preparation and that ommatidia continue to be added to the eye during the postembryonic growth of this animal. To determine the site(s) of ommatidial addition to the eye, a retinal scarring technique was employed to create landmarks that could be distinguished in individual animals before and after molting. An array of 2-3 scars (each destroying 5-10 ommatidia) was made over the anterior edges of eyes of 6th stage juvenile animals and their eyes were photographed before and after molting. Scarring sites were distributed over the anterior margins of the eyes because of observations that anterior ommatidia in juveniles have smaller facet diameters than more posterior ones, that fault lines exist in the hexagonal packing of ommatidia near the anterior edge and that small rows of ommatidia are occasionally evident beneath the translucent carapace of pre-molt juveniles.

The results yielded by comparison of pre- and post-molt eyes are: (1) ommatidia are added to the eyes of these juveniles in vertical strips, (2) new units show dorsoventral size differences with larger units added ventrally, (3) diameters of existing ommatidia increase during growth, and (4) in a single individual, the rate of ommatidial addition may vary between the two eyes. The possibility that ommatidia are also added in other regions of the retina cannot as yet be ruled out.

This study provides a starting point for establishing how added retinal photoreceptors become organized in the lateral optic nerves and optic ganglia. As a result of their postembryonic growth, the eyes of Limulus, like those of the goldfish and hemimetabolous insects, are useful model systems for studying chronotopic aspects of the development of retinotopic projections.

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136.3 MORPHOLOGY AND QUANTITATIVE DIFFERENTIATION OF RETINAL GANGLION CELLS IN THE GOLDFISH. Peter F. Hitchcock and Stephen S. Easter, Jr. The University of Michigan, Ann Arbor, MI 48109 and The Salk Institute, La Jolla, CA 92138.

Retinal ganglion cell morphology was studied using retrograde transport of horseradish peroxidase applied intraorbitally to the severed optic nerve. This produced extensive, Golgi-like filling of ganglion cells that were viewed in the retinal wholemount. Ganglion cells can be placed into at least three classes (types 1-3). Type 1 cells have small somata, 1-3 thin primary dendrites and small dense arbors. Type 2 cells have large somata, 2-5 thick primary dendrites and large moderately dense arbors. Type 3 cells have small somata, 1-3 thin primary dendrites and large sparse arbors. Each type consists of several subtypes based upon the pattern of dendritic stratification within the inner plexiform layer. Infrequent examples of each type are found with their somata displaced into the inner nuclear layer.

Johns has shown that in the goldfish, retinal growth results from stretch of the existing retina and the addition of concentric rings of new neurons at the retinal margin. Thus, retinal position, from disc to margin, corresponds to decreasing neuronal age. As a measure of the developmental changes occurring in the goldfish retina, we have studied quantitatively, using a computer assisted microscope, the morphology of one subtype of type 2 cells as a function of retinal position. Also, to study changes as a function of retinal stretch, comparisons have been made between neurons matched for position in the retinae of a small and large fish. Intraretinal variation (24 cells): Within the central retina (up to approximately 0.7 x maximum distance from disc to margin) of a small fish (3.9 cm body length (bl) 1.6 mm lens diameter (ld)), the total dendritic length, number of branch points, and area of the arbor are constant. More peripherally, all three variables decrease progressively. Interretinal variation: The size of the soma, diameter of the dendrites, total dendritic length, and area of the arbor are all much larger in the retina of a large fish (14.5 cm bl, 4.0 mm ld, 17 cells) than in a small fish (4.0 cm bl, 1.8 mm ld, 19 cells). But within central retina, the number of branch points are similar in the two retinas. Comparisons of average inter-branch point distances show that the increase in dendritic length between small and large retinae results from inter-branch point dendritic growth. Furthermore, the magnitude of this interstitial growth closely matches the stretch with the enlargement of the small retina.

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136.4 Development of the optic tract in a cichlid fish, Haplochromis burtoni. J. Presson, L. Shelton*, and R. Fernald. Neuroscience Institute, University of Oregon, Eugene, Oregon, 97403

In the adult cichlid fish H. burtoni the organization of the optic fiber pathway changes dramatically between the optic nerve and the optic tract (Scholes, '79; Presson et al., '84). The nerve is a retinotopically organized sheet of fibers that undergoes a reorganization caudal to the chiasm to form the 3 components of the optic tract: the marginal (MOT), axial (AxOT), and medial (MdOT) optic tracts. This rearrangement of optic fibers is critical for the establishment of ordered connections between the eye and brain, since it places fibers in appropriate positions to contact their targets in the tectum, pretectum, and diencephalon. In order to better understand the factors that allow this reorganization we have begun a study of the development of optic fiber pathways in H. burtoni. Retinal ganlgion cell axons have been labelled with cobaltous lysine (Springer and Prokosch, '82). Thus far we have successfully labelled optic fibers in animals as young as 6 days, post fertilization. At this age most of the optic fibers are in what will be the lateral-most portion of the dorsomedial MOT. These early fibers enter the superficial layers of the lateral optic tectum. A small number of fibers travel more caudally to enter the lateral tectum via the ventrolateral MOT. Also at day 6, a few fibers are seen medial to the MOT, taking a straight dorsal course to terminate adjacent to the periventricular layer of the optic tectum. These probably constitute the first fibers in the MdOT. The AxOT is not distinguishable at day 6, but is at day 11-12, when there are a few labelled fibers in the AxOT. Fibers accrue to the AxOT during development, but the process does not continue throughout the growth of the visual system, since in adults the AxOT contains fibers from central but not peripheral retina.

These findings suggest that optic fibers are making active pathway choices even in very early development. Further question need to be answered, however, before accepting this conclusion. How precise is the retinotopic ordering among early optic axons? When is the retinotopic reorganization within the MOT first apparent? Are there structural features in the embryo that might account for the apparent pathway choices of optic fibers? Our progress in answering these questions will be reported.

136.5 FIBER ORDER IN THE OPTIC NERVE AND OPTIC TRACT OF THE CATFISH. A.A. Dunn-Meynell* and S.C. Sharma (SPON: A. Rothballer). Ophthalmol. Dept., New York Med. Coll., Valhalla.

In the catfish Ictalurus punctatus, ganglion cell fibers leave the retina via a ring of 10-14 separate optic papillae, then merge into a single optic nerve outside the retina. Each papilla in this ring receives its fibers from a roughly pie-shaped sector of retina. By severing individual papillae before they merged and applying horseradish peroxidase, we followed axons from ganglion cells in each retinal sector through the optic nerve and optic tract.

As the papillae merged, a large glial partition formed extending ventrally from the dorsal edge of the optic nerve, splitting the nerve into a U shape. There was, however, no evidence that fibers were arranged chronotopically from the tip of one arm of the U to the other as in cichlids. Instead young and old fibers from individual papillae tended to remain together. Some individual variability was seen, however the following fiber arrangements were seen at the optic nerve head: Fibers from dorso-temporal papillae appeared to lie along the lateral edge of the lateral arm of the U; Extreme dorsal papilla fibers lay on the lateral arm, but were positioned medial to the dorso-temporal papilla fibers, abutting the glial partition; Adjacent to the glial partition on the medial side of the nerve were fibers from dorso-nasal papillae; Fibers from papillae in the ventral half of the ring lay ventro-laterally (where fibers from ventro-temporal papillae were seen), ventrally, ventro-medially, medially and dorso-medially. As the nerve travelled towards the brain, fiber movements took place so that before the chiasm was reached the ventral retinal fibers were confined to the medial half of the nerve and the dorsal retinal fibers to the lateral half. As the optic nerve neared the chiasm, the U unfolded so that the central edges of the arm became dorsal and the dorsal tips of the arms became medial and lateral edges. Fibers from the dorsalmost papilla therefore lay on the dorsal surface of the lateral half of the nerve. After passing the chiasm, the medial fascicles of the optic tract (serving mostly diencephalic nuclei) seperated out. These fascicles were made almost exclusively from fibers from the dorsal half of the retina, with the largest contribution from the dorsalmost papilla. The rest of the optic tract then rotated 90° as it ran along the wall of the diencephalon so that fibers from the dorsal half retina lay ventrally in the tract and fibers from the ventral retina lay dorsally. These positions were retained as the tract ran into the tectum.

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136.6 DISTRIBUTION OF LAMININ IN THE DEVELOPING VISUAL SYSTEM OF THE CHICK. S.C. McLoon. Dept. of Anatomy, Univ. of Minnesota, Minneapolis, MN 55455.

Evidence from several recent studies strongly suggests that the growth cones of retinal ganglion cell axons grow along the external limiting membrane of the central nervous system. This may have important implications in establishing a retinotopic pattern of axons in the optic nerve and tract. Chemical substrates that might mediate the affinity of retinal axons for the external limiting membrane are yet to be identified. We have examined the developing chick visual system for the presence of various glycoproteins that could mediate this interaction. It was deemed that a likely candidate for this role should be present within the visual pathway during the time the retinal axons are growing, be confined to the external limiting membrane, and have demonstrated ability for supporting retinal neurite growth in tissue culture. So far laminin is the only molecule we have found which meets all these criteria. Previous studies have shown that laminin can suppport neurite outgrowth from retinal cells in tissue culture (Rogers et al, Devel Biol 98:212). Immunohistochemical techniques were used to study the distribution of laminin in the developing visual system. Chick embryos between four and twenty days of incubation were fixed with paraformaldehyde, and the retinas and brains were sectioned frozen. The sections were incubated with affinity purified rabbit antibodies against laminin (from Dr. Leo Furcht) followed by incubation with FITC labelled goat anti-rabbit IgG. At every age examined the external limiting membrane of the retina, optic stalk, optic tract and tectum were positive for laminin. A laminin- positive bridge was present at the top of the optic fissure in the retina connecting the optic fiber layer and surface of the optic stalk. EM immunohistochemistry revealed that the greatest concentration of laminin was in the external basal lamina of the external limiting membrane, but the internal surface of this membrane, which is in contact with the optic growth cones was also positive. Thus laminin is appropriately distributed, both spatially and temporally, to play a role in the adhesion of optic growth cones to its substrate during development.

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136.7 LONG-LASTING CHANGES IN VESICLE DISTRIBUTION WITHIN REGENERATED OPTIC FIBER TERMINALS OF GOLDFISH. Jeffrey D. Radel and M. G. Yoon. Dept. of Psychology, Dalhousie University, Halifax, N.S. Canada B3H 4J1.

The vesicles within optic fiber terminals of normal goldfish cluster near the pre-synaptic membrane of retino-tectal synapses. Newly regenerated optic fiber terminals examined 1 month after optic nerve crush (ONC) show changes in the distribution and number of vesicles within the terminal axoplasm. The present study examines whether these changes would revert back to normal at a later time.

One, 4, 8, 12 and 16 months after unilateral ONC, both tecta were processed for ultrastructural examination of the stratum fibrosum et gresium superficiale layer. Optic fiber terminals were identified using a series of ultrastructural criteria validated by both degeneration and HRP-labelling techniques: irregularly-shaped terminals containing 1) electron-lucent mitochondria with dilated cristae, 2) multiple monadic synapses and 3) clear, round vesicles 50 um in diameter. The area densities of vesicles throughout the entire terminal (axoplasmic vesicle density, AVD) and near each retino-tectal synapse (synaptic vesicle density, SVD) were calculated for each of the 5 time points.

The densities for the terminals in the regenerated side of each pair of tecta were compared with those of the intact side. AVD's were greater in the regenerated side than in the intact side at all time points. The magnitude of the change in AVD's for the 4, 8 and 12 month groups was larger than that of the 1 and 16 month groups. In contrast, the SVD's did not show any significant difference between the two sides at the 1, 4, 12 and 16 month time points. In the 8 month group, however, the SVD for the regenerated side was greater than that of the intact side.

The present results indicate that the changes in the vesicle distribution within regenerated optic fiber terminals persist for at least 16 months after axotomy of the optic nerve. Furthermore, these changes are likely due to an increased influx of vesicles into the regenerated terminals.

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136.8 METAMORPHIC CHANGES IN THE NUCLEUS ISTHMI IN RANA PIPIENS. P. Grobstein, M. Hollyday, and A. Berkowitz.* Dept. Pharm. Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

Tadpoles have laterally placed eyes, while frogs have more frontally placed eyes and a large frontal binocular field. Related differences exist in the organization of nucleus isthmi. In the frog, the cortex of the nucleus consists of several cytoarchitecturally distinct regions which project to different tectal targets. Anterior cortex projects to the ipsilateral tectal lobe. Posterior cortex consists of two subregions, both projecting to the opposite tectal lobe. A dorsalateral subregion projects to loci representing monocular visual field. A ventromedial subregion, having distinctive morphology and termed the "rim cortex," projects to loci representing binocular visual field. We have been studying the transformation from the organization of the nucleus isthmi seen in the tadpole to that seen in the frog.

Total cortical surface area in the tadpole increases slowly until stage XVII, shortly before the onset of dramatic external metamorphic change. During this period, rim cortex represents a roughly constant 10% of total cortex and 20% of posterior cortex. Between stage XVII and the completion of metamorphosis there is a rapid 2-3 fold increase in total cortical surface area. Most of this increase is due to an increase in the area of the posterior cortex and, in particular, of the rim cortex.

^{3}H-thymidine studies indicate that neurons of the post-metamorphic nucleus isthmi are produced at all larval stages. In general, earlier born cells were found more ventrally and later born cells more dorsally. There was however a substantial difference between anterior and posterior cortices, with later born cells found almost exclusively in the latter. Cells born at stages shortly before and during the rapid growth phase were found primarily in the dorsal part of the posterior cortex; cells of the ventrally located rim cortex are born much earlier.

Metamorphic change in the nucleus isthmi involves relatively enhanced growth of the posterior cortex and, within it, of the rim cortex. Sequential addition of newly born cells appears to play a significant role in the growth of the posterior cortex but not of its rim cortex subregion. Our results thus suggest that growth of the rim cortex represents a reorganization of a pre-existing population of cells. Deprivation of binocular experience by unilateral enucleation does not prevent metamorphic increases in rim cortex. It does however produce quantitative alterations in the growth of the various cortical regions. These are currently under study. Supported by NSF BNS 8311929, and the Brain Research Foundation.

136.9 AXON COUNT IN THE DEVELOPING OPTIC NERVE OF THE NORTH AMERICAN OPOSSUM: OVERPRODUCTION AND ELIMINATION.
M. A. Kirby and P. D. Wilson, Dept. of Psychology, University of California, Riverside, Riverside CA 92521.

The number of axons in the developing optic nerve of the opossum (Didelphis virginiana) was determined by electron microscopic examination in nine animals ranging in age from postnatal day five (P5) to postnatal day fifty-nine (P59). (Gestation in this species is 13 days). Animals were anesthetized by hypothermia and/or sodium nembutal, perfused with phosphate buffer (ph 7.2), followed by 4% paraformaldehyde-2% gluteraldehyde in the same buffer. Following dissection of the nerves with eyes attached, the tissue was left in the aldehydes at room temperature for 24 hr. and processed for electron microscopic analysis. Electron micrographs were taken of all grid square openings containing neural tissue. All axons and growth cone profiles were counted for each micrograph and the total number of optic fibers estimated from the cross-sectional area of each nerve. At P5 the cross-sectional area of the nerve is only 1.0% of the adult and contains about 24,000 optic fibers, or slightly less than 1/4 of the mean adult value. Over the next three weeks the number of optic nerve fibers increases rapidly, reaching 87,000 by P9, and 250,000 by the end of the third postnatal week. Maximum counts were obtained in our material at P27, with a population at this time estimated at 267,000, or about 2.7 times adult mean values. During this time axon diameters remain relatively uniform while presumed growth cone profiles, numerous at early postnatal periods, steadily decrease to less than 1% of the optic fiber profiles at P27. Over the next week the number of axons rapidly decreases, reaching 129,000 at P36. From P36 on, the rapid decrease in axon counts slows, obtaining adult values between P50-59. The first myelinated fibers are present around P50 (less than 0.1% at P50), while at P59 axon counts are near adult values (112,000) with 11% of the axons myelinated. Present at early postnatal periods, but absent in the adult, are numerous septa that divide optic fibers into discrete, round fascicles. The number of processes within these fascicles increase until the third postnatal week (100-200), and then decrease in number, until by P50 only a few (10-20) axons are found in each fascicle, by P59 no obvious fasciculation is present. In summary, the data presented here provide evidence that overproduction and elimination of optic nerve fibers occurs in polyprotodont marsupials, and is similar to that observed in several species of placental mammals.

136.10 RAPID POSTNATAL ESTABLISHMENT OF TOPOGRAPHY IN THE HAMSTER RETINOTECTAL PROJECTION. G.E. Schneider and S. Jhaveri. Whitaker College & Dept. of Psychology, M.I.T., Cambridge, MA 02139.

We have previously reported (Schneider et al., Neurosci.Abstr.,'81) the appearance of widely branching single axons in the superior colliculus (SC) of neonatal hamsters. These primitive axons undergo a progressive restriction of extraneous branches while concurrently augmenting and elaborating single terminal arbors, a sequence well underway by the 5th postnatal day (P5). The current study was undertaken to examine how this behavior of single axons translates into the formation of topographic connections by populations of retinofugal axons.

Neonatal hamsters, P1-P7, were anesthetized by hypothermia and subjected to unilateral insertions of small, crystalline pellets of HRP-conjugated wheat germ agglutinin into the SC. Following survivals of 15-20 hours, animals were sacrificed and the brains and contralateral retinae processed for visualization of the HRP-WGA according to the TMB method of Mesulam. During histological processing of the retinae, care was taken to record the proper orientation. The SC was reconstructed, from serial sections, to determine the extent and location of the injection site. Retinae were treated likewise to determine the exact position of retrogradely labelled ganglion cell bodies.

Results indicate that by P2, the spatial distribution of retinotectal axons is established for the upper-lower axis of the eyeball (lateral-to-medial axis of the SC). This corresponds with the appearance of early axons as seen with Golgi methods, since most of the widespread branching occurs in the anteroposterior (and not the mediolateral) extent of individual retinofugal axons.

Nasotemporally distributed retinal ganglion cells give rise to axons which will eventually terminate in caudal-to-rostral order in the SC. Our initial cases indicate that at P2, these axons exhibit a coarse, adult-like nasotemporal topography superimposed on considerable scatter. By P4-P5, the mature organization in both axes appears to be established.

The early appearance of coarse topography in the nasotemporal axis is difficult to explain in terms of the morphology of single axons, whose widespread branching would predict less organization in this axis. The possibility that this observation results from an unequal uptake of the HRP-WGA by different portions of the branching axon is currently being investigated.
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136.11 POSTNATAL TRANSFORMATIONS OF THE CONTRALATERAL RETINO-COLLICULAR PROJECTION IN THE MOUSE M.A. Edwards and V.S. Caviness, Jr. Southard Lab., E.K. Shriver Ctr. and Dept. Neurol., Mass. Gen. Hosp., Boston, MA 02114

Rostrocaudally-oriented axon bundles which course through the superficial gray stratum (SGS) of the neonatal mouse superior colliculus (SC) are eliminated during the first postnatal week (Edwards et al, Neurosci. Abst. 7, 733,'81). In the present study, the rate and magnitude of this process were analysed and correlated with changes in bundle number in underlying stratum opticum (SO) and with the invasion of SGS by collaterals from axons in SO. Analysis is based upon Cajal-de Castro normal fiber impregnations of 3-6 cases each at E17, P0, P2, P4, P6, P10, P14, P20, and >P60. Throughout postnatal development, SO can be distinguished from SGS by its greater bundle density or, on P0, by larger bundle size. These empirical distinctions correspond well with the delineation of SO in Nissl material. Measures of bundle density in SO and SGS in a central transverse sector of SC were expressed per equal-width column through these layers. This method corrects for their dramatic radial growth (>400%), which occurs at a parallel rate in the two stata largely between P0 and P10. Growth in tectal width and length is relatively much smaller (<30%). Between P0 and P6, bundle number in SGS declines steadily to near zero from a maximum roughly 2/3 of that present in SO at maturity. Concurrently, the number of bundles in SO roughly doubles after P0 to approximately adult values on P4. The density of individual, non-fasciculated axons in SGS, measured on the same samples, increases sharply to 80% of adult values by P6, with a slower rate of increase continuing until P20. The retinal origin of the large majority of both axon bundles and individual axons in SO and SGS during the first postnatal week is demonstrated by their virtual absence two days following eye removal on P0 and P4. These observations raise the possibility that the elimination of the set of optic axons coursing through SGS is somehow linked to the invasion of SGS by collaterals from axons in SO. Remaining for future work is to determine whether the axon elimination within SGS is associated with a normal process of ganglion cell death or instead represents a rearrangement in the pattern of projection of individual axons. (Supported by EY04549).

136.12 RETINOTOPIC EXPANSION AND COMPRESSION IN THE RETINOTECTAL SYSTEM OF GOLDFISH IN THE ABSENCE OF IMPULSE ACTIVITY.
R.L. Meyer and L.L. Wolcott. Developmental Biology Center, University of California, Irvine, CA 92717.

Perhaps the most widely offered explanation for how optic fibers achieve topographic order during expansion and compression and other types of plasticity is that a position dependent fiber to tectum chemoaffinity generates rough topography while a fiber to fiber interaction produces retinotopography independently of tectal locus. The strongest candidate for this fiber-fiber interaction is activity dependent sorting. To test the role of impulse related interactions in plasticity, we eliminated impulse activity by periodic intraocular injections of tetrodotoxin (TTX) during the period expansion or compression would normally occur.

For expansion, the temporal half of retina was removed and the optic nerve was crushed. At 2 months, electrophysiological mapping showed that the remaining nasal half of retina had expanded retinotopically across most of tectum. Only the far anterior end of tectum was without apparent innervation. The extent of expansion and the overall orderliness of the projection was essentially the same in fish with TTX blockade and those without TTX. Expansion was confirmed with autoradiography.

For compression, the posterior half of tectum was removed and the optic nerve crushed. At 2-3 months, electrophysiological mapping demonstrated that most of the retina was compressed onto the anterior tectal remnant in topographic fashion, though as previously reported, compression was incomplete in that the periphery of temporal field was missing. Again, the extent of compression and topographic ordering was essentially the same with and without TTX. If the optic nerve was not crushed, compression was minimal with or without TTX.

We conclude that activity is not responsible for generating topography during expansion and compression and suggest that there may be no interfiber interaction with such a role. (Supported by RHS-NS 16319.)

136.13 DEVELOPMENT OF THE OPTIC FIBER PROJECTION TO THE AMPHIBIAN TECTUM: AN HRP STUDY. S. H. Royer and P. Grant. Department of Biology, University of Oregon, Eugene, Oregon 97403.

In Rana optic fibers are known to project into at least 7 laminae within layers 7-9 of the tectum. We have compared development of the retinotectal projections and synaptogenesis within these laminae in Xenopus laevis and Rana pipiens using HRP tract-tracing techniques at the light and electron microscopic levels. Lesioned eyes of Xenopus and Rana at various developmental stages were filled with HRP and reacted with DAB plus Co and Ni to visualize the retinal ganglion cell (RGC) projections. It was necessary to exert care in interpreting the results, since HRP-filled RGC projections varied with developmental stage, survival time, and region of the tectum.

Though the number of optic fiber laminae is about the same in Xenopus and Rana adult tecta, they are much less discrete in Xenopus, and a distinct lamina G is absent. In early Xenopus tadpoles the RGC fibers form a diffuse projection in the tectal neuropil. In later development a single dense lamina of fibers appears deep in layer 9 and the upper part of layer 8 in addition to diffuse projections in layers 7-9. This pattern has been observed in tadpoles as early as stage 48, when the cellular lamination of the tectum has begun, and is well developed by stage 54/55. The more superficial laminae appear during metamorphosis, and the adult pattern is seen in juvenile Xenopus shortly after metamorphosis. These observations suggest a chronotopic ordering of RGC inputs within the optic neuropil of the tectum with the oldest fibers in deep laminae and the youngest ones located more superficially. In contrast, a rudimentary adult pattern of RGC fiber lamination develops much earlier in Rana tadpoles, as early as stage XI (comparable to Xenopus stage 55).

Electron microscopic examination of HRP-filled tectal neuropils at different stages in Xenopus reveals labelled RGC terminals similar in size and morphology to those previously described for Rana. A study of synaptogenesis in layer 9 suggests a rostro-caudal gradient of maturation of optic fiber terminals, with the most mature terminals in rostral tectum. A deep-to-superficial maturation gradient is also seen in layer 9, with the most mature synapses in the deeper regions. These observations are consistent with a chronotopic ordering of the tectal optic fiber neuropil in depth as well as along the rostro-caudal axis.

(Supported by NIH Grant EY02642-05 awarded by the National Eye Institute.)

136.14 THE PATHWAYS OF FIBRES FROM TRANSLOCATED EYES IN XENOPUS. J.S.H. Taylor*, D.J. Willshaw* and R.M. Gaze*(SPON L. Laemle). Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK.

In normal Xenopus the retinal ganglion cell fibres arising in each quadrant of the retina travel in a characteristic fashion to the optic tectum, where they terminate in a retinotopic order (Fawcett, J.W. and Gaze, R.M., 1982, J. Embryol. Exp. Morph. 72: 19-37). We present a recent investigation of the distribution of fibres in the optic tract of Xenopus in which eyes have been translocated in embryonic life from a right to a left orbit without rotation. This operation results in a visuotectal projection which, when recorded electrophysiologically, is normal dorsoventrally but inverted nasotemporally (Gaze, R.M., Feldman, J.D., Cooke, J. and Chung, S.-H., 1979, J. Embryol. Exp. Morph., 53: 39-66).

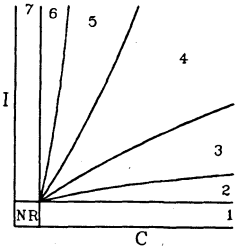
Labelling of small groups of retinal axons with horseradish peroxidase showed that the fibre trajectories from dorsal and ventral retina were normal, whereas fibres from nasally placed retina had diencephalic pathways and tectal terminations typical of temporal fibres, and fibres from temporally placed retina had diencephalic pathways and tectal terminations typical of nasal fibres. Thus from just beyond the chiasm the fibres seem to have already achieved the majour uniaxial rearrangement necessary to establish a normal tract distribution despite the eye translocation. The fibre sorting required to permit the formation of a nasotemporally inverted visuotectal projection appears to occur not on the tectum or in the optic tract, but either within the nerve or in the chiasma.

This research was funded by the Medical Research Council of Great Britain.

VISUAL CORTEX: DEVELOPMENT AND PLASTICITY

137.1 PLASTICITY IN VISUAL CORTEX: CRITICAL PERIOD OR CRITICAL STATE? *P.W. Munro*. Institute for Cognitive Science C-015, U.C.S.D., La Jolla, CA 92093

A new approach is taken toward the analysis of the plasticity of ocular dominance characteristics. The susceptibility of neuronal response characteristics to stimulus manipulation has been correlated with postnatal age [1]. The postponement of the so-called "critical period" in animals deprived of visual input [2] suggests that the *change* in synaptic connectivity is related to the *state* of synaptic connectivity. Consider an ideal neuron in striate cortex having net connectivities to the contralateral and ipsilateral eyes of C and I respectively. These values constitute the "ocular connectivity state" of the neuron. Depending on the transfer (input-output) function of the neuron, the ocular dominance classes can be mapped onto a state space or "ocularity plane" having axes corresponding to C and I as in the figure (NR: no response). Each neuronal state is represented by a point in the plane that traces a path as connections are modified during development. Several factors determine the instantaneous change of the connectivity state. Candidates include the rearing paradigm, norepinephrine concentration [3], and the state itself. The neuronal activity need not be included since its average value is assumed to depend principally upon the ocularity state and the ocularity of the input (i.e. the deprivation paradigm).



The neuronal connectivities C and I are assumed to take on very small initial values. Soon after eye-opening, visual input drives the ocularity state to the region where the ocular dominance categories converge (high plasticity). Patterned stimuli drive the state further from the origin, where migration between categories is relatively difficult. Without input the state travels more slowly if at all, and thereby retains plasticity. Ocular dominance plasticity factors into two components: the time rate of change of the ocularity state, and the change in ocular dominance with respect to the state. This is formalized as follows. Let the preference index p be defined in terms of the neuronal responses to a fixed contralateral stimulus and a fixed ipsilateral stimulus (p is a continuous measure of the ocular dominance). The observed plasticity can be thought of as the change in p which is given by the inner product between two vectors, namely the instantaneous change in the neural state and the gradient of p in the ocularity plane:

$$\frac{dp}{dt} = \frac{\partial p}{\partial C}\frac{dC}{dt} + \frac{\partial p}{\partial I}\frac{dI}{dt} = \frac{d\mathbf{s}}{dt}\cdot\nabla p \qquad \text{where } \mathbf{s} \text{ is the ocularity state } (C, I).$$

[1] D. H. Hubel and T. N. Wiesel (1970) *J. Physiol* 206:419
[2] M. Cynader, N. Berman, and A. Hein (1976) *Exp. Brain Res.* 25:139
[3] T. Kasamatsu, J. D. Pettigrew, and M. Ary (1979) *J. Comp. Neurol.* 185:163

137.2 INTRAVENTRICULAR INJECTIONS OF 6-OHDA DO NOT NECESSARILY PREVENT THE OCULAR DOMINANCE SHIFTS THAT USUALLY OCCUR IN THE VISUAL CORTEX OF KITTENS AFTER MONOCULAR DERIVATION. N.W. Daw, T.O. Videen, R.K. Rader, T.W. Robertson, Physiology Dept., Washington University Medical School, St. Louis, MO 63110.

This experiment had two purposes (1) to see if we could repeat the results of Kasamatsu and Pettigrew (J. Comp. Neurol. 185: 139-162, 1979) concerning the effects of intraventricular injections of 6-hydroxydopamine (6-OHDA) on monocular deprivation, and (2) to test whether the timing of the 6-OHDA injections in relation to the eye suture would affect the results. 6-OHDA is known to have non-specific effects as well as effects specific to the noradrenaline (NA) neurons. It seemed likely that non-specific effects would be more apparent during injections made after the destruction of NA neurons when the specific uptake of 6-OHDA had been abolished.

A cannula was implanted into the lateral ventricle (A11, L3, U6) of 4 to 5-week-old kittens. Daily doses of 6-OHDA were given, increasing from 200 µg to 1.5 mg, until a total dose of 7.4-12.4 mg had been injected. The eyelids of one eye were sutured a few days after starting the 6-OHDA injections. Exactly one week after eye suture, cells in the visual cortex were recorded and an ocular dominance histogram was constructed. Samples of visual cortex tissue were then removed for analysis of NA content by high pressure liquid chromatography. The rest of the brain was sectioned and checked for placement of the cannula and damage around the ventricle.

Three groups of animals were prepared. In four animals the 6-OHDA injections were stopped around the time of eye suture. In four animals, eye suture was done after five days of 6-OHDA injections, and injections were continued until recording. In two animals eye suture was done one day after the start of 6-OHDA injections, which were continued until recording.

In all animals the NA concentration was reduced by approximately 90%. In all animals the ocular dominance histograms were monocular and were similar to ocular dominance histograms from two animals with ascorbate injected instead of 6-OHDA. We conclude that intraventricular injections of 6-OHDA do not necessarily prevent ocular dominance shifts after eye suture in kittens. We were unable to reproduce the results of Kasamatsu and Pettigrew, in spite of trying 3 variations in the relative timing of 6-OHDA injections and eye suture.

137.3 EFFECT OF MONOSIALOGANGLIOSIDE INTERNAL ESTER TREATMENT IN MONOCULAR DEPRIVED KITTENS. G. Carmignoto*, R. Canella*, C. Comelli*, A. Gorio and S. Bisti*° (SPON: M. Vitadello). Fidia Research Laboratories, Dept. of Cytopharmacology, 35031 Abano Terme, Italy. °Istituto di Neurofisiologia, C.N.R. di Pisa, 56100 Pisa, Italy.

We previously reported (Carmignoto et al., 1983, Neuroscience Letters, abstract of the 7th ENA, S54) that monosialoganglioside treatment of molecular deprived kittens reduced ocular dominance shift in the primary visual cortex, suggesting that the treatment could have enhanced the maintenance of the deprived eye cortical projections. In this study we confirm and extend those early observations. In kittens deprived at the 35th day, monosialoganglioside internal ester (AGF2) treated kittens maintain 41.1% of cortical neurons with a binocular input (group 2-6) compared to only 21% of untreated kittens. In animals deprived at 17th day the effect of the treatment seems even more evident: in the AGF2 treated group 21% are binocular units compared to only 4% in the control group. Moreover, a morphometrical study on Golgi-impregnated relay neurons in the dorsal lateral geniculate nucleus (dLGN) suggests that in the deprived A laminae of the untreated kittens Guillery's class 1 and 2 neurons undergo a significant alteration of the dendritic tree and soma size, while the treatment seems to ensure, at least for class 2 cells, an almost normal development of the dendritic pattern and soma size. A diminished cortical plasticity could account for this effect, at least for the reduced ocular dominance shift. Nevertheless since several reports indicate that gangliosides are capable of stimulating neuronal differentiation, neurite outgrowth and sprouting in vivo and in vitro, and of interacting with neuronal membranes modifying the (Na^+-K^+)ATPase and adenylcyclase activities some tentative different hypothesis on gangliosides effects on visual cortical plasticity are discussed.

137.4 DEVELOPMENT OF GABAergic IMMUNOREACTIVITY IN MONKEY VISUAL CORTEX. A. Hendrickson. Depts. of Biological Structure and Ophthalmology, Univ. Washington, Seattle WA 98195.

Immunocytochemical techniques have been used to trace the development of neurons using gamma amino butyric acid (GABA) as a neurotransmitter by use of an antiserum to the GABA synthesizing enzyme glutamic acid decarboxylase (GAD). GAD antiserum was a gift from J-Y. Wu. Primary visual cortex has been examined at fetal days 138 and 160 and at 2, 8 and 52 weeks after birth. Paraformaldehyde-lysine-periodate fixed tissue was frozen sectioned and GAD was visualized by the indirect peroxidase-antiperoxidase technique. The same brains were also stained for the mitochondrial enzyme cytochrome oxidase (CO) by the method of Wong-Riley.

At F138 there is very little staining for GAD in visual cortex, but some cell bodies and processes can be identified in layer 1. CO shows heavy cell and light neuropile stain in all layers. By F160 GAD has increased in all layers so that neuropile label is detectible, especially in 1A, 4C and 6. GAD+ cell bodies and processes also occur widely. CO staining is mainly in the neuropile and differs from adult visual cortex in that 4A is not stained while 4C is subdivided into three bands. The inner and outer band are darkly CO+, but the midband, overlapping the outer half of 4Cβ, is much lighter. Enface sections show the CO+ dots to be present, but lightly stained compared to adult.

By 2 weeks after birth the amount of GAD has increased markedly in both cell bodies and neuropile of all layers. Perisomatic terminals are apparent on many neurons, some of which are GAD+. CO staining now shows 4A clearly, but 4C is still laminated. At 6 weeks the visual cortex is similar to 52 weeks in that there is heavy neuropile label for GAD in all layers, especially 1A, 3, 4A, 4C and 6. GAD+ cell bodies are more prominent at 6 than 52 weeks, occur in all layers and vary widely in size, indicating a variety of neurons are GABAergic in visual cortex. CO stain is similar at 6 and 52 weeks with no banding apparent in layer 4C.

This study indicates that GABAergic neurons and synapses in monkey visual cortex develop with a rapid timecourse, mainly in the first 6 weeks after birth. In contrast, CO staining is heavy well before birth but shows laminar differences from adult CO cortical staining patterns. The distribution of GAD and CO are quite different during development, but both reach adult levels and distributions by 6 weeks after birth. (Supported by EY01208 and the Dolly Green Scholar Award of Research to Prevent Blindness, Inc.)

137.5 STAINING PATTERN FOR CYTOCHROME OXIDASE (CO), ACETYLCHOLINESTERASE (AChE), AND SEROTONIN (5-HT) IN AREA 17 OF LONG-TERM MONOCULARLY DEPRIVED (MD) RHESUS MONKEY. M. Tigges, J. Tigges, J. R. Wilson. Yerkes Regional Primate Research Center and Dept. of Anatomy, Emory Univ., Atlanta, GA 30322.

In area 17 and the lateral geniculate nucleus (LGN), CO staining patterns different from normal have been reported in mature rhesus monkeys following monocular enucleation and TTX injections into one eye (for ref. see Wong-Riley and Carroll, Nature 307, 1984). The results imply that mature neurons in LGN and area 17 respond not only to denervation, but also to blockage of retinal activity in a system that seems structurally intact. We studied a rhesus monkey who had his right eyelid permanently sutured from 2 days after birth to 17 months of age. In electrophysiological explorations of area 17 of the contralateral hemisphere neurons responded predominantly to stimulation of the open eye. Subsequently, brain sections were stained for CO, for AChE (Hedreen et al, personal communication), and immunocytochemically with antibody to 5-HT (courtesy M. Molliver). In CO preparations of area 17, layers IVA and IVC were uniformly and uninterruptedly stained. Layers II/III exhibited the full complement of regularly spaced "puffs in rows". AChE-stained axons were present in all layers with distinct, continuous fiber plexuses in layers I, IVA and IVC, and deep VI. Serotonergic fibers formed a conspicuous laminar network; especially heavy, uniformly dense fiber concentrations were in upper layer IVC, the sparsest accumulations of fibers were in layers I and deep V. Thus, in the laminar staining pattern for CO, AChE and 5-HT of area 17 of the deprived monkey, our methods revealed no inhomogenities in staining intensity; the observed patterns closely resemble those of normal adults. In the LGN, in contrast, MD altered the normal Nissl and CO staining pattern: layers connected to the deprived eye were noticeably paler than those connected to the open eye. Thus, as a consequence of MD, morphological and cytochemical changes are prominent in the LGN, but, in spite of severe functional alterations, no dramatic structural and cytochemical changes were discernible in area 17 of the deprived monkey. These results suggest that area 17 in young rhesus monkeys reacts differently to sensory deprivation via MD than does area 17 in adult monkeys to denervation and impulse blockage.

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137.6 TIMING OF FLASH RESPONSES IN VISUAL CORTEX OF NORMAL AND STRABISMIC CATS: G.Eschweiler*, M.Popp*, J.P. Rauschecker*, W.Schrader* (SPON: U.EYSEL), MPI für biolog. Kybernetik, D-7400 Tübingen, FRG.

Single unit responses to flash stimuli have been studied extensively in the retina and the lateral geniculate nucleus. Not so much is known about their characteristics in the visual cortex. We have analyzed the timing of single neuron responses in area 17 of normal and strabismic cats to stationary flashing bars of light.

About 70% of the cells in striate cortex responded well to stationary flashing stimuli. However, no cell responded better to such stimuli than to moving bars. 20% of the flash responses exhibited very pronounced oscillatory behaviour with a repetition frequency of between 10 and 30 Hz and with an exponential decay of their secondary response amplitudes. In normal cats the response latencies to flashing bars ranged between 50 and 150 msec with a mean value of 70 msec. The scatter of the onset latencies was usually in the order of ± 10 msec or more. Even the sharpest responses showed a scatter of 6-7 msec. This is remarkable, if one considers the precision with which the visual system can evaluate latency differences in psychophysical tasks. It has to be concluded therefore that a temporal sampling mechanism is at work similar to the one postulated for the spatial domain (Barlow, 1979; Marr et al., 1979).

In cats with unilateral squint we found that the deviated eye drives cortical units with on average longer latency and with a larger scatter of response. This was more pronounced in non-granular layers suggesting an intracortical reduction in the efficiency of synaptic transmission. In the few remaining binocular units the response elicited through the squinting eye was always delayed as compared to the normal eye. The increased latency of the deviated eye's response can help to explain the permanent suppression of this eye in amblyopia. Furthermore, the decreased precision of spatio-temporal interpolation mechanisms may be the basis of the well-known phase-related deficits in strabismic amblyopia termed as "crowding".

137.7 INFLUENCE OF STROBE REARING ON THE RECEPTIVE FIELD PROPERTIES IN AREA 17 OF THE CAT. G.A. Orban, J. Cremieux*, J. Duysens and B. Amblard[1]. Laboratorium voor Neuro- en Psychofysiologie, K.U.L., Campus Gasthuisberg, B-3000 Leuven (Belgium) and [1]I.N.P.4 - C.N.R.S., 31 Chemin Joseph-Aiguier, F-13274 Marseille Cedex 2 (France).

Receptive field properties of area 17 cells were compared in normal and strobe-reared (2 Hz) cats over a range of eccentricities (0-45°). Receptive field (RF) width, end-stopping, RF type, binocularity and orientation tuning were measured with handplotting in 128 cells of strobe-reared animals and 453 cells of normal animals. RF width increased with eccentricity in strobe-reared and in normal animals, but at all eccentricities RF width of the strobe-reared cells exceeded that of the normal population. In area 17 of strobe-reared cats, the proportion of binocular cells and of end-stopped cells was strongly reduced. RF types and orientation tuning width were almost normal. Velocity characteristics, direction selectivity, response latency and strength were studied quantitatively with a multihistogram technique (N = 82 in strobes and N = 302 in normals). Direction-selective cells occurred in strobe-reared cats in smaller than normal proportions at all eccentricities. Response strength was not altered by strobe rearing and response latency showed only small changes. Changes in velocity sensitivity were restricted to the part of area 17 subserving central vision. The proportion of velocity low-pass cells was reduced from 57% to 18% in the strobe-reared animals. It is likely that the latter deficit is due to deprivation of long lasting visual stimuli which are required to activate velocity low-pass cells (Duysens et al., Vision Res., 24:17, 1984). Comparison with a previous study on area 18 (Kennedy and Orban, J. Neurophysiol., 49:686, 1983) shows that the changes induced by strobe rearing are less severe in area 17 than in area 18 and that these changes in area 17 are more restricted to the part subserving central vision.

137.8 DEVELOPMENT OF SPATIAL RECEPTIVE FIELD ORGANIZATION AND ORIENTATION SELECTIVITY IN KITTEN STRIATE CORTEX. P.Heggelund and B.O.Braastad*. Neurobiological Laboratory, University of Trondheim, 7055-Dragvoll, Norway.

The spatial organization of the receptive field of neurones in striate cortex of kittens between 8 days and 3 months of age was studied quantitatively by extracellular recordings. A static dual-stimulus technique was used to plot enhancement and suppression zones across the receptive field. Already in the youngest kittens the receptive fields were organized into adjacent enhancement and suppression zones like in adult cells. The relative suppression was as strong as in adult cells. The receptive fields were like magnified adult fields. The width of the various subregions within the receptive field decreased with age in the same proportion. The decrease followed the decrease of the receptive field center diameter of retinal ganglion cells described by Rusoff and Dubin (1977), indicating that the decreased cortical field size was caused by changes in the eyes.

Two kittens were dark-reared until recording at 1 month of age. The spatial receptive field organization in these animals was normal, and the field dimensions were the same as in normal kittens at 1 month. This indicated that the spatial receptive field organization is innate and that visual experience is unnecessary for the organization to be maintained and for the receptive field size to mature normally during the first month.

Orientation selectivity was determined quantitatively with moving slits. The half-width of the orientation tuning curves became narrower during the first 5 weeks. No difference with respect to this development was seen between the dark-reared and the normal kittens, so the orientation selectivity developed independently of visual experience the first month.

The most marked difference between the development of the cells in the normal and in the dark-reared kittens concerned responsivity and latency before firing. In the normal animals these properties improved rapidly over the first 4 weeks. In the dark-reared animals these properties remained at the level of 1-2 week old normal kittens, showing that responsivity and response latency of the cells required visual experience to develop.

137.9 THE POSTNATAL DEVELOPMENT OF ORIENTATION-SELECTIVITY IN AREA 18 OF THE CAT'S VISUAL CORTEX. David Price* and Colin Blakemore, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, England.

The maturation of neuronal function in kitten striate cortex has been extensively studied, but much less is known about the normal postnatal development of area 18, which has its own independent afferent input. We have recorded extracellularly from single units in area 18 in normal kittens, aged from 12 days to 10 weeks, and adult cats. The experimental procedures used were similar to those described by Blakemore and Van Sluyters (J.Physiol.,248:663,1975). Single units were isolated at regular intervals along each electrode track, and the response of each neurone to stationary and moving patterns projected on to a tangent screen was assessed.

Some cells, identified by their spontaneous activity, did not respond to any stimulus presented and were classified as unresponsive. Responsive cells were classed as non-selective, orientation-biased, or orientation-selective, and the preferred orientation of biased and selective cells was determined.

After each experiment animals were perfused and sections of the visual cortex were stained for cytochrome oxidase activity to allow identification of the 17/18 and 18/19 borders and reconstruction of each penetration from electrolytic lesions made along each track.

All types of cell were found at all ages in area 18, although very few responsive cells were identified in older kittens and adult cats. From 12 days onwards the percentages of unresponsive, non-selective and orientation-biased cells fell while the percentage of orientation-selective increased rapidly. By 4 weeks the percentages of unresponsive and non-selective neurones had decreased to the values seen in adult cats. The proportion of orientation-selective units increased rapidly between 12 days and 4 weeks, but a further slow rise occurred over the next 6 weeks until the value approached that seen in the adults. A gradual decrease in the percentage of orientation-biased cells was seen between 12 days and 10 weeks.

As early as 13 days the existence of a rudimentary columnar organization for the orientation domain was suggested by evidence of a progressive shift in the orientational preferences of selective and biased units along electrode tracks. This organization was much less distinct than in older kittens and adult cats. Overall, the maturation of cells in area 18 seemed similar in time-course and extent to that described for striate neurones, despite their separate parallel inputs.

Supported by MRC grant G979/49 to Colin Blakemore.

137.10 VISUAL TEXTURE SEGMENTATION IN NORMAL AND NEONATALLY LESIONED CATS. Frances Wilkinson & Cheryl McCormick*. Dept. of Psychology, McGill Univ., Montreal, P.Q., Canada H3A 1B1.

Remarkable sparing of visual function has been reported following neonatal ablation of cortical areas 17,18,&19 in cats. In the present study, however, we report a marked impairment in the ability of such cats to segment a visual image on the basis of textural discontinuity.

Three kittens sustained bilateral ablation of areas 17-18-19 before 1 mo. of age; two animals had small lesions lateral to area 19; four normal littermate controls were also tested. Prior to texture training, all animals were taught one pattern discrimination and visual acuity was assessed in 2 17-18-19 lesioned animals and their normal littermates, in the same apparatus and under the same lighting as the texture tests. No deficits were seen in the lesioned animals.

DETECTION TASK DISCRIMINATION TASK

Two aspects of texture segmentation were studied: 1) detection of textural discontinuity, and 2) discrimination of visual form on the basis of the virtual contours texture boundaries. The stimuli were composed of high contrast texture pairs (T1, T2) matched for mean luminance but differing in element microstructure or orientation. After pretraining on black/white solid-contour analogues, the texture stimuli were introduced and training continued to a maximum of 500 trials on each of 3 texture problems. Normals and lateral lesioned animals showed immediate transfer to the textured square detection task. With a single exception to be discussed, all 3 17-18-19 lesioned animals failed to acquire any of the texture detection tasks and therefore did not proceed to the discrimination phase. Of the 5 animals trained on square vs triangle discrimination, 3 showed rapid transfer to the textured form variants; one normal and one lateral lesioned animal failed to learn these problems in 500 trials.

Thus the ability to segment a visual scene on the basis of textural cues alone would appear to depend on the integrity of areas 17-18-19 (or some part thereof). Even after neonatal lesions, this ability is not spared.

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137.11 VERNIER ACUITY FOR GRATING STIMULI IN CATS. Sylvie Belleville & Frances Wilkinson. Dept. of Psychology, McGill Univ., Montreal, P.Q., Canada H3A 1B1.

The ability of human subjects to detect small vernier offsets in visual stimuli has received considerable attention recently. Vernier acuity in humans is better than 10" of arc, less than the diameter of a single retinal cone.

Vernier thresholds have rarely been measured in other species. Berkley & Sprague (J. comp. Neurol, 187:679-702, 1979) have reported vernier acuity measures for two normal cats using offsets in short single line targets. Thresholds were found to be 5' of arc in both cats. Vernier detection was found to depend critically on the integrity of cortical areas 17+18.

Vernier acuity in humans is relatively independent of the stimulus configuration used to measure it (single lines, gratings, arrowheads, etc.). In the present study we have assessed vernier acuity in cats using an offset in a square-wave grating as the stimulus.

The subjects were two normal cats, 3 and 5 months of age at the onset of training. A two choice jumping-stand discrimination procedure with food reinforcement was employed. Daily sessions of 40 trials were given. The stimuli were high contrast square-wave gratings produced on a laser printer (resolution: 1/300 in.). At the viewing distance of 47 cm, the spatial frequency of the gratings was .4 c/deg. The negative stimulus was a grating with no offset; the positive stimulus had an offset at the midpoint of each bar. A series of 18 different offsets were used ranging from 52.7' to 2.3' of arc. The area of each grating stimulus was 24 by 30 of visual angle.

In initial training with a very large offset stimulus, the cats required 665 and 435 trials respectively to reach a criterion of 27/30 correct. Thresholds were then tracked using a staircase procedure. Taking 70% correct performance as threshold, vernier acuities of 7' and 6.6' of arc were recorded in the two cats.

This finding confirms the earlier report of Berkley & Sprague using a very different stimulus, thus suggesting that vernier acuity is independent of stimulus configuration in the cat as in the human.

We have also attempted to train two young kittens on this task, so far without success (>2000 trials). One cat with bilateral neonatal ablation of areas 17-18-19 has also failed to learn the task.

Supported by NSERC grant A7551 to F.W.

137.12 THE CONTRIBUTION OF IMAGE MOVEMENT AND DISTANCE TO KITTEN VISUAL CORTICAL DEVELOPMENT: A PRELIMINARY INVESTIGATION. J. D. Daniels, D. J. Kraus* and E. K. Pressman*. Brown University, Providence, RI 02912.

We have shown previously that steady scotopic illumination is adequate to promote the normal cortical ocular dominance (OD) shift in monocularly deprived (MD) kittens while flickering scotopic illumination is insufficient to result in this plastic response (Daniels et al. Exp. Brain Res. 54:186-190 (1984)). One possible explanation for these results is that the visual discontinuity produced by the flicker does not allow a normal OD shift because it limits motion perception.

To test our hypothesis we raised 6 MD kittens viewing a distant, dimly lit suspended mobile that was either moved by a fan or fixed rigidly in place. The cage, the floor, ceiling and walls were ultra flat black and in virtual darkness. In both paradigms less than 50% of the cells examined were driven primarily by the open eye (46% for the moving mobile, N=73; 49% for the stationary, N=98), whereas in our control condition of scotopic illumination 67% of the units (N=136) were dominated by the open eye. The viewing of these distant objects, whether moving or stationary, is, therefore, insufficient stimulation to promote OD plasticity.

To increase the mobile's effectiveness as a stimulus, we halved the distance between it and the center of the cage (150 cm to 75 cm). At this position the mobile appeared four times larger and slightly more luminous while the inside of the cage remained in the shadows. Surprisingly, only 34% of the units (N=138) were open eye dominant. Since there was even less of a shift for the close mobile kittens, some aspect of the mobile arrangement itself may prevent the expected shift. We believe this aspect is that the mobile, which is outside or distant from the cage, is illuminated while the kitten's immediate environment is dark. Thus the kitten light-adapts to the distant mobile and is not adapted to its immediate surroundings.

Preliminary results from one kitten raised with the exact same lighting conditions as the other mobile kittens but with the mobile removed (65% open eye dominant, N=59) are comparable to scotopic rearing. This similarity supports our current hypothesis that lack of adaptation to the near environment disrupts cortical development.

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VISUAL CORTEX: STRIATE AREA I

138.1 LOCAL INTERACTIONS DETERMINE ORIENTATION AND DIRECTION SELECTIVITY OF NEURONS IN CAT VISUAL CORTEX. N.V.Swindale* and M.S.Cynader (SPON:J.D.Bousfield). Dept. of Psychology, Dalhousie University,Halifax, B3H 4J1, Nova Scotia, Canada.

Experiments in which spots and bars of light were flashed on the receptive fields of simple and complex cells in area 17 of the cats visual cortex suggest that both direction and orientation selectivity are achieved by temporally and spatially specific non-linear interactions beteen inputs originating from neighbouring beta ganglion cells in the retina. Interactions between ganglion cells that are not nearest neighbours seem to be relatively less powerful as determinants of orientation and direction selectivity. Our evidence for this is as follows:

1) Direction selectivity, determined from the response to a stroboscopically illuminated moving bright bar, breaks down when the spatial separation between flashes is greater than about a tenth of the receptive field width. The inter-flash distance for which direction selectivity disappears is about twice the spacing of the receptive field centres of ON (or OFF) centre beta ganglion cells in the retina (i.e. the spacing between cells that are nearest neighbours but one). In simple cells this distance is less than the distance between neighbouring ON and OFF subunits of the receptive field, making it unlikely that interactions between these subunits contribute to direction selectivity.

2) Orientation selectivity can be demonstrated with stationary flashed bars that are only 2 to 3 ganglion cell spacings long. Thus the anatomical ordering of ganglion cells is accurately preserved in terms of local cortical interactions that generate orientation selectivity.

3) Two bright spots aligned along the receptive field axis (on-axis) or perpendicular to it (off-axis), and separated by a distance equal to 1 to 3 ganglion cell spacings, were flashed with varying delays (-250 to 250 msecs) between the presentation of each spot. In the on-axis condition there was, in most cells, facilitation for synchrony, and inhibition for delay. In the off-axis condition there was always inhibition for synchrony, facilitation for delays corresponding to movement in the preferred direction, and continued inhibition for delays corresponding to movement in the null direction. For cells that were not direction selective there was equal facilitation for both positive and negative non-zero delays. This spatio-temporal pattern of integration of inputs from adjacent or closely adjacent ganglion cells can account for direction selectivity, and orientation selectivity to both moving and flashed stimuli.

138.2 BEHAVIORAL MEASURES OF ORIENTATION AND SPATIAL FREQUENCY "CHANNELS" IN THE CAT: A COMPARISON OF PSYCHOPHYSICS AND ELECTROPHYSIOLOGY. M.A. Berkley. Florida State University, Tallahassee, FL.

Our current understanding of the neural substrates of vision is based heavily upon comparisons of single neuron data, obtained primarily from cats, with human psychophysical data obtained with paradigms designed to reveal the existence of feature selective "channels". One such paradigm (aftereffects paradigm) measures changes in sensitivity to a test target after viewing an adaptation target of varying degrees of similarity to the test target. To more directly assess the relationship between putative feature selective "channels" and their possible neural substrates, an aftereffects paradigm was used to behaviorally measure spatial frequency and orientation selectivity in cats.

Cats were trained to view an adaptation field for periods up to 10 secs. After the adaptation period, a luminance-matched, bipartite test field (half consisting of a zero-contrast grating and the other half of a variable contrast grating) was briefly presented. The cat was rewarded for pressing a nose-key on the same side (L or R) of the test field which contained the variable contrast grating only when the target was present. The contrast, spatial frequency, and orientation of the adaptation field or the test field were varied and changes in correct detections recorded. From these data, frequency of detection functions were generated and thresholds for detecting the test grating estimated.

The results showed that: 1) detection thresholds for a grating were significantly elevated after viewing the gratings of the same spatial frequency and orientation; 2) the magnitude of the threshold elevation was monotonically related to the adaptation field contrast and adaptation time; 3) the threshold elevation effect was spatial frequency selective with a bandpass (½ width at ½ height) of about 1 octave; and orientation selective with a bandpass of 15°. Comparisons of the behaviorally measured "channel" tuning curves and the adaptation time constant with related published electrophysiological measures of cortical neurons in cats show significant similarities. The present study not only demonstrates the feasibility of employing complex, psychophysical paradigms with animals, but provides an important link in the logical chain relating models of the human visual system to cortical physiology. (Supported by NSF grant BNS 81-18780).

138.3 MECHANISMS UNDERLYING BINOCULAR INTERACTIONS OF CELLS IN THE CAT'S VISUAL CORTEX. I. Ohzawa* and R. D. Freeman. (SPON: L. Cooper) School of Optometry, University of California, Berkeley, CA 94720.

We have used sinusoidal grating stimuli, presented dichoptically, in order to determine the extent of binocular spatial summation for simple and complex cells of the visual cortex. For each cell, gratings of optimal orientation and spatial frequency were drifted such that the right eye was always exposed to a grating of constant initial phase while the initial phase of the left grating, and hence the relative phase between left and right, was varied over 360° randomly from one presentation to another.

Our principal results are as follows. All simple cells which are monocularly excitable through either eye, show marked phase-specific binocular interactions. Furthermore, the majority of simple cells that are strongly dominated by one eye, including those which appear exclusively monocular, also show unexpectedly large interactions. Similarly, reduction of contrast by a factor of 10 of the grating presented to one eye does not appreciably affect the degree of binocular interaction. This is striking since this reduction makes monocular responses from that eye subliminal. It can be shown by a polar representation of response amplitude and phase for these cells, that linear binocular summation followed by a threshold for firing, can account for this type of interaction.

Approximately half of the complex cells also show phase-specific binocular interactions. To test whether they are due to linear summation, optimal gratings for each eye are drifted in opposite directions. The responses show a modulation at twice the temporal frequency of the stimuli, suggesting that "neural images" identical to those of monocularly presented counterphase gratings are created in subunits of the receptive fields by linear superposition. It should be noted, however, that this complex cell model requires a highly specific organization in that the majority of subunits must possess the same optimal relative phase. Most of the remaining complex cells of our sample show interaction only in the form of non-phase-specific facilitation. A small proportion of simple and complex cells which appear monocular show non-phase-specific suppression. Asymmetric inputs from the two eyes seem necessary for cells of this type.

We conclude that linear summation of visually evoked influences from the two eyes is sufficient to account for most of binocular interaction in striate cortex. (EY01175)

138.4 THE CORTICAL SUBSTRATE FOR VISIBLE PERSISTENCE AND TEMPORAL SUMMATION. J. Duysens, G.A. Orban, J. Cremieux* and H. Maes*. Laboratorium voor Neuro- en Psychofysiologie, K.U.L., Campus Gasthuisberg, B-3000 Leuven (Belgium).

For short flashes (< 100 msec) of moderate contrast, human observers systematically overestimate stimulus duration and the error, known as "visible persistence" increases as flash duration shortens (the inverse duration effect). The detection of such brief flashes depends purely on stimulus energy and the interchangeability of stimulus duration and intensity in this domain of "temporal summation" is known as Bloch's law.

In a search for neurophysiological correlates of these psychophysical findings a series of cells from areas 17 and 18 of N_2O anesthetized and paralyzed cats were tested with a stationary light bar presented for different durations and intensities. One quarter of all S family cells responded to the briefest flashes (12.5 msec) with an ON discharge ending 67 msec (median, N = 13) after the onset of ON stimulation. The neural persistence, defined as the part of the response outlasting the stimulus period, decreased as stimulus duration increased in accordance with the inverse duration effect. At high stimulus contrast however, neural persistence was constant because the cells gave ON and OFF responses even when tested over a subregion which previously yielded only ON responses. It is suggested that these high contrast OFF rebounds are responsible for the positive afterimages observed psychophysically under similar conditions.

By manipulating both stimulus duration and intensity within the same test it was found that both parameters were interchangeable to a large extent but deviations from Bloch's law were frequent, especially at the high contrast end.

The remaining S family cells, which were unresponsive to single brief flashes, were tested with a series of such flashes to determine their temporal summation requirements. In the dark adapted cat it was found that these cells could sum the effects of single flashes for interstimulus intervals as large as 500 msec. Long integration times seem useful for nocturnal animals such as the cat because they improve the animals sensitivity to low contrast stimuli.

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138.5 SOMATOSTATIN-LIKE IMMUNOREACTIVE NEURONS IN THE OCCIPITAL CORTEX OF THE ADULT RAT, D.L. Meinecke*, and A. Peters. Dept. of Anatomy, B.U. Medical School, Boston, MA 02118.

Since the 14 amino acid peptide somatostatin (SOM) is widely present in the mammalian cerebral cortex and has been implicated as a possible neuromodulator or neurotransmitter, we used antisera to SOM (DAKO) to examine SOM-like immunoreactive neurons in the rat occipital cortex. Adult Sprague-Dawley rats were anesthetized and perfused with a buffered 4% paraformaldehyde solution. Their brains were removed and 30-40um vibratome sections of occipital cortex reacted with antisera to SOM and immunostained with the Avidin/Biotin system (VECTOR). Immunoreactive neurons were present in all layers of the occipital cortex. The majority were in layers II/III and V/VI, but occasional immunoreactive cells were also seen in the white matter underlying the cortex. Six distinct types of SOM-like immunoreactive neurons were identified in the occipital cortex (including areas 17, 18, and 18a). On the basis of descriptions of neurons from Golgi studies, multipolar neurons made up the largest proportion of immunostained neurons and had long dendrites radiating from the perikarya; axons could sometimes be seen emerging from the cell body. Bipolar neurons were also immunostained. These had thin ascending and descending dendrites, and some had an oblique dendrite arising from the cell body. Somatostatin-like immunoreactive neurons with morphologies suggestive of pyramidal neurons were also encountered, being almost exclusively located in upper layers II/III. They had prominent basal dendrites, but short apical dendrites which branched close to the cell body. Electron microscopic analysis is necessary to establish that these are pyramidal cells, for the presence of SOM-like immunoreactive neurons in the mammalian cortex has been disputed. Lastly, layer I neurons, horizontal multipolar neurons in layer VI, and small multipolar neurons in subcortical white matter were immunostained, although they were few in number. These results indicate that SOM is a good label for diverse cell types in the rat occipital cortex. Some of these neurons have been suggested to be excitatory and others inhibitory. Supported by NIH grants NS 07016 and T32 NS 07152.

138.6 **CALCIUM BINDING PROTEINS IN THE MONKEY VISUAL CORTEX** M.R. Celio, J.H. Morrison, C.W. Heizmann*[1], A.W. Norman*[2] F.E. Bloom. A.V. Qavis Center, The Salk Institute, San Diego Ca 92138, [1]Dept. Biochemistry Univ. Zürich; [2]Dept. Biochemistry UC Riverside.

Ca^{++} ions are involved in a variety of crucial control functions in the nervous system, which include excitability, neurotransmission and axoplasmic transport. It is becoming increasingly clear, that the effects of Ca^{++} occur through the intermediary of soluble, cytoplasmic proteins called Ca-binding proteins (CaBP's). Beyond the ubiquitous calmodulin, three other Ca^{++} binding proteins have been detected in the nervous system: S-100, Vitamin D-dependent Calcium binding protein (VDCaBP) and parvalbumin (PV). S-100 is present in astroglia, whereas PV and VDCaBP display a highly reproducible and distinct neuronal distribution in various regions throughout the brain. We studied the organization of VDCaBP and PV containing neurons in the visual cortex of the primate brain. Immunohistochemical methods were applied to sagittal, coronal and tangential sections through the adult Squirrel- and Rhesus Monkey occipital cortex. VDCaBP+ and particularly PV+ profiles are present in a high density in the visual cortex and are distributed in a highly laminar manner. PV+ cell bodies are found across layers II to VI of area 17 and represent a heterogeneous group of cell types. Cell processes and terminal fields are present in all layers displaying immunoreactive somata; however an extremely dense terminal-like plexus is present in layers IVa and IVc, whereas few processes are seen in layers I and IVb. The transition at the 17-18 border is abrupt; the PV+ neurons in area 18 are distributed more randomly and sparsely across the cortical layers. VDCaBP immunoreactive neurons in area 17 are mainly present in a superficial band corresponding to layer II; they send processes to deeper layers. A similar pattern is found in area 18. In tangential sections, no particular clustering of PV or VDCaBP immunoreactive sites were noticed in any layer. PV but not VDCaBP immunoreactive axons were found in the white matter. The shape of the PV and VDCaBP immunoreactive cells is typical of interneurons; however, the presence of PV immunoreactive axons in the white matter suggests that PV may be present in long projection systems as well.

138.7 LIGHT AND ELECTRON MICROSCOPIC OBSERVATIONS OF GLUTAMIC ACID DECARBOXYLASE AND SOMATOSTATIN IMMUNOREACTIVE NEURONS AND TERMINALS IN THE RAT'S VISUAL CORTEX. C.-S. Lin; S. M. Lu; D. E. Schmechel and R. P. Elde, Depts. of Anatomy and Medicine, Duke University, Durham, NC 27710 and Dept. of Anatomy University of Minnesota, Minneapolis, Minn. 55455

In this study, we have examined the morphology and distribution of neurons and axon terminals that are immunoreactive to glutamic acid decarboxylase (GAD) and somatostatin (SS) in the pigmented rat's visual cortex. Modified fixation and reaction methods allowed us to identify the dendritic and axonal arborization patterns of the immunoreactive cells.

GAD immunoreactive cells are present in all layers of visual cortex and form several distinctive subclasses. For example, GAD positive neurons in layer I resemble the horizontal or Retzius-Cajal cells described previously in Golgi studies. In contrast, GAD reactive cells located in the lower half of layer VI and the adjacent white matter have the morphological characteristics of the "interstitial neurons" originally described by Ramón y Cajal ('11). The GAD reactive cells were identified as neurons by double-labeling them with a second antibody - neuron specific enolase (NSE). The GAD reactive cells constitute from 13 to 15% of the neurons labeled with the NSE.

Neurons that react to the SS antibody are present in layers II/III, V and VI. A few are also found in the white matter. Most of the SS reactive neurons are bitufted although a few are multipolar. The axons labeled with the SS antibody are mainly oriented vertically and have a beaded appearance.

Many GAD positive neurons in the infragranular layers and white matter also react to the SS antibody. Fewer double labeled neurons were found in the supragranular layers.

The GAD immunoreactive neurons were also examined with the electron microscope. Most GAD reactive cells have an infolded nucleus, a small amount of cytoplasm and well-developed cisternae of granular endoplasmic reticulum and Golgi apparatus. The somas of GAD reactive cells are contacted by a few GAD positive terminals. GAD positive terminals are also arranged as pericellular nests and form symmetrical contacts upon the somata and proximal dendrites of pyramidal cells.

The present study indicates that GABA-ergic neurons are heterogeneous and participate in complex arrangements with other neurons in the visual cortex. Some of the GABA-ergic neurons are also immunoreactive to somatostatin.

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138.8 A COMPARISON OF THE NUMBER OF NEURONS IN SEVEN CORTICAL AREAS OF CAT. C. Beaulieu* and M. Colonnier. Department of Anatomy, Laval University, Quebec, Que. G1K 7P4.

The number of neurons per mm^3 (Nv) and the number under 1 mm^2 of cortical surface have been estimated for each lamina of 7 cytoarchitectural areas of the cat, using a method of size-frequency distribution. The areas studied comprised 4 visual areas (17B(inocular), 17M(onocular), 18 and PMLS), a somatosensory area (3B) and 2 motor areas (4γ and $6a\alpha$). The statistical significance of differences reported between areas was determined by means of a one-way ANOVA, followed by an a posteriori Tukey test.

For both series of measurements, significant differences could be demonstrated among the 7 areas studied (ANOVA, $p < 0.001$). The Nv of 17B and 17M (49,000 ± 1,409) are 85% greater than those of each of the other regions (26,744 ± 2,733), at p values < 0.01 (Tukey). The number of neurons under 1 mm^2 of cortical surface is greater in 17B (78,000) than in any other area (p values < 0.01). Other sensory areas (17M, 18, PMLS, 3B) have fewer neurons and the numbers do not vary significantly between regions (59,020 ± 2,367). Areas 4γ and $6a\alpha$ have still fewer neurons (43,872 ± 628), at p values < 0.01. We thus only partially confirm the conclusions of Rockel et al. (Brain, 103: 211, 1980) that there is a basic uniformity of the number of neurons per unit of cortical surface in different cortical areas of the cat. The 7 areas studied fall under 3 different categories. Motor areas have the smallest number of neurons, sensory areas have more, and the greatest number is found in 17B.

The larger number of neurons under 1 mm^2 of cortical surface in 17B is largely but not exclusively due to layer IV in which the number of neurons is significantly greater than in all other area, p values ranging from < 0.05 to < 0.01. The basic uniformity of neurons in the other sensory areas is maintained in spite of a few demonstrated differences at the laminar level. For example, layer IV of 17M has at least twice the number of neurons found in each of the other three areas (p values < 0.01). This appears to be largely compensated by the presence of more neurons in layer III of the other three regions, and perhaps in layer VIA of PMLS and 3B. The similar number of neurons in 4γ and $6a\alpha$ is obtained in spite of the fact that the latter has no discernable layer IV. The difference is completely compensated by an increase in the number of neurons in layer III of $6a\alpha$. Supported by MRC grant MT 3735.

138.9 NONSTATIONARY STOCHASTIC POINT-PROCESS MODELS AND VISUAL CORTICAL NETWORKS. Wm Wren Stine*, Muhammad K. Habib*, Pranab K. Sen*, Michael R. Isley*, and Paul G. Shinkman (SPON: Richard A. King). Univ. North Carolina, Chapel Hill, NC 27514.

We are currently studying neuronal connectivity in kitten and cat visual cortex. Our technique involves analyzing the structure of cross-correlations among simultaneously recorded extracellular spike trains of two or more neurons using one or two microelectrodes. The overall goal of the project is to characterize synaptic plasticity in the developing visual cortex as a model for adaptive change and learning in cortical neuronal networks.

The use of cross- and auto-correlations for the analysis of neural organization has grown steadily. Recent interest has centered on visual cortical connectivity (e.g., Toyama, Kimura, & Tanaka, J. Neurophysiol., 1981; Ts'o, Gilbert, & Wiesel, Soc. Neurosci. Abstr., 1983). Applications of these techniques, however, pose certain difficulties of interpretation. For instance, several distinct neural circuits may engender identical cross-correlations. Our goal was to develop an approach that minimizes the number of cross-correlation equivalent circuits.

Generally, evoked activity in visual cortical cells is nonhomogeneously distributed over time within a single trial or stimulus presentation, and should therefore be treated as a nonstationary process. We present a point-process model of spike trains based upon doubly stochastic Poisson processes (Habib et al., in preparation). When applied to simultaneously recorded spike train pairs, this model allows for the presence of nonstationarities in the data and hence requires the use of three-dimensional correlational surfaces. By studying nonstationarities during single trials, circuit equivalence classes can be partitioned, so that the number of interpretations is reduced. Furthermore, the study of nonstationarity in neuronal activity has implications for modeling developmental plasticity in the synaptic connectivity of visual cortex. Some of these implications are discussed with respect to extant physiological data as well as simulations.

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139.1 AN ANALYSIS OF LOCAL NEURONAL CIRCUITS IN THE VISUAL CORTEX OF THE CAT. S. Reinis, J.P. Landolt and D.S. Weiss*. Dept. of Psych., Univ. Waterloo, Waterloo, Ont., and DCIEM, Downsview, Ont., Canada.

The interactions between several cortical neurons were studied in area 18 of the cat cerebral cortex**. A light bar moving across the visual field of one eye acted as the stimulus. Spike activity was recorded by a single tungsten microelectrode, and subdivided by computer into 5 groups according to spike amplitude, and the interspike intervals between spikes of the same amplitude and those of different amplitudes. Cross correlation histograms were also calculated. The large-amplitude spikes usually represented the activity of single neurons, whereas the low-amplitude spikes were recorded from larger (and more distant) populations of neurons. Spike activity in one cell was often followed by spike activity in several neighbouring cells. The cross-correlation histograms revealed the existence of interactions between neighbouring neurons. The intervals at which these interactions were detected often exceeded 750 ms, but, they were very precise in their entrainment (e.g., entrainment would occur consistantly at 455 ms, and not at 455 $\pm$ 1 ms). The incidence of such entrainment, even when it exceeded the chance level substantially, was usually rather low. In other words, less than 10% of spikes of cell B would be entrained to the spikes of cell A. Such long durations in the correlation times indicate that two neighbouring cells in the visual cortex must interact through long chains of other neurons, and that the configuration of such chains is rather variable. An analysis of spike activity in larger populations of neurons revealed rhythmic (probably reverberating) neuronal interactions that were induced by the visual stimulation. Such cycles of interaction lasted between 200 ms and 2 s. These data indicate that it is possible to construct diagrams of local neuronal circuits in the cerebral cortex. However, these circuits involve large numbers of interacting neurons, are rather variable, and the probability of regular interaction of two particular neurons within them is quite low.

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** All of these experiments were approved by the Canadian Council on Animal Care following their careful analysis and repeated on-site inspection of tne experimental procedures.

139.2 STRIATE AND EXTRASTRIATE AREAS IN THE CEBUS MONKEY: AN ELECTROPHYSIOLOGICAL AND ANATOMICAL TRACER STUDY. R. Gattass*, A.P.B. Sousa* and M.G.P. Rosa* (SPON: C.G. Gross). Instituto de Biofísica, UFRJ, Rio de Janeiro, RJ, 21941, Brasil.

The representation of the visual field in striate cortex and in extrastriate visual areas were studied by means of electrophysiological techniques. Eight Cebus apella anesthetised with N_2O/O_2 and immobilized with pancuromium bromide were studied in repeated recording sessions.

VI contains a continuous representation of the contralateral visual field. The representation of the vertical meridian (VM) forms the anterior border of VI, and that of the horizontal meridian (HM) divides the area so that the representation of the inferior visual field is located dorsally and that of the upper field ventrally. Surrounding VI there is another topographically organized visual area, V2. The representation of VM in V2 forms its posterior border, and that of HM is continuous with that of VI, then it splits and forms the anterior border of V2. In order to determine the eccentricity at which the split of the HM occurs we used combined injections of two different fluorescent tracers (nuclear yellow and bisbenzimide). We injected the tracers along the representatiom of HM in VI at different eccentricities, in two animals. The results showed that the HM is represented at the anterior border of V2, and that the split occurs close to the representation of 0.8 deg. Similar to the macaque, the representation of the central visual field is magnified relative to that of the periphery both in VI and in V2. In these areas receptive field sizes increase with eccentricity; however, for a given eccentricity receptive field size in V2 is larger than in VI.

Anterior to V2, we have recorded from several visual areas. In the superior temporal sulcus, in a region where we found labelled cells after injections of fluorescent tracers in VI, we found a representation of the contralateral visual hemifield. This area is probably homologous to MT of the macaque (Gattass, R. and Gross, C.G., J. Neurophysiol., 46: 621-638, 1981).

(Research supported by CNPq, FINEP and CEPG/UFRJ)

139.3 **PRECISION OF VISUOTOPIC ORGANIZATION OF AREA MT IN THE MACAQUE.** **T.D. Albright and R. Desimone. Dept. Psychology, Princeton University, Princeton, NJ, 08544 and Lab Neuropsychology, NIMH, Bethesda, MD 20205**

Area MT in the macaque receives a direct projection from striate cortex (V1) and, like V1, contains a first order representation of the visual field. Unlike in V1, however, the visual topography in MT has been reported to be crude or irregular. We sampled MT neurons at small intervals and found suprising regularity in the local visuotopic organization. Receptive field (RF) size, RF scatter, magnification factor and point-image size bear the same relationships to one another in MT as they do in V1.

Over 500 neurons were isolated on 20 tangential and oblique penetrations in four monkeys. On each penetration RF location was measured every 50 μm for distances up to 3 mm parallel to the cortical surface. RF eccentricities were primarily within the range of 1° to 25°. Progressions of RF location were highly linear on almost all penetrations at all eccentricities. A linear regression was calculated for each RF trajectory of constant rate of change of field position on each penetration. Both RF scatter (average deviation from the regression lines) and RF size increased as a linear function of eccentricity and both were about 10 times larger than in V1. RF scatter was about one-third RF size, which is identical to the relationship between scatter and RF size on vertical penetrations in V1 (Dow, B.M. et al., Exp. Brain Res., 44:213-228, 1981). Magnification factor (derived from the slopes of the regression lines) was a power function of eccentricity and roughly one-tenth that reported for V1. Because of the proportional increase in RF size and decrease in magnification factor in MT as compared to V1, point-image size in MT (based on RF size, scatter and magnification factor) is similar to that in V1 (Dow et al., op. cit.) at equivalent eccentricities. Thus, the area of the cortex activated by a single point in the visual field is about the same in MT as in V1. At 1° eccentricity the point-image area is approximately 10 mm² but drops sharply in the parafoveal region and levels out to about 1 mm² at 25° eccentricity.

Our results suggest that apparent irregularities in the local visuotopic organization of MT reflect RF size, just as they do in V1. In addition, the point-image size appears to be similar in the two areas. As 400-500 μm of cortex represent 180° of axis-of-motion in MT and 180° of orientation in V1, a single point in the visual field may activate a similar number of 'modules' in MT and V1.

139.4 TWO GROUPS OF NEURONS RESPONDING TO LOCAL AND WHOLE FIELD MOVEMENTS IN THE MACAQUE MT AREA. K. Tanaka, H. Saito, Y. Fukada*, NHK Broadcast. Sci. Res. Labs., Setagaya, Tokyo, K. Hikosaka*, M. Yukie*, E. Iwai*, Tokyo Metropolitan Inst. for Neurosci., Fuchu, Tokyo.

It has been shown that the direction of motion is systematically analyzed in the macaque MT area. However, little is known whether MT cells analyze a local movement or a large field movement. It is suggested that nearly all cells in the owl monkey's MT area do the former analysis (Miezin et al., 1982). We recorded MT cells in anesthetized, immobilized macaques (M. fuscata), and examined their responses to two kinds of visual stimuli; movement of a slit and that of a textured pattern extended over the whole screen (60x80°).

All the cells responded well to a moving slit, mostly in one direction. Movement of the textured pattern also evoked strong or moderate responses in a half of them, but no responses in the remaining cells.

Interactions between the two stimuli were then examined. In the majority of the cells which were unresponsive to the field movement, the response to a moving slit was strongly suppressed (50-100% reduction) when the textured pattern went along with the slit in the same direction and speed. The suppressive effects were weak in the cells which responded to the field movement. The effective area of the suppression extended far beyond a 20x20° area centered at the excitatory receptive field. The exact matching of the direction and speed of motion between the two stimuli was not crucial for the suppression. In many cells, suppressive effects of more than 50% of the maximum strength were obtained even when the field motion was deviated up to 60° in direction or 1/4-4 times in speed from the slit motion.

In a quarter of the whole cells, the responses to the prefered direction of slit motion were facilitated when the textured pattern moved in the opposite direction, although the field stimulus moving in that direction, with or without a stationary slit, elicited no excitations.

The cells with strong background suppression were encountered almost exclusively in the superficial layers, whereas those showed strong responses to the field stimulus were encountered more often in the deep layers.

There are two groups of MT cells. One can analyze local movements, distinguishing them from field movements. The other can analyze the field movement itself. As to the latter cells, there are two possible functions: they supply the informations of field motion to higher cortical areas, or exert inhibitory influences on the other group of MT cells.

139.5 INTEGRATION OF DIRECTION QUES OF STIMULUS MOTION IN MACAQUE STS CORTEX. H. Saito, K. Tanaka, Y. Fukada,* NHK Broadcast. Sci. Res. Labs., Setagaya, Tokyo, M. Yukie,* K. Hikosaka,* E. Iwai,* Tokyo Metropolitan Inst. for Neurosci., Fuchu, Tokyo.

We have studied visual response properties of neurons in the area anteriorly adjoining the MT, using anesthetized and paralyzed macaques (M. fuscata). Since this part of the anterior bank of the superior temporal sulcus (STS) can be considered to be a relay station from the MT to the parietal association cortex (Van Essen & Maunsell, 1983), a special attention was paid to how this area integrates the outputs of the MT.

By systematic electrode trackings of horizontal penetrations forming an angle of 45-60° against the frontal plane, we found a narrow area where three kinds of motion sensitive cells clustered. This area is contiguous to the dorsal half of the MT. One-third of the cells responded to a straight front-parallel movement of patterns (slit or texture) with strong direction selectivity (D-cells). They had much larger receptive field (RF) than MT-cells. Another one-third responded to expanding or contracting stimulus size (S-cells). They responded even to a size change as small as 1° irrespective of both the initial size and position, provided the stimulus was confined within the RF (20-60°). The third class of cells responded to a rotation of patterns in one direction (R-cells). The majority responded to a rotation of patterns in the front-parallel plane (either clockwise or counterclockwise) while the rest responded to a rotation in the three dimensional space. R-cells did not respond at all to the straight movements of patterns in any direction. Rotation-cells with larger RF have been found in the area 7a (Sakata et al., 1984). There were cells which especially favored a movement of textured patterns (movement of a plane) in all the three classes. It seems that D-cells integrate inputs from MT-cells with the same prefered directions, whereas S- and R-cells integrate signals of MT-cells with different prefered directions.

In the region ventral to this area, we scarcely found S- and R-cells. Instead, a population of cells responding to moving stimuli with a fixed or changing binocular disparities intermingled with D-cells. In the region lateral to the area, cells were also direction selective, but motion of a real object with highly complicated configuration was needed to activate them. This feature can not be interpreted solely by the integration of motion informations of the MT but needs qualitatively different informations analyzed in the other areas than the MT.

139.6 DEFICITS IN PURSUIT EYE MOVEMENTS FOLLOWING IBOTENIC ACID LESIONS OF THE FOVEAL REPRESENTATION OF AREA MT OF MACAQUE MONKEY. M.R. Dursteler*, R.H. Wurtz, W.T. Newsome, and A. Mikami. Lab. of Sensorimotor Research, National Eye Institute, Bethesda, MD 20205.

We have previously found that damage to extrastriate area MT in the superior temporal sulcus (STS) produces a deficit in a monkey's ability to initiate smooth pursuit eye movements in response to targets moving in extrafoveal portions of the visual field. We have now made injections that included the foveal representation in MT and determined the effect on the maintenance of pursuit after the monkey acquired the target with his fovea.

Monkeys began each trial by fixating on a spot of light. The spot then went out and another spot came on at a variable location on the horizontal meridian and began to move towards or away from the fixation point at 16°/sec (step-ramp paradigm). The monkeys were required to pursue the moving target to obtain a reward, and eye movements were measured using the magnetic search coil technique. Lesions were produced by injections of ibotenic acid (1µl of 15µg/µl) into the foveal representation of MT.

Foveal lesions produced an asymmetric deficit in the maintenance of pursuit eye movements. The speed of pursuit eye movement for targets moving towards the side of the lesion decreased; pursuit of targets moving away from that side did not. A drift of the eye away from the lesion side was also evident.

We also tested pursuit of stabilized images: in these trials pursuit was dependent upon position information from the stabilized image rather than visual motion. Monkeys successfully pursued such images after the lesion even when the image was stabilized within the damaged portion of the visual field. This indicates that pursuit movements made in response to position rather than retinal motion were not affected by the lesion.

These experiments suggest that lesions of the foveal representation within the STS produce a directionally specific deficit in maintenance of pursuit of moving targets. The directional deficit in pursuit was in contrast to the deficit found with extrafoveal MT lesions which were bidirectional but was similar to deficits following large cerebral lesions in man and monkey. Since the foveal representation lies near the boundary of MT, these direction related deficits in maintenance may result from invasion of adjacent areas.

139.7 MODULATION OF ATTENTIONAL BEHAVIOR BY INJECTION OF GABA-RELATED DRUGS INTO THE PULVINAR OF MACAQUE. Steven E. Petersen*, J. David Morris*, and David Lee Robinson. Laboratory of Sensorimotor Research, National Eye Inst., Bethesda, MD 20205, and Department of Psychology, University of Maryland, College Park, MD 20742.

Our electrophysiological experiments have suggested that some pulvinar neurons are involved in selective visual attention. The present experiments were designed to explore this involvement using an alternative experimental approach. We attempted to modify an animal's attentional behavior by pharmocologically altering the functioning of the pulvinar.

Two monkeys were trained on a cued-detection task developed by Posner. The animals fixated a spot of light and responded with a bar press to a small peripheral target. If the target was preceeded by a cue on the same side (valid), the animals' reaction times were faster than when the target was preceeded by a cue on the opposite side (invalid) -- suggesting that the animal had shifted its attention to the location of the cue.

Bicuculine (a GABA-antagonist) or muscimol (a GABA-agonist) were injected into the pulvinar. The effects of bicuculine and muscimol injections on the perfomance of the task can be summarized as follows:

1) the effects were limited to the contralateral visual field;
2) the effects of the two drugs were opposing in nature; and
3) the effects were primarily related to the cue, and were most apparent on invalidly cued trials.

Specifically, when both cue and target were in the visual field ipsilateral to the drug injection, reaction times were unaffected. When both cue and target were in the contralateral field, reaction times in control and bicuculine conditions were similar; muscimol treatment slowed responding.

For invalidly cued trials, the drugs gave opposing results. Following bicuculine injection, reaction times were faster with the cue in the ipsilateral field, slower with a contralateral cue. Following muscimol injection, reaction times were slower with the cue in the ipsilateral field, faster with a contralateral cue.

These results support an involvement of the pulvinar in selective visual attention. They suggest that muscimol interferes with the shift of attention to the affected field whereas bicuculine exaggerates this shift.

139.8 ANATOMICAL AND PHYSIOLOGICAL ANALYSIS OF INTRACORTICAL CONNECTIONS IN CAT AREA 7. E.C. Callahan* and L.B. Haberly. Dept. of Anatomy and Neurosci. Training Prog., Univ. of Wisconsin, Madison, WI 53706.

Physiological properties of cortico-cortical connections within area 7 of the cat have been studied with microstimulation and intracellular recording and staining techniques. Area 7 provides a convenient preparation in which to study intrinsic "horizontal" connections in neocortex by virtue of their great length as revealed by amino acid autoradiography.

Surface or intracortical stimulation evokes two types of responses: either a depolarization followed by a hyperpolarization or a pure hyperpolarization. In both types the hyperpolarization has properties of a chloride-mediated IPSP: inversion with chloride leakage from microelectrodes, inversion with low level hyperpolarizing current injection, and inhibition of action potential generation. This IPSP is partially or completely blocked during the period of inhibition induced by preceding conditioning shocks, consistent with a disynaptic origin. The initial depolarization appears to be an EPSP since it evokes action potentials when of sufficient amplitude. In contrast to the IPSP, this EPSP is not usually blocked during the period of inhibition following conditioning shocks, consistent with a monosynaptic origin. Many neurons that respond to single shocks with a pure hyperpolarization display an initial period of depolarization following conditioning shocks, suggesting the presence of an initial EPSP that is blocked at the level of the soma by a simultaneous IPSP.

Preliminary anatomical evidence has been obtained on the distribution of intrinsic fibers in area 7. Intracellular injections of HRP have revealed that pyramidal cell axons can give rise to long, horizontally directed collaterals with a large number of boutons and en passage swellings that have the appearance of synaptic terminals. Following small extracellular injections of ^{3}H proline, anterogradely transported label is concentrated in longitudinally oriented, branching columns that extend for many millimeters. Label in these columns is present in all layers.

On the basis of these results we postulate that longitudinally oriented intrinsic connections originating from pyramidal cells in area 7 mediate monosynaptic EPSPs and disynaptic IPSPs. Pyramidal cells appear to directly excite other pyramidal cells over both short and long distances.

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139.9 FIXATION AND SACCADE-RELATED AREA 7A NEURONS RECEIVE VISUAL INPUTS. G.K. Essick*, R.A. Andersen, and R.M. Siegel. (SPON: B.D. BOSS) The Salk Institute, La Jolla, CA 92037.

In the course of examining the effects of eye position on the visual response of cells in area 7a we have found that many of these neurons also have properties of fixation cells, including gaze fields. This finding led us to re-examine the classifications of the fixation and also the saccade neurons, since previous accounts maintained that these cells either did not receive visual inputs, or conversely received only visual inputs and their saccade and fixation related activities were artifacts of visual stimulation.

Of 93 fixation neurons we tested, 71 were light sensitive. In 44 of these cells we tested for the angle-of-gaze effect. Thirty-six were found to have the magnitude of their visual responses modified by eye position. In general, when the animal looked into the gaze field of the cell, the neuron was more visually responsive.

Fifty-eight of the fixation neurons were tested in both light and dark and in 51 of these cases the gaze field was unaltered. To determine the effect of the visual fixation point on the gaze fields, the animal was trained to maintain fixation when the fixation point was turned off for one second in total darkness. Of 46 cells tested, all but 4 showed the same gaze field with or without the fixation point. We also tested these light-sensitive fixation cells in the light by changing the gaze angle with prisms. This approach has the advantage that the animal views the same visual scene but from different angles of gaze. Of 8 neurons tested, all showed the same gaze fields with or without prisms.

Seventy-two saccade-related neurons were examined and found to have both visual and eye movement-related components to their responses. The eye movement component was isolated from visual events by requiring the animal to make saccades to remembered locations in total darkness. Since there was a large variation in the onset times of the saccades in this test, it was also possible to show that the saccade-related responses were synchronized with the eye movements. In some cells this saccade-related response occurred just prior to the eye movement but in most cells it occurred at the very beginning of the eye movement or just after the eye movement. Thus, these cells do not appear to be involved in the initiation of an eye movement but rather are receiving an outflow or inflow signal that an eye movement is taking place.

These results indicate that there are neurons in the inferior parietal lobule that carry true extra-retinal eye position or eye movement information. Interestingly, these same neurons also respond to visual stimuli.

139.10 ROLE OF LOW AND HIGH SPATIAL FREQUENCIES IN THE FACE-SELECTIVE RESPONSES OF NEURONS IN THE CORTEX IN THE SUPERIOR TEMPORAL SULCUS. E.T.Rolls, G.C.Baylis* and C.M.Leonard. Dept. Exptl. Psychol., Oxford Univ., Oxford, England.

There are neurons in the cortex in the anterior part of the superior temporal sulcus of the macaque monkey with visual responses selective for faces (Perrett, Rolls and Caan, Exp. Brain Res., 47: 329-342, 1982). This study analyzes further the information which leads them to respond. The responses of 32 such single neurons were measured to faces which were digitized, lowpass filtered at spatial frequencies of 2, 4, 8 ... 128 cycles per face, highpass filtered at frequencies of 4, 8, ... 64 cycles per face, and presented in random sequence using a video framestore.

It was found that many of the neurons could respond to blurred images of faces, with a mean half-maximum amplitude of the neuronal response to the series of lowpass filtered images of faces of 4.3 cycles per face. Almost all the neurons had lowpass cutoff frequencies defined in this way below 8 cycles per face. Many of the neurons could also respond to images of faces in which the only information present was a limited amount of edge information. The mean half-maximum amplitude of the neuronal response to the series of highpass filtered images of faces was 24.3 cycles per face. Almost all the neurons had highpass cutoff frequencies above 8 cycles per face. Thus, many of the neurons could respond to a lowpass and a highpass filtered image of a face even when these had no frequencies in common. The mean separation between the lowpass and highpass cutoff frequencies was 2.5 octaves.

For comparison, face recognition in man can be performed with images which contain only information up to 8 cycles per face, or with highpass filtered images which contain only information down to 8 cycles per face (Fiorentini et al, Perception, 12: 195-201, 1983).

The response of the neurons was not always a smooth function of frequency, but could decrease as higher frequencies were included in the lowpass filtered images of faces, or as low frequencies were included in the highpass filtered images of faces. This indicates that information in certain frequency bands was able to inhibit these neurons. This was particularly likely to occur for the non-optimal face stimulus for a given neuron, indicating that the selectivity of these neurons to different faces was a combination of the excitation produced by some information in faces and inhibition produced by other.

139.11 THE AFFERENT AND EFFERENT CONNECTIONS OF THE INFERIOR TEMPORAL CORTEX IN THE MACAQUE MONKEY: A PRELIMINARY ANALYSIS. S. Demeter, K. Flynn*, K.M. Cote*. Ctr. for Brain Res. and Dept. of Neurology, Univ. Rochester Med. Ctr., Rochester, NY 14642.

The inferior temporal cortex (IT) is an extensive neocortical territory, stretching from the occipitotemporal transition posteriorly to the temporal polar cortex anteriorly and from the depths of the superior temporal sulcus dorsolaterally to the occipitotemporal and rhinal sulci ventromedially. It is a modality-specific region forming the most distal part of the visual-cortical system that is thought to be concerned with form analysis. The ablation of IT produces a higher order visual disturbance, which corresponds in part to the visual component of the Klüver-Bucy syndrome ("psychic blindness"). The response properties of single units in IT are very complex and have defied conclusive analysis. These observations have given rise to the belief that IT is concerned with the "highest" levels of visual form analysis.

It is likely that IT, as prestriate cortex, is composed of subdivisions which receive incompletely analyzed visual information sequentially and in parallel from more proximal visual areas. Since electrophysiological and behavioral approaches have not, thus far, succeeded in identifying the subdivisions of IT to an adequate extent, we have begun a systematic attempt to analyze its intrinsic connectional organization as well as its remote connections in an effort to arrive at an anatomic subdivision.

In a series of adolescent monkeys (Macaca fascicularis), we have pressure-injected either mixtures of tritium-labeled leucine and lysine or horseradish peroxidase (HRP) into IT and visualized the resultant labeling by autoradiography (ARG) and tetramethyl-benzidine histochemistry, respectively. In a preliminary review of three ARG and three HRP cases, we have confirmed most previous observations based on ablation-degeneration methods on the connectivity of this region. For example, we have observed projections to IT from the temporal pole, parahippo-campal gyrus, prestriate cortex, frontal lobe, amygdala, pulvinar and other subcortical structures. In turn, we have observed reciprocal projections from IT to all these structures and additional efferents to the caudate and putamen. These observations indicate that the techniques employed can demonstrate the known connections of this cortical territory reliably.

140.1 LABELING AND CHARACTERISTICS OF RNA AND PROTEIN IN FRESH PUNCHES OF RAT BRAIN REGIONS. Bruce S. McEwen, Mariann Blum, Lenore Snyder*, James L. Roberts and Ljubica Bogic*. The Rockefeller University, New York, N.Y. 10021; Department of Biochemistry, Columbia University College of Physicians and Surgeons, New York, N.Y. 10032; and Boris Kidric Institute, Belgrade, Yugoslavia.

The punch sampling method introduced by Palkovits allows removal of discrete anatomically-defined brain regions for biochemical analyses. Because it utilizes frozen tissue, this method does not permit the study of incorporation of radioactive precursors into macromolecules such as RNA and protein. However, samples punched from fresh, chilled but not frozen, sections of brain tissue will synthesize radiolabeled RNA and protein when incubated in vitro. Rat brains are removed rapidly after decapitation and chilled on ice for 3-5 minutes. With a single-edge razor blade, 2-3mm coronal sections are cut according to landmarks in a standard rat brain atlas. These sections are placed on a large rubber stopper, chilled on ice. Under a magnifying lens, punches are made using a 1mm stainless steel needle and gently expelled into an incubation vessel. Thus far we have routinely sampled medial preoptic area(mPOA) and ventromedial nuclei(VMN) by this method. Arcuate nucleus(ARC) is removed by razor cuts after the VMN has been punched. Fresh punches of ARC, mPOA and VMN have levels of estrogen-induced(E) and uninduced(C) progestin receptors which are comparable to those reported elsewhere from frozen punches (Parsons et.al.J.Neurosci. 2:1446,1982). Values (fmoles/mg protein) after labeling with 0.4nM 3HR5020: mPOA, C-9.9±1.1, E-32.9±4.8; VMN, C-6.0±2.4, E-26.6±2.4: ARC, C-9.3±1.2, E-46.3±3.1. Incorporation of 3H uridine into RNA is linear for 4h in an oxygenated Krebs-Ringer bicarbonate medium containing glucose, and gradual accumulation of labeled cytoplasmic RNA is observed with time. From analysis on oligo(dT) cellulose, cytoplasmic RNA has around 3-5% poly A+ RNA, while nuclear RNA contains around 13-18% poly A+ RNA. Incorporation of 35S methionine into protein is also linear for 4h in a methionine-free RPMI1540 medium under continuous gassing with 95%02,5%C02. Tissue samples dissolved in O'Farrell lysis buffer and subjected to two-dimensional gel electophoresis routinely give more than 100 labeled spots after autoradiography. Studies are currently underway to begin to characterize regional as well as hormone-induced differences in labeling of specific RNA's and specific proteins. (Supported by NS07080, RF81062 and AN27484).

140.2 ASSOCIATION OF PERIPHERAL-TYPE BENZODIAZEPINE RECEPTORS WITH ENDOCRINE CELLS IN RAT PITUITARY, ADRENAL AND TESTIS. R.R.H. Anholt*, E.B. De Souza, M.J. Kuhar and S.H. Snyder. Neuroscience Department, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

We have used [^{3}H]Ro5-4864, a ligand selective for peripheral benzodiazepine receptors to identify and localize such receptors in several endocrine organs. The density of [^{3}H]Ro5-4864 binding sites is higher in the adrenal gland than in any other tissue examined. The pituitary gland has high levels of [^{3}H]Ro5-4864 binding sites when compared to the brain. Binding levels of [^{3}H]Ro5-4864 in the testis are substantial and similar to those observed in many other peripheral tissues. Drug displacement studies confirm that the benzodiazepine receptors in these endocrine tissues are pharmacologically of the peripheral type. Autoradiographic studies reveal a discrete and differential localization of [^{3}H]Ro5-4864 binding sites in all three endocrine organs. There is an uniform distribution of [^{3}H]Ro5-4864 binding sites throughout the anterior, intermediate and posterior lobes of the pituitary gland, with highest concentration in the posterior lobe. In rat adrenal gland, specific binding sites for [^{3}H]Ro5-4864 are found exclusively in the cortex with the highest density in the zona glomerulosa and significant concentrations in the zona fasciculata and the zona reticularis. [^{3}H]Ro5-4864 associated silver grains in the testis appear highly localized to the interstitial tissue, present at only low concentrations over the epithelium of the seminiferous tubules and absent in the tubular lumen. After hypophysectomy, the degeneration of the adrenal cortex and of the interstitial tissue in the testis is accompanied by a parallel reduction in the amounts of peripheral-type benzodiazepine receptors. Thus, peripheral-type benzodiazepine receptors in these tissues appear to be associated with cells which are subject to trophic control from the pituitary. Our observations suggest that previously reported effects of benzodiazepines on endocrine function may be mediated directly via peripheral-type benzodiazepine receptors present on these endocrine cells. This work was supported by a grant of the International Flavors and Fragrances Corporation.

140.3 THE EFFECT OF INTRACAROTID GLUCOSE INFUSIONS ON THE GLUCOSE COUNTERREGULATORY HORMONES DURING INSULIN-INDUCED HYPOGLYCEMIA. P.Cane*, R. ARTAL*, R.N. Bergman* (Spon: D.Lindsley). USC Los Angeles, CA 90033.

The purpose of this study was to determine the role of the central nervous system during a systemic hypoglycemia, but a normoglycemic condition in the segments of the brain most likely responsible for glucose regulation. Is the counter-regulation of insulin-induced hypoglycemia entirely controlled by the CNS or do the splanchic organs possess an autoregulatory mechanism? In conscious dogs, two types of experiments were performed. I) CNS clamp; glucose was infused in both carotid arteries to maintain euglycemia in blood entering the brain, while peripherial glucose dropped due to the constant infusion of insulin (150 mU/min). II) Matched glucose infusion; During systemic insulin infusion, 150mU/min.,glucose was infused into the periphery via the cephalic vein, matching the intracarotid glucose infusion during the CNS clamp experiments. Results: During intracarotid glucose infusion, jugular vein glucose (Gj) was maintained at 100 mg/dl+3 while peripherial blood glucose (Gp) fell to 54.8 mg/dl+3 (P<.001). During matched glucose infusion , Gj= 50 mg/dl+3.6, Gp= 50 mg/dl+2.5 (N.S.). Glucagon was 343 pg/ml +118 when the head was normoglycemic and 281 pg/ml +53 when the head was moderately hypoglycemic (N.S.). Liver production was 87.4 mg/min+14 during intracarotid infusion and 52.2 mg/min +11 during matched CNS glucose infusion. Epinephrine and norepinephrine went from 612 pg/ml +199, and 655 pg/ml +192, respectfully, during intracarotid infusion, to 658 pg/ml +147 and 548 pg/ml +90 during matched glucose infusion. Conclusion: There is no difference in the counter-regulatory hormones and liver glucose production when the CNS was maintained in a normoglycemic range while the peripheral organs were moderately hypoglycemic. Apparently, direct CNS control has little effect on maintaining euglycemia during moderate, insulin-induced hypoglycemia. With a larger hypoglycemia there is possibly a greater hormonal response from the CNS .(NIH AM27617)

140.4 EFFECTS OF AGE AND ESTRADIOL ON BRAIN AND PITUITARY GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND 6-PHOSPHOGLUCONATE DEHYDROGENASE ACTIVITIES. MN Gordon, CV Mobbs, DG Morgan, and CE Finch, Andrus Gerontology Center and Dept. of Biological Sciences, Univ. of Southern California, Los Angeles, CA 90089-0191.

The activities of brain and pituitary glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6-PGDH) were measured after treating female C57BL/6J mice with estradiol (E_2) at various stages of reproductive aging. In Expt. 1, ovariectomized mice aged 7, 12, and 19 mo were implanted chronically (21 d) with a graded series of E_2-containing capsules which produced physiological plasma E_2 levels. In Expt. 2, 5-, 9-, 15- and 22-mo-old ovariectomized mice were injected once with 500 µg 17β-E_2/kg sc. Control mice received implants or injections containing vehicle alone.

Overall, old mice possessed 10-20% higher pituitary G6PDH and 6-PGDH specific activities compared to young mice. These age-related increases were not found if old mice were ovariectomized 12 mo previously. In addition, male C57BL/6J mice do not display elevations of pituitary enzyme activities during their lifespan (3-29 mo).

In both experiments, E_2 administration elevated pituitary G6PDH specific activity. However, the induction of G6PDH by E_2 was not altered during aging. Young and old mice showed equivalent 2-fold increases in G6PDH specific activity after chronic E_2 implants. In addition, the rate of increase in G6PDH after a single E_2 injection was not delayed with age. All age groups displayed a maximum increase of 30% 48 h after the injection. Thus, the regulation of G6PDH by E_2 is unaffected during aging in the female pituitary gland, despite an age-related increase in enzyme activity.

In contrast to the pituitary, brain G6PDH and 6-PGDH specific activities were not altered during aging or by E_2 in mediobasal hypothalamus, corticomedial amygdala, corpus striatum or cerebral cortex.

This work was supported by NIA grant AG-00446 (CEF), NIA training grant AG-00093 (MNG; DGM) and NIA training grant AG-00037 (CVM).

140.5 HYPOTHALAMIC OPIATE RECEPTORS (μ, κ, δ) ARE REFRACTORY TO TESTOSTERONE. C.R. Clark*, A.J. Ball*, J. Hughes* (SPON: P.D. Evans). Parke-Davis Research Unit, Addenbrookes Hospital, Cambridge CB2 2QB, UK

Opiates such as morphine and endogenous opioid peptides exert a naloxone-reversible, tonic inhibitory action on LH release - an effect dependent on the presence of circulating gonadal steroids (Bhanot et al, 1983 Endocrinol. 112:399). In view of the ability of gonadal steroids to up- or down-regulate receptors for other ligands, studies were undertaken to ascertain whether hypothalamic opiate receptors are sensitive to feedback control by testosterone. Male Sprague-Dawley rats (200-250g) were either left intact (I) or castrated and injected (s.c.) for 14 days with testosterone propionate (2.5 mg/kg) (CT) or castrated and injected for 14 days with vehicle (C). Rats were decapitated on day 15, the hypothalamus dissected out and membranes prepared in Tris buffer (50mM pH 7.4). Membranes were incubated for 150 min at 0°C with varying concentrations of either: (a) ^{3}H-etorphine (0.05-5nM) in the presence of D-Ala^2-D-Leu^5-enkephalin (DADLE; 200nM) and D-Ala^2-Mephe^{4}Gly-ol^5 (DAGO; 200nM) to measure kappa receptors; (b) ^{3}H-DAGO (0.1-5nM) to measure mu receptors and (c) ^{3}H-DADLE (0.1-5nM) in the presence of unlabelled DAGO (1-50nM) to measure delta receptors. Non-specific binding was determined in the presence of 10^{-6}M etorphine, 10^{-6}M DAGO and 10^{-6}M DADLE respectively. Reactions were terminated by rapid filtration. Scatchard analysis yielded the following results;

	KAPPA		MU		DELTA	
	Bmax	K_D	Bmax	K_D	Bmax	K_D
I	109	1.31	128	0.67	56.7	1.59
C	113	0.95	116	0.60	50.9	1.65
CT	120	1.07	114	0.68	61.5	2.04

Bmax expressed as fmol/mg protein; K_D as nM

Castration of male rats or administration of testosterone to castrated rats did not alter the number or affinity of μ, κ, or δ opiate receptors, indicating refractiveness of hypothalamic opioid receptors to regulation by androgen.

140.6 ESTROGEN EFFECTS OF TUBERO-INFUNDIBULAR GABAERGIC ACTIVITY IN MALE RATS. F. Nicoletti* and J. L. Meek. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

There is evidence for the existence of a tubero-infundibular GABAergic system projecting from the arcuate nucleus of the hypothalamus to the external layer of the median eminence. GABA released from this pathway into the portal circulation apparently acts on specific pituitary receptors to inhibit prolactin (PRL) release. Hence, GABA has been implicated as a putative physiological PRL-inhibiting factor. Estrogens increase PRL secretion and influence GABAergic transmission in discrete brain regions. Here we present evidence that repeated administration of estradiol benzoate (10 g/kg, s.c., once a day for 8 days) increases GABA concentration in the anterior pituitary (+602%) in the median eminence (+56%) of male rats but it fails to induce changes in other brain regions, including arcuate nucleus, hippocampus, lateral septum, olfactory tubercle and cerebellum. It has been recently suggested that the GABA present in the anterior pituitary is produced by the central nervous system and conveyed to the gland by way of the hypophyseal portal blood supply (Racagni et al., 1979). Accordingly, the large increase in anterior pituitary GABA concentration that we observed after estradiol benzoate suggests that repeated estrogen administration increases the activity of the tubero-infundibular GABAergic system and the increase in GABA concentration found in the median eminence is consistent with this hypothesis. If GABA inhibits PRL secretion, then the observed effects cannot be a cause of the hyperprolactinaemic action of estradiol benzoate. Rather, we can hypothesize that PRL may mediate the effects of estrogens on the tubero-infundibular GABAergic system. It is consistent with this hypothesis that anterior pituitary GABA concentration is increased during suckling-induced hyperprolactinaemia (Racagni et al., 1983) and that conditions of estrogen-independent hyperprolactinaemia mimic the effects of estradiol benzoate on GAD activity in the medial basal hypothalamus (Nicoletti et al., 1983).

G. Racagni et al., Nature 281:1575-1578, 1979.
G. Racagni et al., in: Integrative Neurohumoral Mechanisms (E. Endroczi et al., eds.) pp. 337-339, Elsevier/North Holland Biomedical Press, 1983.
F. Nicoletti et al., Neuroendocrinology 36:13-16, 1983.

140.7 ESTROGEN (E) INHIBITION OF THE RAT BRAIN DOPAMINE (DA)-SYSTEM IS AREA SPECIFIC AND REVERSIBLE. H. Sakamoto*, C. Leranth*, N. MacLusky*, C. Hurlburt* and F. Naftolin* (Spon. C.F. Stevens) Dept. Ob/Gyn, Yale Univ. Sch. Med., New Haven, CT 06510.

Sustained elevations of circulating E induce arcuate nucleus lesions, adenomatous changes in the pituitary gland, hyperprolactinemia and anovulation (Brawer, J., et al. Endocrinol. 103:501, 1978). This involves decreased hypothalamic DA synthesis (Casanueva, F. et al. Endocrinol. 110:590, 1982) and is partially reversible by treatment with DA agonists. However, neither the specific site nor mechanism of pathogenesis is known. We have measured activities of tyrosine hydroxylase (TH-A), L-DOPA decarboxylase (DDC-A), and DA in striatum, arcuate-median eminence (AMe), A13, substantia nigra (SNR) and dorsal raphe of ovariectomized (OVX) animals implanted with silastic capsules containing estradiol-17β (controls, no implant) and sacrified 2-8 wks later. TH-A was measured by tritium release (Nagatsu, T., et al. J. Biol. Chem. 239: 2910, 1964), DDC-A by product identification by HPLC (Okuno, S. et al. Anal. Biochem. 29:412, 1983), and DA by HPLC with electrochemical detection (Anderson, G. et al. J. Chromatogr. 223:315, 1981). Immunocytochemically stained TH neurons and fibers in AMe and A13 were studied by light and electron microscopy.

Results: By 2 wks, TH-A and DDC-A decreased in AMe (OVX vs OVX+E:115.2±17.1 vs 28.8±2.3 TH-A, and 622.4±96.0 vs 314.9±58.5, DDC-A, n=6, pmol/mg/min., $p<0.01$) but not in other areas. DA in AMe of OVX+E fell to 50% of control. AMe enzyme activity and DA levels progressively decreased until the 8th wk. Despite the markedly decreased TH-A, AMe TH immunopositive neurons did not show ultrastructural evidence of damage. 2 wks after E capsule removal, AMe DA levels were restored (OVX vs OVX+E; 42.8±2.1 vs 65.3±18.1, n=4; ng DA/mg, protein $P>0.05$, no statistical difference). DA levels remained unchanged in SNR.

Conclusions: (1) among the DA containing brain areas, only the tuberoinfundibular DA system was inhibited by E; (2) Despite decreased enzyme activity following OVX+E, the ultrastructure of TH immunoreactive DA synthesizing neurons did not show obvious alteration; and (3) the ability of AMe to synthesize DA is not irreversibly inhibited and can be restored by removing the E stimulation. (Supported by HD13587 to F.N. Both H.S. and C.L. and are Mellon Fndn. Fellows in the Reproductive Sciences.)

140.8 RADIOIMMUNOASSAY OF HIPPOCAMPAL CELL-NUCLEAR ALDOSTERONE AND CORTICOSTERONE. B. G. Yongue and E. J. Roy. Dept. of Psychology, Univ. of Illinois, Champaign, IL, 61820

Aldosterone (ALD) and corticosterone (B) bind to cytosol and nuclear receptors in limbic neurons of rodent brain. Receptor binding assays and autoradiography have described the biochemical characteristics and regional distribution of these receptors, but cannot measure the concentrations of either hormone in the brain. Radioimmunoassay (RIA) has revealed that B secreted by intact rats is concentrated in cell nuclei of the brain in a pattern that parallels the distribution of corticosteroid receptors (McEwen, et al., J. Neurosci. Meth. 3:57, 1980). Moreover, the nuclear concentrations of B in the brain were altered by changes in plasma B levels. However, since the relative affinity of each of the corticosteroid receptor-types for B is higher than for ALD, it is questionable whether ALD would be found in brain cell-nuclei in the presence of B.

We are examining the concentrations of ALD and B in purified nuclei of rat hippocampus (HPC) by RIA to determine whether ALD is localized in brain cell-nuclei in the presence of B, whether nuclear levels of ALD are altered by physiological changes in serum ALD, and whether ALD and B compete for nuclear uptake in vivo.

HPC cell-nuclei were prepared from pooled rat-HPC (3-8/assay) as for a nuclear exchange assay. The purified nuclear pellet was extracted in ethanol and the dried extract was reconstituted in buffer for assay of ALD and B by RIA. Adrenalectomized (ADX) rats (n=7/group) were injected with 5mg/kg of either ALD, B, both hormones (A+B), or saline (CON). The nuclear concentrations (pg/μg DNA) of ALD and B, 1 hour later are presented below. These results indicate that ALD can be measured in the presence of B. Cross-reaction is minimal as indicated by the similarity of the values for the B and CON groups in the ALD assay and the ALD and CON groups in the B assay. Similar to McEwen, et al., (1980) we found a B-immunoreactive factor in HPC nuclei of ADX rats.

	ALD	B	A+B	CON
ALD	0.82	<0.07	0.63	<0.07
B	0.53	3.00	5.00	0.74

Experiments are in progress to examine the physiological levels of ALD and B in HPC cell-nuclei. Varying dietary salt, circadian rhythms, and the stress response provide physiological changes in serum ALD and B. Initial results from these experiments indicate that physiological levels of serum ALD and B result in nuclear uptake of both hormones in HPC (ALD ∿ 10-100 pg/mg DNA; B ∿ 100-1000 pg/mg DNA).

140.9 APPLICATION OF A GLUCOCORTICOID RECEPTOR EXCHANGE ASSAY TO BRAIN TISSUES: SOME CAUTIONS. B.B. Turner. Dept. of Physiology, College of Med., East Tenn. State Univ., Johnson City, TN 37614.

In order to assay glucocorticoid receptors in intact animals or to study feedback effects of corticosteroids on receptor number, steroid-bound receptors as well as the unoccupied receptors must be measured. For these studies an exchange assay is required in which endogenous steroid is removed and replaced with 3H-labelled steroid. Such an exchange assay was recently published based on data from liver cytosols.

In attempting to replicate the results, we found that the DTT must be added after the endogenous steroid has been removed with DCC if "exchange" is to occur. We homogenized tissues 1:10 (wt./vol.) with buffer containing 25mM HEPES, 10 mM molybdate, and .25M sucrose, pH 7.4. Aliquots of cytosol were immediately incubated (4h at 4C) in the presence of 5mM DTT plus 3H-dexamethasone (25 nM) with/without 500X excess unlabelled steroid. Additional aliquots of cytosol were pre-incubated (1h at 4C) in the presence of corticosterone (CORT) or dexamethasone (DEX). Pre-incubation was terminated by incubated (10 min) with dextran-coated charcoal (DCC). Following pre-incubation, aliquots were incubated with 3H-DEX for varying periods of time and bound 3H-DEX was recovered following incubation with DCC. Under these conditions no loss of binding occurs for at least 72 hrs.

We have found several points of difference between brain tissue and liver with respect to this exchange assay. First, high protein concentrations are necessary to avoid substantial protein loss with DCC since protein (including receptor) loss varies inversely with protein concentration. Brain cytosols sustained 70%-80% protein loss in the course of two sequential DCC treatments. We find that pre-absorbing BSA to the DCC eliminates receptor loss and reduces protein loss by 60% in cytosols (2.5 mg/ml). Second, the time required for complete exchange and the completeness of exchange varied among the 4 tissues examined: hippocampus, cerebral cortex, hypothalamus and liver. Following pre-incubation with CORT (100nM), cortex, hypothalamus, and liver showed complete exchange by 24 h (100% of initial binding). In contrast, by 48 h the hippocampus reached only 80% of initial binding (100nM CORT) and 65% following 1uM CORT. It was expected that cytosols pre-incubated with DEX would be more difficult to exchange. Liver cytosols exposed to 100 nM DEX required 72 h for complete exchange. Hippocampus and cortex exposed to the same concentration of DEX required only 48 h for complete exchange, but hypothalamus showed only 83% exchange at 48 h. These results suggest that validation of this assay for the brain region of interest may be appropriate.

140.10 DEXAMETHASONE SUPPRESSES BETA-ENDORPHIN IN HUMANS. D. E. Krantz* and W.A. Brown* (SPON: R.G. Mair). Veterans Administration Medical Center and Brown University, Providence, RI 02908.

Considerable evidence indicates that the regulatory mechanisms governing the synthesis and secretion of adrenocorticotropin (ACTH) from the pituitary are common to the opiate peptide beta-endorphin (B-END). Contrary to these findings is the report that dexamethasone (DEX) failed to suppress B-END plasma concentrations in humans (Kalin, N.H. et al, Science, 209:827-828, 1980).

The response of B-END to DEX was recently investigated in our laboratory through the administration of a midnight oral dose of 2 mg DEX and placebo to 6 healthy male volunteers in a double blind balanced design. Blood samples were drawn for measurement of B-END and cortisol between 8:00 and 9:30 a.m. before and after both DEX and placebo. Plasma B-END immunoreactivity was determined by way of affinity gel extraction (Sepharose anti-B-END) followed by radioimmunoassay ($< 5\%$ cross reactivity to beta-lipotropin). B-END levels (mean $\pm$ SEM pg/ml) were significantly suppressed following DEX (pre-DEX 15.3 $\pm$ 2.0, post-DEX 9.1 $\pm$ .5, $t = 3.46$, $p < .01$) but not following placebo (pre-placebo 17.8 $\pm$ 2.9, post-placebo 17.2 $\pm$ 1.5, $t = .27$). Cortisol was also suppressed following DEX but not following placebo.

Prior to this study, B-END levels in 4 of these subjects had been measured using a different B-END assay system, and did not appear to suppress with DEX. This earlier assay was marked by unusually high B-END concentrations ($\geq$ 100 pg/ml) for all baseline and experimental samples; furthermore, plasma samples stripped of B-END by silicic acid yielded spurious B-END levels $\geq$ 100 pg/ml. The previous study by Kalin et al which failed to demonstrate DEX suppression of B-END was also characterized by unusually high B-END levels (pre-DEX and post-DEX > 140 pg/ml).

The failure of DEX to suppress human plasma B-END in this study by Kalin et al and in our own initial study appears to be accounted for by an artifact of the B-END assay system, possibly the non-specific binding of labelled B-END to high molecular weight plasma components (Orf, J.W. et al, Clin. Res., 27:680A, 1979). In the present study, this artifact was not encountered and B-END was clearly suppressed by DEX.

140.11 PRESENCE OF CORTICOTROPIN-RELEASING FACTOR AND GROWTH HORMONE-RELEASING FACTOR IN RAT BRAIN AND EXTRA-BRAIN TISSUE. T.O. Bruhn*, R.T. Mason*, and W.W. Vale. (SPON: M.E. Baker) Peptide Biology Laboratory, The Salk Institute, La Jolla, CA 92037.

Corticotropin-releasing factor (CRF), a 41-amino acid peptide, and growth hormone-releasing factor (GRF), a 43-amino acid peptide, have been recently isolated from rat hypothalamus and sequenced. The availability of specific antisera allows study of the distribution of these peptides. In the present paper, we describe the distribution of CRF-like immunoreactivity (CRF-LI) and GRF-LI in rat brain and peripheral tissues.

Twenty to 40 fragments of the tissues listed below were acetone acid extracted, defattened and subjected to sephadex chromatography (G-50 fine) or to purification using disposable reverse phase C-18 columns. CRF-LI was measured using an N- and a C-terminal directed antiserum (C-24 and RC-68). GRF-LI was measured using antiserum RG-71. The highest concentrations of CRF-LI were found in the hypothalamus (297 fmoles/region; 21.2 fmoles/mg dry tissue weight) followed by brain stem (180 fmoles/region; 3.4 fmoles/mg). Significant amounts of CRF-LI were also found in adrenals (45 fmoles/region; 2.4 fmoles/mg) and duodenum (112 fmoles/region; 2.1 fmole/mg). In other parts of the gastrointestinal tract (GI) only low amounts of CRF-LI could be detected: fundus 0.29, antrum 0.18, ileum 0.2, colon 0.2, pancreas 0.32 and liver 0.045 fmoles CRF-LI/mg dry weight.

GRF-LI was predominantly present in the hypothalamus (455 fmoles/region; 32.5 fmoles/mg dry weight) while only small amounts were detected in the brain stem (79 fmoles/region; 1.49 fmoles/mg). From all the extra-brain tissues listed above, significant amounts of GRF-LI were only found in the duodenum (111 fmoles/region; 2.15 fmoles/mg). On both sephadex G-50 and on HPLC, CRF and GRF from duodenum and hypothalamus coeluted with the respective synthetic peptides suggesting that brain and extra-brain CRF and GRF are similar if not identical. We also analyzed systemic rat plasma and cerebrospinal fluid (CSF) for the two peptides. Levels in systemic plasma are below 1 fmole/ml. Significant amounts of CRF-LI (9.8 fmole/ml) were extracted from CSF, while GRF-LI was undetectable.

The presence of CRF-LI in the GI tract and the reported direct effects of CRF on mesenteric blood flow and stomach acid secretion support the possibility that this peptide may have local physiologic actions within the GI tract.

140.12 THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN) HAS A PIVOTAL ROLE IN COMPENSATORY ADRENAL HYPERTROPHY. J.Z. Kiss * É. Mezey * and F.A. Antoni *. (Spon: C.W. Sharp) Laboratory of Cell Biology, NIMH, Bethesda,MD 20205.

It has been previously observed that removal or mechanical manipulation of one of the adrenals causes hypertrophy of the gland on the contralateral side. This response,"compensatory adrenal hypertrophy", is thought to be mediated by hormonal factors, primarily pituitary pro-opiomelanocortin derived peptides, as well as through a direct neural efferent mechanism. The neurons of the PVN produce corticotropin releasing-factor (CRF) and vasopressin (VP), which are the key regulators of pituitary corticotrope function. Moreover, PVN neurons may influence autonomic nervous function through direct projections to centers of autonomic regulation in the lower brainstem and the spinal cord. The aim of the present study was to investigate whether these morphologically distinct groups of neurons are involved in the compensatory adrenal hypertrophy response. Removal of the left adrenal gland of young male albino rats (LA) increased the wet weight and the RNA content of the remaining right adrenal 24 h after surgery. Microsurgical lesioning of the PVN, hypophysectomy or transection of the pituitary stalk all abolished the effects of LA. These data strongly suggest that stimulation of the secretory activity of pituitary corticotropes, presumably by CRF and VP released from PVN neurons, is essential for the hypertrophy response. This is in contrast with earlier reports which indicated that a direct neural mechanism alone an provide sufficient stimulus for compensatory hypertrophy.

The possibility that a neural efferent pathway is indeed involved was tested in initial experiments by administering the ganglion blocker chlorisondamine (CI). Injection of CI to intact rats produced increased adrenal weight and RNA content comparable to that after LA. When given to rats subjected to LA , CI did not alter the hypertrophy response. Further work is in progress to reveal the possible role of direct neural efferents to the adrenal gland in the mediation of compensatory adrenal hypertrophy.

140.13 ANGIOTENSIN II AFFERENTS FROM CIRCUMVENTRICULAR ORGANS TO THE SUPRAOPTIC NUCLEUS. L.D. Mitchell, L.D. Wilkin, A.K. Johnson. Depts. Psychology, Anatomy, Pharmacology, Univ. of Iowa, Iowa City, IA 52242.

Systemic angiotensin II (AII) and plasma osmolarity are known to stimulate neuronal activity in the supraoptic nucleus (SON) of the hypothalamus as well as the release of vasopressin from the magnocellular-pituitary system. It has been postulated that this activity may be mediated by receptors in the circumventricular organs of the forebrain and by intrinsic AII neurons projecting to the SON.

Forebrain afferents to the SON were investigated in the present study using combined retrograde tracing and immunohistochemistry. The fluorescent antibiotic doxorubicin was used as a retrograde tracer in combination with fluorescein isothiocyanate (FITC) immunohistochemistry utilizing an antibody to AII. Doxorubicin (1.0 - 1.5 µl, 5% or 10%) was injected into the SON of male Sprague Dawley rats 2-4 days prior to perfusion with 4% formaldehyde in buffered saline. Following removal of the brains and 5-6 h fixation, alternating sections were incubated for 48 h in an antiserum to AII (D. Ganten) and then processed for fluorescence immunohistochemistry. Using the same emission (360 nm) and excitation (480 nm) filters, cells labeled retrogradely by doxorubicin fluoresced orange, while cells containing AII immunoreactivity fluoresced green. Cells containing doxorubicin were identified in the subfornical organ, the median preoptic nucleus, and the organum vasculosum of the lamina terminalis. Cells containing AII immunoreactivity were also identified in each of these three regions. A few cells in the subfornical organ and in the organum vascululosum of the lamina terminalis contained both doxorubicin and AII immunoreactive fluorescence. Thus, it may be concluded that a pathway exists by which AII-containing cells of the circumventricular organs may transmit information directly to the SON.

140.14 ORGANIZATION OF AFFERENTS FROM BRAINSTEM NORADRENERGIC CELL GROUPS TO THE SUPRAOPTIC NUCLEUS. L.D. Wilkin, L.D. Mitchell and A.K. Johnson. Depts. Psychology, Anatomy and Pharmacology, Univ. of Iowa, Iowa City, IA 52242.

Brainstem afferents to the magnocellular zones of the paraventricular nucleus (PVN) have been well defined for some time. It has been assumed that brainstem afferents to the magnocellular regions of the supraoptic nucleus (SON) generally parallel those to the PVN, but this has not been well demonstrated. Afferents to the SON from the brainstem noradrenergic cell groups A1, A2 and A6 were investigated in the present study by the use of lesions, retrograde tracing, and fluorescence histochemistry. Doxorubicin, a fluorescent dye that is transported retrogradely, was injected into the SON of Sprague Dawley rats 3-5 days prior to perfusion fixation with 4% formaldehyde. Brainstem sections were examined for the presence of doxorubicin and formaldehyde-induced catecholamine fluorescence. In some brains, alternating sections were incubated with antiserum to tyrosine hydroxylase (T. Joh) and processed for fluorescence immunohistochemistry. Cells labeled retrogradely by doxorubicin were found in the A6 region as well as in the A1 and A2 regions. Some of the labeled cells in each of these regions were also found to contain formaldehyde -induced fluorescence or tyrosine hydrosylase immunoreactivity. Within the A6 region, labeled catecholamine cells were found ipsilateral to the doxorubicin injection. Within the A1 and A2 regions, however, labeled catecholamine cells were found predominantly contralateral to the injection, indicating a crossed projection from these cells to the SON. This was confirmed by examining glyoxilic-acid induced catecholamine fluorescence in the SON following lesions of either A2 or the ventral bundle. Catecholamine terminals showed a decrease within the SON on the side contralateral to the lesions.

It may be concluded that noradrenergic innervation patterns to magnocellular neurons differ between the PVN and the SON, a finding with possible functional considerations.

140.15 CELL COUPLING AND ELECTRICAL ACTIVITY IN THE CORPORA ALLATA OF THE COCKROACH DIPLOPTERA PUNCTATA. C.S. Thompson, D.J. Lococo*and S.S. Tobe* Dept. of Zoology, Univ. of Toronto, Toronto, Ontario, Canada M5S 1A1.

In the adult cockroach Diploptera punctata the corpora allata (CA) are endocrine glands, 200 to 300 microns in length, which synthesize juvenile hormone (JH). The number of cells in the CA is age dependent, ranging from 5000 to 9000 cells. The CA cells are columnar or spindle-shaped, 5 to 8 microns in length. We used intracellular microelectrodes to study the electrical properties of the CA cell membranes. Three observations indicate that these cells are extensively coupled by gap junctions. First, when Lucifer Yellow dye is injected into one CA cell it rapidly spreads to neighboring cells. Second, when current is injected into one cell, voltage responses may be recorded in other gland cells. Finally, freeze fracture electron microscopy reveals extensive aggregations of particles in the plasmalemma of the CA cells.

When current is injected into one cell the magnitude of the passive voltage response in nearby cells is determined by the junctional resistance as well as by cell membrane resistance. Voltage responses were linear to both depolarizing and hyperpolarizing current pulses, up to about 15 nanoamps. Secondary membrane responses were not elicited by depolarizing current, thus, under our experimental conditions the cell membranes are electrically inexcitable. When glands producing maximal amounts of JH (day 5 mated females) were compared with glands producing minimal amounts of JH (day 1 virgin females) no differences in current spread or dye spread were observed.

Electrical stimulation of axon tracts innervating the CA elicited all-or-none depolarizing potentials which resemble chemically mediated excitatory postsynaptic potentials (EPSPs). EPSPs were 1 to 4 millivolts in amplitude and showed no short-term facilitation. Simultaneous recording from two CA cells showed the EPSPs to be of the same amplitude from cell to cell. Experiments are under way to determine the function of this innervation.

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140.PO TARGET SITES FOR $1,25(OH)_2$ VITAMIN D_3 IN THE BRAIN. W.E. Stumpf, Depts. of Anatomy and Pharmacology, Univ. of North Carolina, Chapel Hill, NC 27514.

With thaw-mount autoradiography - a technique developed earlier in our laboratory - genomic sites of uptake and retention of ^3H $1,25(OH)_2$ vitamin D_3 were discovered and anatomically defined. Two hours after injection of 0.19ug/100g bw of ^3H $1,25(OH)_2$ vitamin D_3 to vitamin D-deficient rats and mice, brains were frozen and 4um serial sections mounted on photographic emulsion coated slides. Photographic development after exposure times of several months revealed nuclear concentration of radioactivity in certain neurons in the nucleus centralis of the amygdala and the nucleus interstitialis striae terminalis (pars dorsolateralis septalis), as well as in neurons of motor nuclei of cranial nerves, and of the nucleus ambiguus.

When unlabeled $1,25(OH)_2$ vitamin D_3 was injected prior to ^3H $1,25(OH)_2$ vitamin D_3, nuclear labeling is diminished or abolished. This is not the case with 25(OH) vitamin D_3. The results indicate specific nuclear binding of ^3H $1,25(OH)_2$ vitamin D_3 at select sites, similar to the nuclear binding of this steroid hormone in intestine, kidney and bone. Together with our previous observations of $1,25(OH)_2$ vitamin D_3 target cells in the pituitary, parathyroid and endocrine pancreas, the data suggest direct effects on different neuronal systems that include forebrain sites which are probably part of a vitamin D regulated brain-pituitary-endocrine axis, similar to other steroid hormones.

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141.1 TERMINATION OF PROPRIOCEPTIVE PRIMARY AFFERENT FIBERS IN CAT CUNEATE NUCLEUS. J.P. Pierce, R.J. Weinberg, R.E. Fyffe*, and A. Rustioni, Depts. of Anatomy and Physiology, Univ. of North Carolina Medical School, Chapel Hill, NC 27514.

The injection of HRP into identified nerve fibers allows one to examine the relationship that exists between the physiological response properties of a single axon and the morphology of its terminal arbors. This approach has proven to be particularly useful in the examination of primary sensory fibers, since the response parameters of these neurons are easily characterized. We have studied the terminations of forelimb primary afferent fibers with proprioceptive properties in the cuneate nucleus of the nembutal-anesthetized cat.

Nerve fibers were recorded in the cuneate fasciculus and nucleus. A search stimulus was applied through a cuff electrode placed on the radial nerve. Units were further characterized by mechanical stimulation and manipulation of the limb. When successfully impaled, as demonstrated by the abrupt appearance of a resting potential of over -30 mV, the fibers were iontophoretically injected with HRP using 5-50 nA square wave current pulses. At least one hour after injection, the animal was perfused, and serial sections were processed for light and electron microscopic analysis.

Previous work from our lab (Cheema et al., Neurosci. Abst. 9) found that proprioceptive fibers from the forelimb muscles terminate both in a marginal zone around the edge of the cuneate nucleus and in the ventral reticular zone. Our present results suggest that the marginal terminations are densest along the lateral edge of the nucleus, as suggested by some physiological work (e.g., Rosén, Brain Res. 16). The lateral marginal terminations are relatively focused, but the reticular terminations of these fibers are more diffuse. This suggests that the pattern of termination for proprioceptive units may be dependent on the local region of termination.

We are currently examining the regional variations of these terminals, in respect to branching pattern, size of terminal area, bouton density, form, and arrangement. Preliminary findings suggest that the terminal branching pattern in an individual collateral is closely related to the pattern in other collaterals from the same parent axon, but generally different from the pattern in collaterals from other proprioceptive fibers. Thus, the branching pattern appears to be both non-random, and partially fiber-specific.

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141.2 IMMUNOHISTOCHEMICAL ANALYSIS OF 5HT INPUT TO DIENCEPHALIC-PROJECTING DORSAL COLUMN NUCLEI (DCN) IN THE RAT. S.M. Carlton, H.H. Willcockson*, and W.D. Willis. Marine Biomed. Inst., and Depts. of Anat. and of Physiol. & Biophys., Univ. Texas Medical Branch, Galveston, TX 77550-2772.

Several lines of evidence indicate that the DCN are intimately involved in the central processing of innocuous somatosensory information. Anatomical studies show that the DCN receive 1^{o} afferent input as well as input from postsynaptic dorsal column pathways, which is further relayed on to the ventrobasal thalamus. This convergence of input on the DCN may contribute to such complex functions as sensory discrimination. The cellular and synaptic organization in the DCN responsible for such functions is unknown. However, the DCN contain a variety of morphologically different cell types presumably with corresponding differences in physiology. Also, physiological studies demonstrate that these cells are subject to inhibitory influences arising from the cortex, PAG and various raphe nuclei. The present work identified 5HT contacts on cells in the DCN which project to the diencephalon, and may provide a partial explanation for the complex processing which can occur at this level. HRP injections were made into the thalami of 5 rats. After 48 hrs and pretreatment with pargyline, rats were perfused. Sections (25 µm) were reacted for HRP with the $CoCl_2$-DAB protocol. Free floating sections were then immunohistochemically (IHC) stained for 5HT (Bowker et al., 1982). HRP labeled cells contained black punctate granules while IHC stained varicosities had an amber color. All data were analyzed using a 100X oil immersion objective. 5HT contacts were identified by focusing on varicosities or fibers found within 1 or 2 µm of the focal plane of labeled cells.

Analysis of the sections verified that 5HT fibers and varicosities were present throughout the substance of the DCN (Steinbusch, 1981). Preliminary analysis of individual labeled neurons in the DCN demonstrated that different morphological cell types received 5HT input. Single and multiple contacts were identified on cell somas as well as dendrites. Based on physiological evidence, the nucleus raphe magnus and/or the midbrain raphe are the most likely sources of the 5HT input. Thus, the widely accepted concept that the descending 5HT inhibitory system is specific for nociception should be modified to include modulation of innocuous input to the DCN. Modulation at this level may enhance the recognition of a noxious stimulus. (Supported by NS11255, NS09743, NS07062.)

141.3 THREE-DIMENSIONAL RECONSTRUCTION OF THE BODY REPRESENTATION WITHIN SOMATOSENSORY NUCLEI IN THE RAT MEDULLA. S.E. Knowles, C.-H. Shin, W.K. Smith, D.S. Schlusselberg, D.J. Woodward, and J.K. Chapin, U. Tx. Hlth. Sci. Cntr., Dallas, TX 75235

We have developed a computer based system for 3-dimensional graphic manipulation of somatosensory receptive field (RF) mapping data. Our aim here was to reconstruct in 3-D the sensory representation of the body in the gracile (nG), cuneate (nC), external cuneate (nCE), and spinal trigeminal (nSTT) nuclei in the rat. To precisely define the somatotopic organization of these nuclei, repeated microelectrode penetrations into the dorsal medulla were made in rostro-caudally or medio-laterally oriented arrays. When cells were encountered, the body location of their RF's, and also their somatosensory submodality (i.e. cutaneous, joint, etc.) were determined. 3-D reconstruction of such mapping data was carried out by: 1) making histological sections of the recorded brain, 2) drawing these sections into the computer using a graphics tablet, 3) entering the X-Y-Z positions of cells whose RF's were located on a given body part, and 4) generation of pictures showing 3-D reconstructions of the representation of that body part. These pictures revealed a continuous representation of the cutaneous periphery of the body which extended through the nG, nC, and nSTT. In general, the representation of a given body part formed an oblique, rostro-caudally directed column, which tended to move ventrolaterally at successively more rostral levels. Most of the body map was visible in a typical coronal section taken through the obex. At this level, the back and trunk appeared dorsally; the hind quarters medially; the large upper lip representation laterally; and the distal extremities and rostral mouth parts ventrally. These studies also revealed a rostro-caudal organization. Most of the whisker representation was caudal to the obex, the ventral ("E"-row) whiskers being most caudal. The upper lip was represented most rostro-laterally, with the lower lip just medial to it. Forelimb joint RF's in the nEC were rostral and dorsal to forepaw cutaneous RF's in the nC. The tongue and mouth representation extended rostrally, but also medially into the reticular formation near N. Solitarius. The complexity of this body representation in the rat medulla demonstrates the importance of using 3-D reconstructions for evaluating mapping data. Supported by NS18041, AA0390, and the Biological Humanics Foundation.

141.4 PATTERNS OF RESTING DISCHARGE IN NEURONS OF THE RACCOON MAIN CUNEATE NUCLEUS. Benjamin H. Pubols Jr. Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, Portland, OR 97209.

Neurons of the raccoon main cuneate nucleus (MCN), all having cutaneous peripheral receptive fields (RFs) located on the ipsilateral forelimb, were examined for the presence and nature of resting discharge. Microelectrodes were used to record the extracellular activity of 102 single neurons, of which 66 were activated, either antidromically or synaptically, by electrical stimulation of the contralateral thalamic ventrobasal complex (VB), and 36 were activated by stimulation of the ipsilateral cerebellum (CB).

Principal results were as follows:

1. Approximately 42% of VB-activated and 25% of CB-activated neurons displayed a resting discharge.
2. Most neurons activated from VB and showing a resting discharge fired in bursts of 2-5 spikes, while those activated from CB and showing a resting discharge generally fired as single, irregularly-spaced spikes, with occasional bursts in some neurons.
3. Differences in the proportions of neurons displaying a resting discharge did not vary significantly as a function of type of preparation: methoxyflurane anesthesia, pentobarbital sodium, or decerebrate (the latter CB-activated only).
4. Neurons exhibiting a resting discharge were more likely to show a bursting pattern in methoxyflurane-anesthetized preparations (81% of neurons with a resting discharge) than were neurons in either decerebrate (50%) or pentobarbital-anesthetized preparations (36%).
5. The overall mean rate of firing did not appear to be different for bursting versus non-bursting neurons.
6. In bursting neurons, modal interspike intervals (ISIs) varied between 1.3 and 2.5 msec. Each neuron also had a characteristic minimal interburst interval (IBI), varying between 45 and 80 msec from neuron to neuron. Distributions of within-burst ISIs and minimal IBIs had comparable coefficients of variation, varying between .035 and .202.
7. The application of a mechanical stimulus to a neuron's RF led to an increase in the number of spikes/burst, a decrease in the minimal IBI, an increase in the occurrence of single spikes, or some combination of these changes.

These differences in resting discharge patterns of VB-activated and CB-activated MCN neurons imply fundamental differences in the mode of somatosensory information processing by neurons of cuneothalamic versus cuneocerebellar systems. (Supported by grant NS-19486,USPHS.)

141.5 THE INTERSTITIAL NUCLEUS OF THE SPINAL V TRACT: ANATOMICAL ORGANIZATION AND PATTERNS OF CONNECTIVITY. W. M. Falls and K. D. Phelan. Dept. of Anatomy, Michigan State University, East Lansing, MI 48824-1316.

The spinal V tract (SVT) in its course alongside the spinal trigeminal nucleus contains among its axons patches of neurons and neuropil collectively referred to as the interstitial nucleus of Cajal (IN). Using the methods of Nissl, retrograde transport of HRP and Golgi, this study demonstrates that, based on differences in overall cell morphology and patterns of connectivity, IN in the adult rat consists of morphologically and functionally distinct portions. Associated with the rostral 1 mm of the medullary dorsal horn (MDH) are two distinct groups of neurons projecting to the contralateral thalamus. One group, situated dorsomedially in SVT, is a small cluster of spiny multipolar neurons while the other group, located ventromedially, contains a network of cells with oval to fusiform-shaped somata (∿9 μm in diameter). Extending rostrally from the obex through nucleus interpolaris (Vi) and into caudal nucleus oralis are a diffuse collection of neurons situated within SVT and the adjacent spinocerebellar tract (SVT) as well as wedged between these two tracts and along their medial and lateral borders, respectively. The majority of these neurons have axons which project to the ipsilateral cerebellum via SCT and are characterized by their oval to fusiform-shaped somata (12-18 μm) that give rise to one to four thick primary dendrites. Large islands of neuropil containing 15-30 μm pale staining somata (paratrigeminal nucleus; PT) occupy the dorsomedial portion of SVT as well as adjacent regions of Vi, from the obex through the caudal one-half of Vi. PT neurons include a few Golgi Type II interneurons and many projection cells. Dendritic fields of some projection cells are oriented rostrocaudally and are characterized by tortuously coursing aspiny dendrites while the dendritic arbors of other projection neurons have a dorsoventral orientation and are composed of long primary and secondary dendrites (up to 320 μm in length) with spines prominent on distal dendritic branches. Axons of PT projection neurons do not innervate either the thalamus, the cerebellum or the spinal cord. Based on these results IN should no longer be considered a single homogeneous nucleus. Instead, IN represents a composite of morphologically and functionally distinct regions each differing not only in the overall morphology of its neurons, but also in the projections of these neurons along the neuraxis. Supported by N.I.H. Grant DE06725.

141.6 RED NUCLEUS EFFECTS ON TRIGEMINAL SUBNUCLEUS ORALIS NEURONS. Karen D. Davis* and Jonathan O. Dostrovsky (SPON: F. Coceani) Dept. of Physiology, University of Toronto, Toronto, Ontario M5S 1A8, Canada.

It is widely accepted that the red nucleus (RN) is involved in the neural control of distal muscles. Recently, Gray and Dostrovsky (Soc. Neurosci. Abstr. 8: 91, 1982; 9: 247, 1983) have shown that electrical stimulation applied to RN modulates the transmission of sensory information in the spinal cord dorsal horn, dorsal column nuclei and medullary dorsal horn. Anatomical studies have reported that the red nucleus projects to these and other somatosensory relay nuclei such as the trigeminal subnucleus oralis (V oralis). Hence we have chosen to study the effect of RN stimulation on the responses of cells in V oralis.

Experiments were performed on chloralose anesthetized cats. Arrays of bipolar stimulating electrodes were inserted into the contralateral thalamus and both the contralateral and ipsilateral RN. Extracellular single unit recordings were made in V oralis with tungsten microelectrodes. Cells were excited to just suprathreshold levels by electrical stimulation within their receptive field. Some cells were also excited by electrical stimulation of the mandibular and/or maxillary tooth pulp. RN influences were studied by applying 100ms, 500Hz, trains of conditioning pulses to the contralateral or ipsilateral RN 130ms prior to the peripheral test stimulus. The responses of most cells (40/42), both trigeminothalamic and interneurons, were inhibited following RN stimulation. 7 of these cells were excited by RN stimulation prior to the inhibition. The responses to tooth pulp stimulation were inhibited as readily as those to cutaneous stimulation. In many cases stimulation of the ipsilateral RN was found to be as effective as stimulation of the contralateral RN in reducing V oralis cell activity. Since many stimulation sites were in the medial part of the nucleus we may have been activating rubral efferents as they crossed over from the contralateral side. These results provide further evidence suggesting that the RN provides a modulatory influence on the transmission of somatosensory information at various relay sites in the somatosensory system. These modulatory effects may become active during movements.

Supported by the USPHS DE05404

141.7 GOLGI-EM STUDIES OF DENTAL RELAY SITES IN SPINAL TRIGEMINAL NUCLEUS. L.R. Johnson*, L.E. Westrum and L.M. O'Neil* (Spon: M. R. Byers). Depts. of Neurological Surgery and Biological Structure, Univ. of Washington, Seattle, WA 98195.

Pilot studies are being carried out on the cat brain stem trigeminal nuclei using the Golgi-EM method of Farien et al., (1977). We are emphasizing the regions shown to receive afferents from the teeth; ventral pars interpolaris at the periobex level. This area demonstrates transganglionic degeneration after dental surgery or exfoliation. It is also a subregion of increased immunoreactivity (Westrum, et al. - this meeting). It is the purpose initially to characterize in normals the cell types in the subnucleus and their light microscopic (LM) dendritic patterns. Then the electron microscope (EM) will be used to identify and describe the synaptic populations associated with these impregnated profiles in the same preparations. This will be followed by similar correlative, LM-EM studies in subjects after dental surgery or tooth shedding, in order to characterize the class of cells, dendritic arrangements and synaptic relationships (degenerating terminals) associated with the specific afferents.

Thus far, in the controls, at least three cell types can be identified in the subnucleus with LM: 1) Large (~30 μm) soma with few thick, smooth branches, and an elongated dendritic field; 2) Small (~15 μm) soma with sparsely-spined dendrites emerging usually from one pole of the soma (mitral-like) and; 3) Small (10-15 μm) soma with shorter, branching dendrites containing spines and excrecenses (complex or Golgi II-like). The dendritic fields vary in radius from the full width of the nucleus (cell type 1) to as little as 50 μm (type 3). EM of the dendrites of these cells show terminals with either flat (F) or round (R) synaptic vesicles and clearly symmetric or asymmetric synaptic contacts. The quality of preservation will permit ready identification of even subtle or atypical degeneration, in addition to dense types, all seen in the area with dental lesion.

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141.8 "SUICIDE TRANSPORT" IN FELINE DENTAL AFFERENTS. M.A. Henry*, L.R. Johnson*, and L.E. Westrum (Spon: J.D. Loeser). Depts. of Neurological Surgery and Biological Structure, Univ. of Wash., Seattle, WA 98195.

Transganglionic transport and degeneration techniques have shown two different projection patterns from teeth to brain stem with light microscopy (LM). Argyrophilic degeneration following dental surgery is seen bilaterally in ventral partes interpolaris and caudalis whereas HRP is transported from teeth ipsilaterally to the dorsal and middle portions of all the nuclei.

Toxic ricin, in the form of Ricinus communis agglutinins [RCA I (120) or RCA II (60)] (E-Y Labs) has been shown to be retrogradely transported to the cell body, where the toxic ricin causes neuronal death by inactivating ribosomal function. The degenerating terminals within the CNS associated with such cell death have been demonstrated by LM reduced silver stains (Fink-Heimer). We have used this procedure to study the projection patterns of dental afferents.

Toxic ricin (RCA I or II) was injected (1-3 μl/tooth) into the pulpal chambers of either; 1) the maxillary and mandibular cuspids unilaterally or 2) all teeth unilaterally (from the cuspid posterior to the molar). Following a 1-2 week survival time the animal was sacrificed and the trigeminal ganglia and brain stem were processed for LM. Cresyl violet stained ganglion sections demonstrated cell bodies that have chromatolytic changes with peripherally displaced nuclei and nucleoli. These cell body changes were restricted to the ganglion regions previously identified as containing cell bodies from V_2 or V_3. Serial (1 in 10) frozen sections through the brain stem trigeminal nuclear complex, stained by the Fink-Heimer method, were studied by LM. Degenerating fibers and terminals were identified in the spinal tract and each of the nuclei. Although somewhat variable in numbers, degeneration was seen in both ventral and dorsal regions, the areas previously identified as receiving dental afferents, but by different methods. Our results show that the ricin technique is clearly applicable to this specific primary afferent system and is a useful adjunct to the identification of the class of cells and the combined CNS projection sites. It further confirms a dual projection pattern for dental afferents.

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141.9 TRANSNEURONAL AND TRANSCELLULAR TRANSPORT OF HORSERADISH PEROXIDASE-WHEAT GERM AGGLUTININ (HRP-WGA) IN RAT TRIGEMINAL SENSORY NEURONS. C.F. Marfurt and C.E. Adams*. Pennsyl-State University College of Medicine, Hershey, PA 17033

HRP-WGA conjugate is used widely for studying neuronal connectivity and the superior sensitivity of the tracer as compared to "free" HRP is well documented. However, there is some evidence that the conjugate may, in certain pathways, be transported transneuronally. In the present study, we report that HRP-WGA injected into the rat trigeminal ganglion (TG) is transported transneuronally and transcellularly in large quantities at both the central and peripheral endings of trigeminal primary sensory neurons.

HRP or HRP-WGA was injected into the TG of adult rats. Five hours to 14 days later, the animals were perfusion-fixed and the brain, TG, cornea, and dental tissues processed for HRP histochemistry. Tissues were examined critically at both the light and electron microscopic levels. The results showed that HRP-WGA was taken up avidly by virtually all neurons in the injected TG. The conjugate was then transported anterogradely in massive amounts into both the central and peripheral processes of these cells.

At 5 hours postinjection, intensely-labeled terminal fields filled the entire ipsilateral trigeminal brainstem nuclear complex (TBNC). At 20 hours, large numbers of transneuronally-labelled cell bodies were seen embedded within the terminal fields at all rostrocaudal levels of the TBNC. By 24 hours, faint terminal labeling was present also in the VBm nucleus of the contralateral thalamus. Terminal fields and neuronal perikarya in the TBNC remained intensely labeled at 2,3, and 5 days postinjection; terminal labeling in VBm was maximal at 2 days and disappeared by 5 days. At 7 and 10 days postinjection, the labeling intensity in the TBNC terminal fields and perikarya was decreased significantly; by 14 days terminal labeling was negligible and only a few transneuronally-labeled somas remained.

Twenty-four hours postinjection, linear arrays of reaction product filled completely also the peripheral axons and terminals of the corneal and tooth pulp sensory fibers. In addition, reaction product was seen wedged in the extracellular spaces between the corneal nerve terminals and the surrounding epithelial cells, within the extracellular spaces of the pulp chamber, in odontoblasts, and occasionally within pulpal macrophages.

"Free" (non-conjugated) HRP injected into the trigeminal ganglion was never transported transneuronally or transcellularly. (Supported by USPHS DE06093 & EY04923.)

141.10 TRANSGANGLIONIC PROJECTION OF THE SUPERIOR LARYNGEAL NERVE TO CAT BRAINSTEM. R. Egizii* and G. E. Lucier (SPON: S. Roth). Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada, T2N 1N4.

The internal branch of the cat superior laryngeal nerve (SLN) conveys sensory information from receptors of the laryngeal mucosa and supporting structures. Previous anatomical studies (Lucier and Dostrovsky, Neurosci.Abstr., 5:2401, 1979) showed that the SLN afferent cell bodies are mainly concentrated in the rostral end of the nodose ganglion. The purpose of this study was to determine the projection of these cells into the brainstem.

Five adult cats were anaesthetized with ketamine hydrochloride, 40 mg/kg I.M. The left SLN was exposed and the internal sensory branch isolated from the external motor branch. The peripheral cut end was placed in a small cup containing a concentrated solution of horseradish peroxidase (HRP) (Boehringer Mannheim Grade I) in saline and encased in low-melting point wax. Following a survival period of 48-72 hours, the animals were re-anaesthetized and perfused with Karnovsky's Aldehyde fixative. Coronal 40u frozen sections were cut from a point 5 mm rostral to 4 mm caudal to obex and reacted according to the tetramethylbenzidine method of Mesulam (Histochem. Cytochem., 26:106-117, 1978). Serial sections were examined using dark field microscopy for presence of HRP-reaction product. These particles were first seen at the entrance of the vagus nerve and were traced caudally, passing through the spinal trigeminal nucleus and entering the lateral subnucleus of the solitary tract (STN). At 1.9 mm rostral to obex, the HRP-containing fibers entered the medial, ventral, intermediate and interstitial subnuclei of STN. At 1.3 mm rostral to obex, the fibres are seen in the lateral subnuclei. At 1.0 mm rostral to obex, most of the reaction product in the medial subnuclei has disappeared. Fibres continued to leave the tract and join the commissural nucleus to a point approximately 2.5 mm caudal to obex. We did not observe HRP-containing fibres branching into any other nuclei at any of these levels. (Supported by the Canadian MRC).

141.11 TRIGEMINOTECTAL PROJECTIONS: COMPARISONS AMONG SEVERAL MAMMALS. J.G. McHaffie*, L.L. Bruce, and B.E. Stein (Spon: A.J. Szumski). Dept. Physiology & Biophysics, Medical College of Virginia, Richmond, VA 23298.

The superior colliculus (SC) contains a topographical somatosensory representation which is in register with its visuotopy. Although this organizational scheme is present in a variety of animals, the relative amount of neural tissue devoted to the face, or portions of the face, varies among species. The distribution of the trigeminotectal cells within the trigeminal complex also varies. These experiments were directed specifically at detailing intra-species trigeminotectal distinctions which may underlie functional differences among these animals.

HRP techniques were used to reveal the distribution of trigeminal cells innervating the SC. Injections of 25% HRP were made in Nembutal anesthetized cats, rats, and hamsters. The tissue was reacted using TMB as the chromagen.

Each species studied had a distinct pattern of trigeminotectal cells. In cats, the densest concentration of labeled cells was in the rostral pole of pars oralis with substantially fewer cells in principalis and fewer still in pars interpolaris and pars caudalis. In marked contrast, most trigeminotectal cells in rats were in caudal pars interpolaris, somewhat fewer in pars oralis and least in principalis and pars caudalis. In contrast to a previous report (Van Buskirk, R.L., Brain Res. Bull., 10: 583, 1983), hamsters appeared fundamentally the same as rats with most cells in pars interpolaris and progressively fewer in pars oralis, principalis, and pars caudalis. The proportion of ipsilateral cells also varied between rodents and cats with rodents having significantly more ipsilateral trigeminotectal cells than cats.

Regardless of the species, there is a tendency for labeled cells to be concentrated more ventrally within their respective nuclei: this was particularly marked in rodents. Moreover, trigeminotectal cells are generally amongst the largest cells within a given region of the trigeminal complex, usually being multipolar, fusiform, or triangular in shape.

These data support earlier suggestions that trigeminal cells projecting to the SC are a distinct population by virtue of both their morphology and their relative position within the nucleus. The absolute distribution of trigeminotectal cells appears to be species specific and may reflect specialized ecological adaptations.

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141.12 ORGANIZATION OF THE HAMSTER TRIGEMINAL COMPLEX: PROJECTIONS TO SUPERIOR COLLICULUS, THALAMUS, AND SPINAL CORD. L.L. Bruce, J.G. McHaffie*, and B.E. Stein. Dept. of Physiology/Biophysics, Medical College of Virginia, Richmond, VA 23298.

Fluorescent dyes (fast blue and diamidino-dihydrochloride yellow) were used to: a) compare distributions of trigeminotectal, trigeminothalamic, and trigeminospinal cells; and b) determine if some of the same trigeminal cells project to the superior colliculus (SC) and to other somatosensory structures. In each Nembutal anesthetized hamster, one dye was injected into the SC and the other into either rostral thalamus, caudal thalamus, or spinal cord.

Trigeminotectal cells comprised a distinct group of cells within the trigeminal complex characterized by both their morphology and position. The largest populations were located ventromedially in the caudal pole of pars interpolaris although many were also located ventromedially in pars oralis. In contrast, trigeminothalamic cells were densely distributed throughout principalis and, to a lesser extent, throughout the dorsolateral caudal aspect of pars interpolaris. However, only a few labeled cells were found in rostral pars interpolaris, pars oralis, and pars caudalis. Trigeminospinal cells formed a third distinct distribution. Most of these cells were located in pars caudalis, whereas rostral pars interpolaris contained a slightly smaller population of labeled neurons. Principalis and pars oralis contained the least labeled neurons.

No double-labeled trigeminal cells were seen after SC and rostral thalamus injections. Similarly, after SC and caudal thalamus injections, only a few double-labeled trigeminal cells were found in pars oralis and pars interpolaris, and still fewer in pars caudalis. All double-labeled cells were observed in regions where both trigeminotectal and trigeminothalamic cells were interspersed. Following SC and upper cervical spinal cord injections, the results were similar: a few isolated double-labeled cells were found only in caudal interpolaris and rostral caudalis.

Thus, there appear to be at least three separate populations of neurons within the trigeminal complex that can be characterized according to their projections to SC, thalamus, or spinal cord. This anatomical segregation suggests that there may also be three independent lines of somatosensory information reaching these structures.

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141.13 ANATOMICAL LOCALIZATION OF SOMATOSENSORY RESPONSES IN THE VENTROBASAL COMPLEX OF THE RAT. E.C.Cropper and J.S.Eisenman. Dept. Physiology,Mt.Sinai Sch.Med., New York, NY 10029.

Anatomical studies have divided the ventrobasal (VB) complex into ventral posterolateral (VPL) and ventral posteromedial (VPM) nuclei,and distinguished it from the posterior (PO) complex.The purpose of this study was to compare unit response characteristics from these different divisions in the rat.

120 extracellular single unit recordings were made in 16 male albino rats anesthetized with 1.2-1.3 gr/kg urethane, using glass pipettes filled with 2% Niagara Sky Blue dye in 0.5 M sodium acetate.Dye spots were made iontophoretically and electrode placements verified.Histologically,VPL was distinguished by the laminar arrangement of its cells and its reticulated appearance;VPM by its large,round,densely packed somata.The PO complex was considered to include the area dorsomedial to VPM and ventral to the lateral nuclei, and the region posterior to the VB complex and medial to the medial geniculate.

Most recorded units were biphasic (pos-neg,av amp 370 uV, av dur 1.5 msec).In VPM and VPL,49/97 units responded to LT in small (e.g.,1x 3 cm) contralateral receptive fields;47 with an increase in activity,2 with a decrease.In VPM most (22/22) LT units had facial receptive fields while in VPL most (21/27) responded to bodily stimulation.In VPM and lateral VPL,LT units were somatotopically organized as has been reported.In medial VPL,most units had forepaw receptive fields giving the forepaw the largest (14/27) VPL representation.38/97 VPL and VPM units responded to nociceptive stimulation in large bilateral receptive fields (e.g.,whole body);37 with an increase in activity,1 with a decrease.Nociceptive and LT responses were interspersed except in anterior VPL and medial VPM where responses to nociceptive stimulation were most common.6/97 units responded to both LT in a small receptive field and nociceptive stimulation in a large area (e.g.,whole body).In the PO complex,16/23 units were excited by nociceptive stimulation;2/23 were inhibited. None were effected by LT.

The results of this study indicate that unit response characteristics can be correlated with anatomical divisions of the rat somatosensory thalamus.Units responding to LT of facial areas lay almost exclusively within VPM,units responding to LT of the body in VPL.In the VB complex,responses to both nociceptive and LT stimulation were recorded,but LT units were more numerous than nociceptive.In the PO complex no LT responses were encountered.

141.14 LIGHT AND ELECTRON MICROSCOPIC ANALYSIS OF PHYSIOLOGICALLY CHARACTERIZED, INTRACELLULARLY-LABELED NEURONS IN THE SOMATOSENSORY THALAMUS OF THE CAT. H.J. Ralston III and P.T. Ohara. Department of Anatomy, University of California, San Francisco, CA 94143

The morphology of physiologically characterized neurons in cat ventrobasal thalamus (VB), posterior group (PO) and the border between ventrolateral nucleus (VL) and VB has been examined to determine whether cells with particular physiological properties have unique morphological attributes. Animals were anesthetized with intravenous pentobarbital (20-30mg/Kg) I.V. until they no longer withdrew from a pinch to the paw. No paralytic agents were used, thus permitting continuous assessment of anesthetic level. Blood pressure and expired CO_2 were monitored and the body temperature maintained at 37-38°C. The head was held in a stereotaxic frame. A hole was drilled in the skull and bone removed to expose the cortex overlying the somatosensory thalamus (AP + 3.0 to 11.0), as well as the pericruciate cortex, for the stimulation of the somatosensory cortex. Metal microelectrodes were used to search for cells which responded to nonnoxious and/or noxious cutaneous stimuli. Once a region of thalamus containing somatosensory neurons was localized, glass micropipettes containing 4% HRP in 0.1M Tris in 0.5M-KCl at pH 8.0 were used to record intracellularly from neurons. Following physiological characterization of the neurons, HRP was iontophoresed into the cells using 50 Hz pulsed current at 1nA for 5-10 mins. In this manner we have recorded from over 24 units which have responded to noxious, or nonnoxious, or both noxious and nonnoxious stimuli. Seven neurons have been successfully labeled with HRP.

The labeled neurons have all exhibited "tufted" dendritic trees, with varying numbers of dendritic spines. The axons of the thalamic cells gave rise to collaterals which branched in the nucleus reticularis, but never within the thalamus. Electron microscopic analysis of a neuron which responded to hair movements on the forearm revealed non-spiny dendrites and a myelinated axon. The cell body was not contacted by synapses; primary and secondary dendrites were contacted by large principal afferent profiles with round vesicles (RL), flat vesicle profiles (F) and presumed presynaptic dendrites (PSD). Tertiary dendrites were contacted almost exclusively by small profiles with round vesicles (RS). The sampled synaptic population contacting the labeled neuron was: RL-13%; F-22%; PSD-8%; and RS-55%. Descriptions of the synaptic relationships of other types of neurons will be presented. (Supported by NS-11614).

141.15 ANATOMICAL STRUCTURE OF PHYSIOLOGICALLY IDENTIFIED NEURONS OF THE RAT VENTROBASAL THALAMUS. R. M. Harris. Department of Biological Structure, University of Washington, Seattle, WA 98195.

Distinct classes of thalamocortical relay cells have been distinguished in thalamic nuclei of several animals. To determine if similar classes exist in the ventrobasal thalamus of the rat, neurons were first identified by intracellular recordings. Receptive fields and somatosensory modalities were determined by stimulation of the body surface. Horseradish peroxidase (HRP, 4% in 0.5 M KCl) was injected into the neuron by iontophoresis. Sections 100 μm thick were then reacted with diaminobenzidine intensified by cobalt. To date, 8 physiologically identified cells plus 9 cells of uncertain physiology have been recovered. These cells have been drawn and measured on a computer-assisted microscope system.

Morphologically, all eight identified cells look similar. They have cell bodies averaging 16 μm in diameter with short, thick proximal dendrites which often develop "whorls" of many secondary dendrites branching from a single point. There are numerous spines, both short and stubby and long and thin, over the dendrites. Little dendritic beading is seen. The maximum dendritic length is about 250 μm. For these cells, the cross sectional area of the soma appears to be related to the receptive field size: a cell responding to a single whisker had an area of 100 μm^2, while another responding to a large part of the belly had an area of 350 μm^2.

The axons of these cells could be followed through serial sections into the internal capsule. On 5 out of 7 axons, collaterals were given off to the nucleus reticularis thalami, about 500 μm rostral to the cell body. In some cases these collaterals had extensive branching oriented in a medial direction.

No convincing evidence for distinguishable classes of neurons in this nucleus has been yet found. Since there are few, if any, local circuit neurons in rat ventrobasal thalamus, this may be an example of a pure "relay" nucleus. (Suypported by USPHS Grant NS-19073 from the NIH.)

142.1 THE MAKE-UP OF SPINAL CORD CIRCUITS WHICH PROCESS INPUT FROM FEMORAL VENOUS AFFERENTS. B. J. Yates, F. J. Thompson and J. P. Mickle*. Depts. of Neuroscience and Neurosurgery and the Center for Neurobiological Sciences, Univ. of Florida College of Medicine, Gainesville, FL 32610.

Both anatomical (Woollard, 1926; Hinsey, 1928; Truex, 1936; Millen, 1948) and electrophysiological (Thompson and Barnes, 1979; Thompson et al., 1982; 1983) studies have indicated that peripheral veins of the cat receive afferent innervation. These afferents appear to be sensitive to vein distention (Thompson et al., 1983) and, when stimulated, elicit a powerful segmental reflex (Thompson et al., 1982). However, until recently little was known about the spinal circuitry which processes input along venous afferents. Spinal cord processing of input along afferents innervating the femoral vein was studied through the intracord mapping of field potentials. The circuitry in both the L_6 segment, the main input segment for these afferents (Thompson and Yates, 1984), and the L_7 segment, the main output segment for the reflex elicited by stimulation of these afferents (Thompson et al., 1982), was considered in these studies.

Evidence for three pools of interneurons processing input from the femoral vein was noted in L_6. One pool, active at short latency, was focused in Rexed's lamina V. A second pool of interneurons appeared to be activated by neurons in the first pool at long latency (8.9 ± 1.9 [mean ± Stand. Dev.] msec from field potential onset); the second pool was focused dorsal and lateral to the initially-active pool, in Rexed's laminae III and IV. A third pool of interneurons processing femoral venous afferent input was localized to the intermediate zone. The interneuronal pools in L_7 involved with processing input from the femoral vein appeared to be more diffusely organized than those in L_6. The pools were focused in Rexed's laminae V and VII.

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142.2 SLOWLY CONDUCTING TRACT FIBERS RECORDED FROM LATERAL FUNICULUS OF RAT SPINAL CORD. J.J. WEI* AND R.P. TUCKETT (SPON: J.W. WOODBURY). Dpt. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84108.

A high concentration of 1.0-0.1 μm unmyelinated fibers, as well as myelinated fibers as small as 0.2 μm, have been located histologically in the lateral funiculus of rat sacral spinal cord (Chung, K. and Coggeshell, R.E., J. comp. Neurol., 214: 72 and 217: 47, 1983). We wished to determine whether the microdissection method (Wei, J.Y., J. Neurosci. Meth., 4: 431, 1981) could be used to record from these slowly conducting tract fibers.

The experiments were carried out on nembutalized rats (3 WF and 2 DA). A pair of stimulating ball electrodes were placed on the dorsolateral part of the white matter at L4-L5. The searching electrical stimulation rate was 1/sec. Evoked potentials were recorded from filaments dissected from T7-T9 lateral funiculus, displayed on the storage oscilloscope and sent to the computer for averaging. With the use of a graphics terminal the final averaged record could be displayed on line, with the option of increasing the number of repititions, or storing the data for later analysis. Many of the evoked slowly conducting potentials were either not visible or barely distinguishable on the oscilloscope display; however, after averaging 100 to 500 times, they stood out clearly above the noise level. Increasing the stimulation rate to between 10 and 20/sec was shown to slow the conduction of the averaged evoked potential and decrease its amplitude. This suggests that the ability of these fine fibers to follow different stimulation rates is similar to peripheral polymodal nociceptive fibers (Tuckett, R.P., J. Invest. Dermol., 79: 368, 1982). So far we have isolated 25 filaments containing 94 slowly conducting elements, with conduction velocities ranging from 0.34 m/s to 9.0 m/s of which 57 elements were slower than 1 m/sec. It is interesting to note that both species of rat exhibited slowly conducting tract fibers in T7 to L5 lateral funiculus, and we have preliminary data showing similar results from different segments of cat spinal cord.

In conclusion, by combining signal averaging with the microdissection method, it is possible to record from slowly conducting tract fibers in the spinal cord.

142.3 POSTSYNAPTIC POTENTIALS OF LAMINA 3,4 NEURONS OF THE CAT SPINAL CORD EVOKED BY SINGLE ACTION POTENTIALS IN SLOWLY ADAPTING TYPE 1 (SA1) AFFERENT FIBERS. D.N. Tapper, P.B. Brown and L.A. Ritz. Dept. Physiol., Cornell U., Ithaca, NY 14853 and Dept. Physiol., West Virginia U., Morgantown, WV 26506.

In decerebrate or α-chloralose anesthetized cats postsynaptic potentials (PSPs) were evoked in dorsal spinal neurons of the sacral-1 segment by eliciting single or pairs of action potentials in individual SA1 fibers. The PSPs varied in size and duration and were often much larger and longer than those described for motoneurons. The longer durations (up to 60 ms) between dorsal horn and motoneuron PSPs may reflect the widespread dendritic connections made by a single SA1 fiber as has been observed for hair follicle afferents. As found for both spinocerebellar and spinocervical tract cells, the peak amplitudes ranged up to 19 mv. In instances where several SA1 fibers were monosynaptically connected to the cells, the characteristics of the PSP varied markedly with input fiber. These differences presumably reflect the number and spatial distribution of synapses between the afferent fiber and central cell. The time course of the PSPs is consistent with that observed for unit responses (recorded extracellularly in previous studies), however, the early responses corresponded in time with the rising phases of the potential; the late responses, when present, occurred at the times of additional components of the prolonged PSPs. The responses to pulse pairs were also determined for some cells. The conditioning (first) stimulus produced an initial facilitation followed by a prolonged inhibition as predicted by extracellular unit response studies. The peak depolarization was facilitated at 10 ms, was inhibited markedly at 30 ms and remained slightly inhibited at 100 ms. In most instances there was little or no evidence of postsynaptic inhibition, however. The reduction in PSP size following the initial facilitation is most likely due to presynaptic inhibition, a result consistent with previous studies using direct stimulation of central terminals. The statistical properties of spontaneous PSPs were also evaluated. (Supported by USPHS Grant # 07505.)

142.4 INTRA-AXONAL RECORDING FROM PRIMARY AFFERENT FIBERS IN RAT SPINAL CORD SLICE PREPARATION. S. Jeftinija and M. Randic (SPON: W. W. Kaelber). Dept. of Vet. Physiology and Pharmacology, Iowa State University, Ames, IA 50011.

Using electrical excitability testing of single sural primary afferent A- and C-fibers, we have reported that gamma-aminobutyric acid (GABA), 5-hydroxytryptamine (5-HT), norepinephrine (NE) and substance P modulate axonal excitability (Randic, in Spinal Cord Sensation, 1981; Jeftinija et al., Brain Res., 219:456, 1981; Carstens et al., Brain Res., 221:151, 1981). In order to examine further membrane actions of these putative neurotransmitters, a method was developed to study intracellular potentials from axons in dorsal roots of rat spinal cord slice preparation.

Rats 14 to 18 days old were used. After lumbosacral laminectomy, 300 μm thick horizontal dorsal horn slice with attached dorsal rootlets was maintained in an oxygenated Ringer solution according to the described technique (Murase et al., Brain Res., 234:170, 1982). The lumbar dorsal roots were mounted on wire stimulating electrodes, such that the root of origin of each fiber could be determined. Recordings were made from fibers at the dorsal root entry zone and in the dorsal horn using microelectrodes filled with 2M KCL (resistance 70-110 MΩ). Primary afferent fibers were identified by their short latency, short duration spikes which followed stimulation frequencies in excess of 300 Hz. Since the conduction distance was short, latencies were usually less than the time that would be required for a synaptic delay.

Stable intracellular recordings for up to 2 hrs have been made from primary afferent fibers. The mean membrane potential was -63.6 ± 5.2 mV; the mean action potential amplitude was 73.8 ± 10.3 mV (S.D.). Bath application of GABA (10^{-3} to 10^{-4} M), NE bitartrate (10^{-3} M), and 5-HT hydrochloride (10^{-4} to 10^{-3} M) caused a reversible, dose-dependent depolarization. While the depolarization produced by GABA and NE was associated with a fall in axonal resistance, in the case of 5-HT, the similar effect was less consistently observed. These effects might explain some of the electrical excitability changes observed at the central terminals of single sural primary afferent fibers during local application of GABA, NE and 5-HT near these terminals.

These experiments indicate that the in vitro rat spinal cord slice preparation can be successfully utilized for further physiological and pharmacological studies of the modulation of axonal excitability by potential neurotransmitters. Supported by NIH grant (NS 17297).

142.5 RESPONSE PATTERNS TO SURAL NERVE STIMULATION IN DORSAL HORN NEURONS WITH AND WITHOUT A RECEPTIVE FIELD IN THE SURAL NERVE REGION. L.M. Pubols, M. Foglesong and C. Vahle-Hinz, Neurological Sciences Inst., Good Samaritan Hosp. and Med. Ctr., Portland, OR 97209.

As reported earlier (L. Pubols, Neurosci. Abstr. 9:260, 1983) a population of neurons that responded to electrical stimulation of the sural nerve (SN), but not to mechanical stimulation of the skin innervated by that nerve (SN region), was identified in the lumbar dorsal horn of cats, indicating the presence of latent projections from the nerve to these neurons (Wall, Phil. Trans. Roy. Soc. B 278:361, 1977). The patterns of responses to SN stimulation have been further analyzed (1) to determine what mechanisms might underlie them, and (2) to compare the responses of neurons that had a response to natural stimulation in the SN region with those that did not. In a sample of 157 L6 and L7 dorsal horn neurons in anesthetized cats, 58 had excitatory responses to stimulation of Aαβ SN fibers. 21 cells had both an early and a late response, and 37, only a late response. The early responses consisted of one or two spikes occurring with a fixed latency on every trial. Late responses consisted of an irregular burst of impulses with variable latencies and durations of up to 40 msec. All cells that had an early response had a receptive field (RF) in the SN region. About half (18/37) of the cells with only a late response had RF's in the SN region and half had RF's outside it. Aside from this, there were no obvious differences between these latter two subgroups in their responses to natural or electrical stimuli.

Previous investigators found that similar early and/or late responses could be elicited by single impulses in A fibers, evoked by either natural or electrical stimuli (Brown et al. J. Neurophysiol. 36:827, 1973; Tapper et al. J. Neurophysiol. 44:1190, 1980), and that electrical stimulation of A fibers in the sural nerve produced EPSP's in dorsal horn neurons for up to 40 msec (Price et al. Exp. Neurol. 33:291, 1971). Thus, it appears that both the early and late responses recorded in the present study are due to excitatory effects of A fiber stimulation, and that the former is mediated by monosynaptic, and the latter by polysynaptic pathways. The similarity in the late responses between cells with a receptive field in the SN region and those without suggests that under altered circumstances, for example, after lesions, the latter could exhibit responses to natural stimulation of the SN region. (Support: NIH NS-19523)

142.6 DESCENDING MODULATION OF SPINAL DORSAL HORN LOW THRESHOLD NEURONS. J.G. Collins. Dept. of Anesthesiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

The use of a newly developed recording technique has made it possible to study extracellular single neuron activity in the dorsal horn of the spinal cord of physiologically intact, awake, drug free cats. This study was carried out to establish a data base which adequately describes the normal physiologic response profile of low threshold (LT) neurons in the dorsal horn of the spinal cord in physiologically intact, awake, drug free animals.

Methodology. Using a surgically implanted chronic recording chamber which allows for daily placement of tungsten microelectrodes (Frederick Haer & Co.) through the intact dura into the dorsal horn of the spinal cord within a 12 mm long x 6 mm wide window, activity was recorded from LT neurons in physiologically intact, awake, drug free cats. Animals must be comfortable and pain free during all aspects of the experiment. Natural stimulation was used to identify receptive fields and typical neuron responses. Spontaneous activity was recorded with the receptive fields isolated from stimulation.

Results. Many characteristics of LT neurons in the intact preparation are similar to those reported in anesthetized animals. Typically, receptive fields on hairy skin of the hindlimb were contiguous, oval areas with a central area of greatest sensitivity. Many neurons responded with both slowly and rapidly adapting characteristics. In spite of those similarities, however, there was one striking difference. LT neurons in the intact animal have little or no spontaneous activity. Most cells had spontaneous rates of less than 1 per second. Although spontaneous activity is lacking, they responded briskly to receptive field stimulation.

Discussion. LT dorsal horn neurons in decerebrate, spinal cord transected animals have spontaneous rates of 10/20 impulses per second. The results of the present study indicate that in the intact animal, there are descending supraspinal influences that are capable of modulating LT sensory input at the level of the spinal cord. Such modulation could be responsible for, among other things, filtering LT sensory input, maintaining a balance between low and high threshold sensory input in the spinal cord or influencing spinal reflexes.

Supported by NIH Grant GM 29065

142.7 HABITUATION AND DEHABITUATION OF THE SPINAL POLYSYNAPTIC REFLEX RESPONSES: MODIFICATIONS PRODUCED BY NALOXONE AND OPIATES. F. Pellicer Graham*, J.M. Calvo y Otálora*, and A. Fernández-Guardiola. Depto. de Investigaciones Biomédicas Instituto Mexicano de Psiquiatría. Calz. México-Xochimilco No. 101. Tlalpan, C.P. 14370, México, D.F. and Facultad de Psicología, UNAM.

The sustained inhibitory action of spinal endorphines could be responsible for the habituation of polysynaptic responses in the spinal cord. To test this hypothesis acute spinalized cats without anaesthesia (decerebrated and curarized) were used. The sural nerve was stimulated every 5 sec. with suprathreshold single electric shocks of 0.3 msec. duration. We analyzed: a) the afferent volley at the dorsum of the spinal cord; b) the field potential of the grey matter (recorded at ± 1500 µm, Rexed lamina V); and c) polysynaptic reflexes recorded at the ventral root (S_1). The arterial pressure was monitored through the experiments. The potentials were averaged (8) and integrated every 5 min. A progressive decrease in the reflex response of the sural nerve was found (habituation). Sometimes dehabituation appeared spontaneously but it was always provoked by the interruption of the stimuli. The ventral root reflex responses returned to amplitude control or higher values during dehabituation. The field potential (lamina V), on the contrary, progressively increased during the iterative periodical stimulation, reaching its maximum amplitude when the reflex showed its highest habituation. Without interrupting the stimuli a marked dehabituation was produced by the I.V. administration of naloxone (0.8-1.0 mg/kg) towards the end of the habituation process. On the other hand, the previous administration of a similar dose of naloxone prevented habituation to occur.

Habituation appeared faster when pentazocine I.V. or spinal cord microinjected fentanyl were administered.

142.8 SYNAPTIC CONNECTIONS BETWEEN PRIMARY MUSCLE AFFERENTS AND DORSAL SPINOCEREBELLAR TRACT NEURONES IN THE CAT SPINAL CORD. B. Walmsley, E. Wieniawa-Narkiewicz*, M.J. Nicol* and D.J. Tracey*. Experimental Neurology Unit, John Curtin School of Medical Research, Australian National University, Canberra, Australia.

The excitatory post-synaptic potential (EPSP) evoked in cat spinal motoneurones by impulses in a single muscle spindle (Ia) primary afferent, fluctuates in peak amplitude from trial to trial. These fluctuations occur between discrete levels separated by an incremental voltage of approximately 100 µV. This 100 µV incremental EPSP has been interpreted as arising from all-or-none transmission at a single synaptic terminal (Redman and Walmsley, J. Physiol., 343: 117-145, 1983).

Primary afferents from muscle spindles (and tendon organs) also make monosynaptic connections with neurones situated in Clarke's column which give rise to the dorsal spinocerebellar tract (DSCT). Light microscopic observations have revealed that many of the synaptic boutons arising from these afferent fibres are extremely large (up to 20 microns long). We have examined, using electron microscopy of serial sections, the fine structure of identified primary afferent boutons in Clarke's column. Group I primary muscle afferents were penetrated in the dorsal columns near the L3/L4 boundary using an HRP filled microelectrode. In addition, DSCT neurones in the same region were labelled intracellularly by ionophoresis of HRP. Labelled boutons contacting labelled neurones were examined under the electron microscope. Multiple pre-and post-synaptic densities with clusters of pre-synaptic vesicles were observed in single labelled boutons, indicating that these boutons probably contain multiple transmitter release sites.

Analysis of group I single fibre EPSPs in DSCT neurones indicates that fluctuations also occur between discrete amplitudes but with an increment of approximately 180 µV. This increment was independent of EPSP peak amplitude which ranged from 250 µV to 6 mV. We propose that synaptic transmission between group I muscle afferents and DSCT neurones occurs with discrete all-or-nothing EPSPs associated with transmitter release sites, rather than boutons per se, since some boutons may contain multiple transmitter release sites.

142.9 STUDIES OF THE AREA AROUND THE CENTRAL CANAL: EFFERENTS AND AFFERENTS. R.L. Nahin, A.M. Madsen, P.E. Micevych, F. Haist, G.W. Terman, and G.J. Giesler, Jr. Neuroscience Program, Dept. of Anatomy and Dept. of Psychology, University of California, Los Angeles, CA 90024 and Dept. of Anatomy, University of Minnesota, Minneapolis, Minn. 55455

Evidence suggests the gray matter surrounding the central canal plays a role in nociception. The purpose of the present paper was to identify those ascending tracts in the rat spinal cord originating from around the central canal and to specify their termination sites in the reticular formation. In addition, double-labeling studies using immunohistochemical techniques sought to define the anatomical and neurochemical nature of synaptic terminations onto projection cells around the central canal.

Large pressure injections of HRP were made to fill the caudal medulla unilaterally, a procedure previously shown to produce heavy retrograde labeling of cells located around the central canal. Thirty minutes prior to the injection, lesions were made of spinal cord white matter. We have found that lesions of the dorsal columns or the dorsal portion of the lateral funiculus failed to reduce labeling of the neurons under study. Lesions destroying the ventral portion of the lateral funiculus (VLF) reduced labeling by as much as 80%. Ventral funiculus destruction in conjunction with VLF lesions completely blocked labeling.

To determine the termination sites of these projections, small HRP injections were made into various reticular nuclei, with the spinal cord subsequently reacted for labeling. Moderate labeling of cells around the central canal was seen after injections into N. Ret. Paragigantocellularis Lateralis, the Lateral Reticular Nuclei or the N. Ret. Gigantocellularis and the subjacent pars Alpha region.

In seperate experiments, serotonin (5-HT), Substance P (SP) and met-enkephalin (M-ENK) immunohistochemistry was combined with the retrograde True Blue method in attempts to elucidate the anatomical relationship between projecting cells around the central canal and the abundant array of neurochemical-containing fibers found in the same area. Preliminary light microscopic studies revealed that fibers and boutons containing SP and M-ENK are in close association with some cells projecting at least to the caudal medulla. (Supported by NIH grants NS07628 and NS17540, and a gift from the Brotman Foundation)

142.10 ULTRASTRUCTURE OF THE SUBSTANCE P (SP) INNERVATION OF LAMINA X IN MONKEY SPINAL CORD. B.E. Rodin and C.C. LaMotte. Sections of Neuroanatomy and Neurological Surgery, Yale Univ. Sch. of Med., New Haven, CT 06510

The distribution and ultrastructural localization of SP was examined in lamina X of monkey cervical cord with the PAP method. SPLI was located throughout lamina X; its density was greatest dorsolateral to the central canal, and was relatively sparse in ventrolateral, dorsal and ventral regions. Labeled varicose axons crossed the gray matter in the dorsal and ventral commissures.

The majority of SPLI terminals contained small round clear vesicles. Others had larger round or pleomorphic vesicles, and a few labeled terminals in dorsal regions contained granular vesicles. Most often, labeled terminals formed asymmetrical or symmetrical junctions with varying sizes of unlabeled dendrites and dendritic spines. In lateral regions, symmetrical axosomatic synapses were also common. Large dendrites or somas that were postsynaptic to a SPLI terminal usually were also postsynaptic to a number of unlabeled terminals containing round, flattened or pleomorphic clear vesicles. Occasionally, unlabeled terminals formed junctions with labeled terminals, and the SPLI terminal appeared to be postsynaptic. Glomerular complexes, consisting of a central terminal and a number of postsynaptic dendrites, were found in dorsal lamina X, but the terminals were not immunoreactive; these glomeruli resembled those described in studies of the superficial dorsal horn.

The SP innervation of lamina X contrasts with that of the superficial dorsal horn where many SPLI terminals are of primary afferent origin. In the dorsal horn, SPLI terminals often contain granular vesicles, form either the central terminals of glomeruli or simple axodendritic synapses, and their junctions are asymmetrical. In lamina X the variety of SPLI terminal types probably indicates that its SP innervation arises from a number of sources, including primary afferent, intrinsic spinal and supraspinal neurons. Direct impingement of SPLI terminals onto somata and large dendrites in lamina X, as opposed to the more complex glomerular circuitry in the dorsal horn, suggests a simple and powerful control of certain lamina X cells. Our preliminary experiments reveal that some of these neurons are spinothalamic cells possibly involved in nociception. Axo-axonic contacts onto SP terminals in lamina X may thus provide a morphological substrate by which other neurochemical systems (e.g. ENK, 5HT) modulate the effects of SP on intrinsic or projection neurons. (Supported by NS13335)

142.11 A STUDY OF PEPTIDERGIC* AND SEROTONINERGIC* IMMUNOREACTIVE ELEMENTS IN THE NEONATAL RAT SPINAL CORD. M.C. Langan and R.H. Ho. Department of Anatomy and Neuroscience Research Laboratory, The Ohio State University, Columbus, Ohio 43210.

This study describes the distribution of cholecystokinin (CCK), vasoactive intestinal peptide (VIP), α-melanocyte stimulating hormone (MSH), bombesin (BOM), molluscan cardioexcitatory peptide (FMRF-amide), neurotensin (NT), somatostatin 28(1-14) ($SS28_{1-14}$), methionine-enkephalin (ENK), serotonin (5HT), and substance P (SP) elements in the spinal cord of the day 2 neonatal Sprague Dawley rat. Animals were perfusion fixed with Zamboni's solution and transverse cryostat sections from representative spinal cord levels were processed by Sternberger's indirect antibody peroxidase-antiperoxidase technique. The immunostaining appeared as fibers and varicosities cut in various planes of section. The densest distribution for CCK, VIP, BOM, NT, SS28(1-14) and SP immunoreactive fibers is in the superficial laminae of the dorsal horn. The ventral gray matter only exhibited negative to moderate immunostaining. MSH, FMRF-amide, ENK, and 5HT exhibit their respective characteristic pattern of distribution ranging from sparse to moderate in the gray matter. MSH, BOM, NT, ENK, 5HT and SP immunoreactive fibers are also present in the white matter. Preliminary studies designed to examine the acute effects of capsaicin on the above chemically identified elements were performed. Day 2 neonates were given capsaicin (50mg/kg of body weight) subcutaneously and were perfused 3 hours post injection. The spinal cords of these animals demonstrated a decreased density of immunostaining for CCK, VIP and SP in the superficial laminae of the dorsal horn. In conclusion, the chemically identified neuronal elements studied are present in the neonatal spinal cord. While their function is not certain, studies with capsaicin indicate that like SP primary afferents, other peptide containing elements are also susceptible to its neurotoxic effects.
(Supported by NIH NS-17080 and NIH NS-10165) (We thank Dr. R. Elde for the FMRF-amide, ENK, 5HT, AND SS28(1-14) antibodies).

*A substances's immunoreactivity is referred to by its name.

142.12 SEROTONIN-LIKE IMMUNOREACTIVE FIBERS IN FROG SPINAL CORD. G.L. Yuen, D.S. Adli, B.M. Rosenthal, R.H. Ho and W.L.R. Cruce. Neurobiology Program, N.E. Ohio Universities College of Medicine, Rootstown, OH 44272 and Department of Anatomy, Ohio State University, Columbus, Ohio 43210.

Physiological and pharmacological studies of neurotransmitter function have frequently used the spinal cord of frogs, yet little is known of the anatomical distribution of neurotransmitters in this tissue. Serotonin (5-HT) is of special interest to us because of its possible role in nociceptive function in mammalian spinal cord. As a part of our investigation into the anatomical organization of frog spinal cord, we have studied the distribution of 5-HT-like immunoreactivity.

Adult leopard frogs (Rana pipiens) weighing 45-65gm were anesthetized with MS-222 and fixed by transcardial perfusion with Zamboni's solution. Transverse sections of the cord were cut 50-60um thick on a freezing microtome and processed for the localization of 5-HT immunoreactivity by the indirect antibody PAP method of Sternberger.

Immunostaining appeared as a sparse to moderate number of fibers and varicosities throughout the gray and white matter. The least number of 5-HT fibers was found in the dorsal funiculus and entering dorsal roots. The greatest number of 5-HT fibers was found in the lateral funiculus of the white matter, including the sub-pial zone and Lissauer's tract, and in the dorsal gray field of Ebbesson. An intermediate number of 5-HT fibers was found in the lateral and ventral gray fields of Ebbesson and in the ventral funiculus of the white matter. Supported by the United Way of Stark County and NIH grant NS17080. We thank Dr. R. Elde for the 5-HT antibody.

142.13 ENKEPHALIN-LIKE IMMUNOREACTIVE ELEMENTS IN LISSAUER'S TRACT AND OTHER REGIONS OF FROG SPINAL CORD. D.S. Adli, G.L. Yuen, B.M. Rosenthal, R.H. Ho, and W.L.R. Cruce. Neurobiology Program N.E. Ohio Universities College of Medicine, Rootstown, OH 44272 and Department of Anatomy, Ohio State University, Columbus, OH 43210.

The spinal cord of frogs has been used frequently in studies of neurotransmitter function. Enkephalin (ENK) is of special interest to us because of its possible role in nociceptive processing and because it is found in relatively high concentrations in fibers of Lissauer's tract in mammals. As part of our investigation into the organization of Lissauer's tract in frogs we have studied the distribution of ENK-like immunoreactivity in the spinal cord. Adult leopard frogs (Rana pipiens) weighing 45-65gm were perfusion-fixed with Zamboni's solution. Transverse sections of the cord were cut 50-60um thick on a freezing microtome and processed for the localization of ENK immunoreactivity by the indirect antibody PAP method of Sternberger. Immunostaining appeared as fibers and varicosities throughout the gray and white matter. The least number of ENK fibers was found in the dorsal funiculus and entering dorsal roots. ENK fibers were especially numerous in Lissauer's tract, which is most prominent as a narrow band located just ventral to the entering dorsal root fibers and stretching from the lateral edge of the cord to the lateral edge of the dorsal horn. The dorsal part of the lateral funiculus, located ventral to Lissauer's tract contained ENK fibers in greater density than the rest of the lateral and ventral funiculi. ENK reacting fibers were also present in substantial numbers in the sub-pial zone or lateral neuropil of Ebbesson, especially its dorsal half. A few scattered ENK reacting cells were found in the gray matter. ENK fibers were sparse in the dorsal and ventral gray fields of Ebbesson. However a moderate number of ENK fibers was found in a narrow band of the gray matter located on the border between Ebbesson's dorsal and lateral fields. Laterally this band merged with Lissauer's tract and the dorsal part of the lateral funiculus; medially it was continuous with a concentration of ENK fibers around the central canal. A small number of ENK fibers was also found in a narrow band capping the dorsal horn, especially towards the midline of the cord. Supported by the United Way of Stark County and NIH grant NS17080. We thank Dr. R. Elde for the ENK antibody.

142.14 **IMMUNOCYTOCHEMICAL LOCALIZATION OF TRH-LI IN DORSAL HORN NEURONS OF THE MOUSE SPINAL CORD.** J.A. Coffield*, E.M. Zimmermann*, M.J. Hoffert, V. Miletic, B.R. Brooks. Dept. of Structural & Functional Sciences, School of Veterinary Medicine, Madison, WI 53706, and Dept. of Neurology, Univ. of Wisconsin Medical School, Madison, WI 53792.

Thyrotropin-releasing hormone-like immunoreactivity (TRH-LI) has been reported in neuronal cell bodies, fibers, and axon terminals of the hypothalamus and brain stem of rodents using both immunofluorescence and peroxidase antiperoxidase (PAP) immunocytochemistry. Both of these techniques have been used to localize TRH-LI in the rat spinal cord in nerve fibers and varicosities of the ventral horn closely associated with motor neurons.

In the present study we localized TRH-LI in dorsal horn neurons of the mouse cervical and lumbar cord. We employed the PAP immunocytochemical technique using a new TRH antiserum (Institut Pasteur Paris, France). This antiserum appears to be highly specific for TRH with cross reactivity of less than .01% with other TRH analogs and no known cross reactivity with other CNS neurotransmitters. This antiserum has been used to localize TRH-LI in areas of the hypothalamus known to contain TRH. Six normal mice (NIH:N strain) were studied for TRH-LI in the spinal cord. Fifty micron vibratome sections of both cervical and lumbar cord were incubated in TRH antiserum at dilutions of 1:250-1:600 for 24-60 hours at 4°C. Light microscopic examination has shown a band of dorsal horn neurons demonstrating TRH-LI in all the animals. The immunoreactivity was confined primarily to the cell soma with an occasional process projecting dorsally. By direct measurement these neurons were located within the superficial laminae (100-200 μm from the dorsal white-gray matter border) with a concentration along the laminae II-III border (150-200 μm). The number of cells present per 50 μm cervical section ranged from 12-30 and cell soma size ranged from 10.6 μm to 15.9 μm (long diameter). The number of cells per lumbar section ranged from 6-26 with cell soma size ranging from 10.6 μm to 13.3 μm (long diameter). These cells were not seen in mouse spinal cord tissue immunocytochemically processed for Serotonin or Substance P. To our knowledge, this is the first description of TRH-LI in neurons found in the mouse spinal cord. The change in immunoreactivity of these cells following motor neuron damage induced by either sciatic neurectomy or murine neurotropic retrovirus infection is currently under study.
(Supported by grants from ALSSOA and MDA.)

142.15 EFFECTS OF THE AMINO ACIDS, ASPARTATE AND GLUTAMATE, ON NEURONS IN THE SPINAL DORSAL HORN STUDIED IN VITRO. S.P. Schneider and E.R. Perl. Dept. of Physiology, Univ. of North Carolina Sch. of Med., Chapel Hill, NC 27514.

Recent experiments suggest that [^{3}H] D-aspartate, a substance thought to be taken up by neurons utilizing aspartate (ASP) or glutamate (GLU) as a neurotransmitter, is retrogradely transported by small dorsal root ganglion cells and by some neurons within the superficial dorsal horn (laminae I and II). To further test the role of ASP and GLU in this region, we investigated their effects on neurons in the in vitro spinal cord.

Horizontal slices of the lumbosacral spinal dorsal horn with dorsal roots attached were obtained from hamsters 3 to 4 weeks old. Extra- and intracellularly recorded activity was identified by recording site and excitatory afferent input; in some cases, the neurons from which recordings were obtained were intracellularly stained. In contrast to earlier reports indicating that nearly all spinal neurons are sensitive to GLU, we found 32% (51/159) of the dorsal horn units to be especially responsive to ASP and GLU. The firing rate of these units increased in response to bath (< 1mM) or iontophoretic (< 10nA) application of ASP and GLU. Intracellular recordings indicated that repetitive firing was accompanied by a depolarization and an increase in membrane conductance. The excitation by ASP and GLU persisted in low [Ca^{++}] for the majority of units tested (26/44). Excitation of the remaining units was blocked or significantly reduced, suggesting the possibility of both pre- and postsynaptic mechanisms of action.

Most dorsal horn units (100/159) were not excited by either amino acid in concentrations up to 10mM or when they were iontophoresed by currents up to 30nA. On the other hand, very high concentrations of ASP and GLU (> 1mM) also depolarized these neurons and increased their membrane conductance.

Most units excited by ASP and GLU (36/51) were located within lamina I or II, and 63% (32/51) were activated by C fibers. Only 30% (32/105) of the units insensitive to ASP and GLU responded to stimulation of C fibers; they were located throughout the dorsal gray matter.

Our results are consistent with the concept that certain fine diameter afferent fibers and/or neurons in the dorsal horn may utilize ASP or GLU as a neurotransmitter.

Supported by NIH grant NS10321 and USPHS Fellowship NS07317.

142.16 RESPONSES OF $[K^+]_o$ AND SUBSTANCE P-RELATED SLOW EPSPs IN SPINAL CORD SLICES IN VITRO. L. Urbán*, P.G. Aitken and G. G. Somjen (SPON: J.G. Nicholls). Department of Anatomy, Medical University of Debrecen, Hungary and Department of Physiology, Duke Univ. Med. Center, Durham, NC 27710, USA.

Extra- and intracellular recordings were made in the dorsal horn of transversely cut spinal slices from 10-17 day old rats. Dorsal root stimulation could evoke extracellular field potentials (FP) which consisted of an early, apparently presynaptic component and a later postsynaptic component. The postsynaptic FP was negative and had maximum amplitude in laminae 2 and 3, and was positive in the intermediate zone (lamina 6). Evoked FP were not detected in the ventral horn.

Accumulation of extracellular potassium ($\Delta[K^+]_o$) in response to repetitive dorsal root stimulation was measured with double-barreled K^+-sensitive microelectrodes. The magnitude of $\Delta[K^+]_o$ was correlated with the amplitude of the extracellular FP, and was dependent on stimulus intensity, frequency, and train duration. The decay of $\Delta[K^+]_o$ was independent of stimulus parameters. Maximum $\Delta[K^+]_o$, +6.6mM from the baseline level of 3.1mM, was evoked by 50 Hz, 5 second trains of 0.5msec pulses at 2.5x the minimum intensity needed to evoke postsynaptic FP's with single shocks.

When slices were bathed in a solution containing no Ca^{2+} and elevated (12mM) Mg^{2+}, postsynaptic FP's disappeared and $\Delta[K^+]_o$ was reduced to approximately 50% of its control value. Returning to normal bathing medium reversed these effects. The regions of the dorsal horn where the FP and $\Delta[K^+]_o$ were largest coincided with the region previously found (Urbán & Randić, Brain Res.,290, 1984, 336-341) to contain the largest number of neurons generating substance P-related slow EPSP's. The timecourse of stimulation-induced $\Delta[K^+]_o$ was, however, much shorter than that of the slow EPSP or of the substance P-induced neural depolarization. Adding substance P (5μM) to the bathing medium caused only very small (0.5mM) $\Delta[K^+]_o$. We conclude that the slow EPSP of dorsal horn neurons is not caused by the liberation of K^+ ions into the interstitial fluid. (Supported by USPHS grants NS 17771 and NS 18670.)

142.17 ALPHA$_2$-ADRENOCEPTOR BINDING AND MONOAMINE CONTENT IN THE CAT LUMBAR SPINAL CORD AFTER INTRATHECAL 6-HYDROXYDOPAMINE OR CERVICAL HEMISECTION.
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Spinopetal fibers were selectively lesioned in adult cats. Two,7,or 21 days later the cats were sacrificed and the L4-L6 spinal cord was assayed for specific ^{3}H-rauwolscine(^{3}H-RAUW) binding (Bmax (fmol/mg prot) and K_D(nM) values from 4-6 pt Scatchard plots) and for norepinephrine(NE) and serotonin(5HT) content(ng/g tiss). We have previously established the alpha$_2$-nature of specific ^{3}H-RAUW binding sites in the cat lumbar spinal gray. NE and 5HT content were assayed by HPLC. Intrathecal 6-hydroxydopamine(6-OHDA:200µg) produced a marked, time-dependent reduction of lumbar cord NE content. Lumbar 5HT content was not significantly altered. Alpha$_2$-adrenoceptor density was increased significantly(50%) 7 days after 6-OHDA. Results for dorsal(below) and ventral horns were similar.

	^{3}H-RAUW BINDING		MONOAMINE CONTENT	
	K_D	Bmax	NE	5HT
CONTROLS(4)	0.66	78	219	601
2 DAYS(3)	0.73	94	81	544
7 DAYS(3)	0.88	119	57	657
21 DAYS(3)	0.63	74	14	471

Cervical(C1) hemisection reduced NE content 60 and 55% in the ipsilateral, 52 and 45% in the contralateral,dorsal and ventral cord respectively(7,21 days). ^{3}H-RAUW binding in either dorsal or ventral quadrant 2,7 or 21 days after hemisection was not different from control values for the respective quadrant. There were also no left vs right differences in any group. We conclude that alpha$_2$-adreno-ceptors on terminals of noradrenergic or other spinopetal fibers do not constitute a significant fraction of alpha$_2$-adrenoceptors in the cat lumbar spinal gray. The increase in alpha$_2$-adrenoceptor density after 6-OHDA may contribute to the greater analgesic potency of intrathecal noradrenergic agonists after 6-OHDA which we reported before.

PAIN: CENTRAL PATHWAYS I

143.1 SUBSTANCE P MODULATION OF SPINAL 5-HT$_1$ RECEPTORS AND NOCICEPTION.
R.M. Murphy* and F.P. Zemlan (SPON: A. Michaelson). Dept. Psychiatry, University of Cincinnati Sch. Med., Cincinnati, OH 45267-0559.

Serotonin (5-HT) and substance P (SP) have been shown to coexist in the same bulbospinal neurons and colocalized in the same spinal cord dense core vesicles. The present receptor binding study suggests that SP modulates the binding of ^{3}H-5-HT to spinal cord 5-HT$_1$ binding sites. The present behavioral studies suggest that the SP/5-HT$_1$ coupled binding sites are associated with the control of spinal pain reflexes implying a physiological function for the SP/5-HT$_1$ receptor complex.

Behavioral study. Two days after spinal transection, 5-HT$_1$ receptors were stimulated by ip administration of the 5-HT agonist 5-MeO-DMT (1.5 mg/kg) which resulted in the previously reported expansion of the receptive field (RF) area for all 3 spinal reflexes tested (ventro-, dorsi- and lateral flexion; average 387% increase after 5-MeODMT). After intrathecal SP administration (0, 0.25, 1.25, 7.5 ng in a 30 ug/ml bacitracin solution) a dose-response related reduction of pain reflex RF area occurred, decreasing from 814±91 mm^2 to 501±84 mm^2 after the 7.5ng SP dose ($p<0.01$).

Receptor binding study. The effect of SP added to the incubation media (including bacitacin) on ^{3}H-5-HT binding to spinal cord 5-HT$_1$ receptors was examined. Similar to the behavioral experiments, SP decreased specific ^{3}H-5-HT binding (2nm) in a dose response related manner (0nM SP=16.88±0.62 fmoles/mg, 10nM SP=14.14±0.69, 100nM SP=11.96 ±0.48, p's<0.01) while a significant 24% increase was observed in dorsal horn ^{3}H-5-HT binding. Interestingly, in the absence of bacitracin similar results were obtained in dorsal horn but a 30% increase in ventral horn ^{3}H-5-HT binding was observed. The opposite affect of SP on ^{3}H-5-HT to 5-HT$_1$ receptors in the presence or absence of bacitracin binding suggests the existence of two subtypes of SP/5-HT$_1$ coupled receptors in spinal cord which are presently being characterized. (Supported by USPHS grant NS18326).

143.2 SUBSTANTIA GELATINOSA NEURONS WITH AXONS PROJECTING TO OTHER DORSAL HORN LAMINAE.
A.R. Light and A.M. Kavookjian*. Univ. of North Carolina Sch. of Med., Chapel Hill, NC 27514.

The spinal substantia gelatinosa (SG) has been described as a closed system, i.e., axons originating from neurons in the SG terminate in this same layer. Recently, investigators have challenged this description, proposing that many SG neurons are excitatory interneurons which send their axons to the marginal zone (lamina I) to excite spinothalamic tract neurons (Price et al., J. Neurophysiol. 42: 1590, 1979).

In studies of physiologically characterized SG neurons that were intracellularly stained with horseradish peroxidase (HRP), certain neurons projected out of the SG to terminate in deeper dorsal horn laminae. In anesthetized cats, neurons were characterized by their responses to dorsal root volleys and adequate stimulation. The effects of descending volleys from n. raphe magnus and periaqueductal gray were also determined. Intracellular iontophoresis of HRP from the recording pipette marked the neurons. After aldehyde perfusion and histochemistry, vibratome sections were embedded in plastic. Neurons were drawn and photographed at the light level, then cut into ultrathin sections for electron microscopic analysis. One such SG neuron was particularly well characterized at both light and EM levels. It responded to electrical stimulation of dorsal roots at Aαβδ strength and was excited only by noxious pinch of the most lateral toe. The cell soma was located in outer lamina II of the L-7 segment, and had short, spiny dendrites oriented rostrocaudally in laminae I and II. The axon originated from a primary dendrite and was myelinated. The axon traversed ventrally through the dorsal horn and eventually turned rostrally and ran for several mm. It distributed small myelinated and unmyelinated collaterals, some of which terminated in outer lamina II and others in lamina III. In lamina III, boutons of this axon contacted both dendrites and cell somas. The boutons contained clear, round vesicles and a few large dense-core vesicles. They usually established a single asymmetric contact with dendrites or cell somas. This type of neuron demonstrates that: 1) SG neurons may have myelinated axons; 2) SG neurons have axons that may terminate in other parts of the dorsal horn; 3) SG neurons may provide excitatory input to neurons in more ventral laminae of the dorsal horn. Such neurons may account for the nociceptive input to some neurons in laminae III and IV.

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143.3 ANATOMICAL AND PHYSIOLOGICAL DEMONSTRATIONS OF CENTRAL PATHWAYS OF PULP AFFERENTS OF DECIDUOUS TEETH. E.Shohara*,Y. Lenz*,L.Tam*,J.Hu* and B.J. Sessle (SPON: H.Kwan). Fac. of Dentistry, Univ. of Toronto, Toronto, Canada M5G 1G6.

Although there has been a considerable focus on the central projections of pulp afferents from permanent teeth of adult animals, little study has been made of the central pathways of pulp afferents originating from deciduous teeth. We have used anatomical tracing and electrophysiological recording techniques to delineate the projection of these afferents to the brainstem and the properties of brainstem neurones activated by stimulation of the afferents. The study was carried out on 2-4 month old kittens which had firmly rooted deciduous canines and unerupted permanent canines. In some of the kittens, HRP was applied to the maxillary or mandibular deciduous canine pulp, and 1-2 days post-operatively, the trigeminal (V) ganglia, superior cervical ganglia and brainstem were removed for processing. In other kittens anaesthetized with chloralose, single neurones were recorded in the V brainstem complex (oralis and caudalis) and responses tested to tactile, pressure and electrical stimulation of oral-facial tissues and to electrical stimulation of the deciduous canine pulps.

HRP labelling was noted in neurones of the ipsilateral V and superior cervical ganglia and in axons at all levels of the ipsilateral V brainstem complex; these structures were not labelled contralaterally. In the brainstem, labelling of axon terminals was densest in layers I/II and V of caudalis but also occurred rostral to caudalis in the medial aspect of the mandibular and maxillary representations in the complex. Electrophysiological data were consistent with these demonstrated projections to the complex of pulp afferents: 38 of 136 oralis neurones could be excited at short latency by deciduous pulp stimulation; all were low-threshold mechanoreceptive (LTM) neurones except for 4 which could not be excited by oral-facial mechanical stimuli but which were excited by pulp stimulation. In caudalis, pulp stimulation excited 18 of 105 LTM neurones tested and 3 of 7 cutaneous nociceptive neurones; no neurones excited only by pulp stimulation were found.

These studies have established that (i) deciduous pulp afferents project to the ipsilateral V brainstem complex, (ii) all levels of the complex may receive this projection, and (iii) the anatomical and physiological features of the projections show many similarities with those previously described for pulp projections from permanent teeth.

143.4 TOOTH PULP DEAFFERENTATION: CHANGES IN RECEPTIVE FIELD PROPERTIES OF TRIGEMINAL (V) BRAINSTEM NEURONES. J.W. Hu*, Y. Lenz*, J.O. Dostrovsky and B.J. Sessle. Fac. of Dentistry, Univ. of Toronto, Toronto, Canada M5G 1G6.

Our recent studies have shown that endodontic removal of the tooth pulp can lead to changes in somatotopic organization and functional properties of neurones in the V spinal tract nucleus of adult cats. This study was undertaken to quantitate these latter changes. Single neurone activity was recorded in systematic microelectrode penetrations of the V spinal tract nucleus (subnucleus oralis) of chloralose-anaesthetized cats at a single post-operative time that varied from 3 days to 2 years after removal of the coronal pulp of the maxillary or mandibular canine, premolar and molar teeth. Electrical, tactile and noxious stimuli were applied to various orofacial sites. An electronically controlled mechanical stimulator capable of delivering precise, reproducible square or sine wave indentations of the skin was also used.

Compared with control animals, animals deafferentated for 7-15 days showed statistically significant reductions (50%) in the proportion of neurones with receptive fields localized within the V division containing the deafferented pulps. There was also a marked increase in the incidence of neurones with abnormal responses to orofacial stimuli (14%, in contrast to 1% in controls): some of these neurones had different adaptation characteristics in different zones of the receptive field (e.g. rapid adaptation in one zone, slow adaptation in another), and other neurones responded only to a brisk tap applied to the receptive field and exhibited marked habituation. 83% of these 'tap-sensitive' neurones could not follow stimuli > 1 Hz and showed no capacity to 'tune' sinusoidal mechanical indentations of the skin. These features contrasted with those of neurones which had receptive fields with rapid adaptation characteristics typical of normal animals: 85% could faithfully follow stimuli applied at > 1 Hz and had tuning curves indicating optimal sensitivity to sinusoidal mechanical indentations of frequencies $\geq$ 5 Hz (e.g. Greenwood & Sessle, Brain Res. 117, 1976). Whereas by 6 months the proportion of neurones with a localized receptive field had returned to control levels, the proportion of 'tap-sensitive' neurones was still statistically higher than that in control animals. Thus, dental deafferentation may lead to changes reflecting an impaired ability to localize and code orofacial stimuli by V brainstem neurones. (Supported by NIH Grant DE04786).

143.5 ALTERATIONS IN THE DISTRIBUTION OF RAT SPINOTHALAMIC NEURONS AS A RESULT OF POSTNATAL CAPSAICIN TREATMENT. S. Saporta, Dept. of Anatomy, Univ. of South Florida Coll. Med., Tampa FL 33612

Administration of capsaicin to neonatal rats during a critical period from 1-5 days after birth has been shown to destroy up to 93% of spinal unmyelinated primary afferent fibers and cause a marked depletion of substance-P within the spinal cord. When tested as adults, these animals are clearly deficient in their ability to appreciate noxious stimuli, though the exact nature of this deficit has not fully been explored. These data may be interpreted as indicating that some type of physiological and anatomical reorganization has occurred within the somatic sensory system. The organization of neurons within the spinal cord of the rat which project to the thalamus was studied in untreated or capsaicin treated animals using the retrograde transport of horseradish peroxidase (HRP). Rat pups were injected with 50 mg/kg of capsaicin on day 1,2,7 or 15. An additional group of pups received capsaicin injections (50 mg/kg) on days 1,3 and 5 (1-5). Vehicle control and untreated animals were included in each litter. Multiple HRP injections which would provide uptake of the tracer from throughout the mediolateral extent of the thalamus or single thalamic injections were made at the level of the ventrobasal complex when these animals were 50 days of age. Multiple injections in all animals labeled neurons in the ipsilateral and contralateral nucleus proprius and the lateral cervical nucleus. There was a consistent absence of labeled neurons in laminae I, II and III of the contralateral spinal cord of treated animals and an increase of neurons in lamina VII. In addition, there appeared to be an increase of labeled neurons in the ipsilateral spinal cord as compared to untreated or vehicle control animals. This pattern of labeling closely paralleled the distribution seen following single medial thalamic injections in untreated or vehicle control animals and was similar to that seen after single medial thalamic injections in treated animals. These results lend support to the hypothesis that an anatomical reorganization of neurons within the central nervous system which are concerned with conveying information from nociceptors occurs following postnatal treatment with capsaicin.

143.6 EFFECTS OF NERVE APPLICATION OF CAPSAICIN ON SPINOTHALAMIC TRACT CELLS. K.H. Lee*, J.M. Chung, Y. Hori* and W.D. Willis Marine Biomed. Inst. and Dept. of Anat. and of Physiol. and Biophys., Univ. of Tex. Med. Br., Galveston, TX 77550.

Capsaicin is a neurotoxin that appears to affect unmyelinated nociceptive sensory fibers selectively. We examined the effects of capsaicin applied to the sural nerve on peripheral nerve volleys and on the responses of neurons belonging to the spinothalamic tract (STT) in the monkey. The responses examined included those following electrical stimulation of the sural nerve and also those produced by more natural forms of noxious and innocuous stimuli applied to the skin.

Eight anesthetized and two decerebrate monkeys (Macaca fascicularis, 2.0-3.5 kg) were used in this study. All distal hindlimb nerves were cut except the sural nerve to ensure that the effects of stimuli applied to the periphery were due to conduction in the sural nerve. The afferent volley following sural nerve stimulation was monitored with bipolar recording electrodes placed on the sural nerve proximal to the stimulating electrodes. In addition, single unit activity was recorded from identified STT cells ("STT-like" cells in case of decerebrate preparations) in the lumbosacral spinal cord. Responses of tract cells were evoked by electrical stimulation of the sural nerve. Graded mechanical stimulation and noxious heat were applied to the skin in the receptive field. Evoked responses were compared before and after application of capsaicin to the sural nerve.

Capsaicin (1% solution) applied onto the sural nerve for 15 min. resulted in a reduction in the sizes of Aδ- and C-fiber afferent volleys. These changes paralleled the reduction of A- and C-fiber responses of the STT cells elicited by electrical stimulation of the sural nerve. During capsaicin application onto the sural nerve, the background activity of STT cells increased for 5-10 min. After capsaicin treatment, the responses of STT cells to noxious mechanical stimuli applied to the cutaneous receptive field were somewhat decreased. However, topical capsaicin application almost eliminated the responses of STT cells to noxious heat stimulus. The results of the present study suggest that topical capsaicin application onto a peripheral nerve produces a transient nociceptive response followed by a decrease in sensitivity to noxious stimuli, particularly to noxious heat. These changes are due to conduction block of the nerve fibers at the site of capsaicin application. (Supported by NIH grants NS09743, NS11255 and NS18830 and grants from the Moody Foundation and the American Heart Association, Texas Affiliate.)

143.7 THE FUNICULAR COURSE OF ASCENDING NEURONS OF THE LUMBAR SPINAL CORD. Apkarian, A. V., Hodge, C. J., Jr. and Stevens, R. T. Dept. of Neurosurgery, Upstate Med. Ctr., Syracuse, NY 13210.

The anterolateral spinothalamic tract is thought to be the major nociceptive pathway originating mostly from contralateral dorsal horn cells of laminae I, IV, V, VII and VIII. Recent physiologic studies have shown, however, that the majority of ascending lamina I cells project through the dorsolateral funiculus. The purpose of this study was to identify the laminar location of cells whose axons are located within the dorsolateral funiculus (DLF), the ventrolateral funiculus (VLF) and the ventromedial funiculus (VMF).

In nine cats, horseradish peroxidase (HRP) pellets were placed in the spinal cord white matter (T8 to T10) in either the DLF, VLF or VMF. Concurrently, various lesions (T12 to T13) were made caudal to the injection. These lesions were designed to limit the retrograde transport to a single funiculus. After a 3 day survival time, the animals were perfused, the lumbar enlargement was removed, sectioned and reacted with the tetramethyl benzidine technique.

The results of experiments in which the HRP pellet was placed within VLF were similar to experiments in which the VMF was injected. With these injections the lamina I cells constituted less than 1% of the HRP labeled cells, while 50%-60% of the HRP labeled cells were located within the contralateral lamina VII and VIII. The remainder of the labeled cells were located within laminae IV, V, VI and X (primarily contralaterally).

When the HRP pellet was placed within the DLF, the largest population of labeled cells was located within lamina I (25%-30% of total). Of the labeled lamina I cells, 60%-70% were located contralateral to the HRP pellet. Lamina VIII was labeled mainly contralaterally and constituted 14%-16% of the total population of HRP labeled cells. The remainder of the labeled cells were located within laminae IV, V, VI and VII (mostly ipsilaterally).

While the lamina VII, VIII projection is mainly through the contralateral VLF and VMF, these results show that the primary projection for ascending lamina I cells is through the DLF (bilateral with a contralateral predominance). Since it has been shown that there is a lamina I projection to the thalamus, this ascending lamina I pathway must travel within the dorsolateral funiculus.

143.8 A NEW DORSOLATERAL SPINOTHALAMIC TRACT ORIGINATING WITHIN LAMINA I. M. W. Jones*, C. J. Hodge, A. V. Apkarian and R. T. Stevens. Dept. of Neurosurgery, Upstate Med. Ctr., Syracuse, NY 13210.

Recent anatomical investigations in this laboratory have demonstrated that ascending lamina I projections travel almost exclusively through the dorsolateral quadrant (DLQ) of the spinal cord. Since lamina I cells are known to contribute to the spinothalamic tract, these results raise the question of whether the spinothalamic tract is limited to the ventrolateral quadrant as classically described. This study was designed to determine if the DLQ projection from lamina I terminates in the thalamus.

In adult cats, follewing a lesion of the ventrolateral quadrant (VLQ) of the low thoracic cord, horseradish peroxidase was injected ipsilaterally into medial and lateral nuclei of the thalamus to include nucleus submedius, the central lateral nucleus and the ventrobasal complex. Following a 3 or 5 day survival period cats were perfused and 80 um spinal cord sections were reacted with tetramethylbenzidene. Control experiments (no spinal lesion) with 3 and 5 day survivals were also done. Cell distribution plots were made of retrogradely labeled cells in the lumbar enlargement.

In animals with thoracic VLQ lesions, a distinct lumbar enlargement population of contralaterally labeled cells was present in lamina I. Clusters of cells consisting of three to six directly juxtaposed lamina I cell bodies, just medial to the dorsal root entry zone, occurred at intervals of 200-400 um. Other labeled lamina I cells were interspersed between these clusters. The average number of labeled lamina I cells per section in the lumbar enlargement ranged from 0.12-1.8 and comprised 88-100% of the contralaterally labeled population. The remaining contralaterally labeled cells were distributed between laminae V-X. Five per cent of the lamina I cells projected ipsilaterally. The distribution of cells in control experiments was consistent with previous reports.

These results demonstrate a contralateral spinothalamic projection through the DLQ which has not been previously described. This dorsolateral spinothalamic tract originates predominately within lamina I. This pathway likely represents an additional nociceptive input to the thalamus and its existence has implications regarding the clinical treatment of pain.

143.9 **UPPER THORACIC SPINOTHALAMIC TRACT NEURONS WITH VISCEROSOMATIC CONVERGENT INPUTS PROJECTING TO MEDIAL THALAMUS.** W.S. Ammons, M.-N. Girardot*, and R.D. Foreman. Dept. of Physiol. & Biophys., Univ. of Oklahoma. HSC. Okla. City, OK 73109.

Spinothalamic tract (STT) neurons in the T_2-T_5 segments projecting to medial thalamus were studied for responses to cardiopulmonary visceral and somatic inputs. Characteristics of these medial spinothalamic tract (M-STT) neurons were compared to cells projecting to the ventral posterior lateral nucleus (L-STT cells). Extracellular unit recordings were obtained from 38 STT cells in 12 monkeys (Macaca fascicularis) anesthetized with α-chloralose. Stimulation of a bipolar electrode in the medial thalamus, usually nucleus centralis lateralis or centralis medialis, antidromically activated 18 cells. Stimulation in the ventral posterior lateral nucleus activated 12 cells, while 8 cells were activated from both sites (LM-STT cells). Antidromic conduction velocities were 14.1 ± 1.9 m/s for M-STT cells, 20.3 ± 1.9 for L-STT cells and, 22.7 ± 3.8 for LM-STT cells. The M-STT group included 6 high threshold (HT) cells, 7 wide dynamic range (WDR) cells, and 3 with only muscle input (Deep). One M-STT cell had only an inhibitory field and one had no demonstratable field. The L-STT group included 3 HT cells, 8 WDR cells, and 1 Deep cell. Two LM-STT cells were HT and 6 were WDR. Somatic fields of M-STT cells were usually complex and often bilateral whereas fields of L-STT cells were simple and never bilateral. Electrical stimulation of cardiopulmonary sympathetic afferent fibers excited 15/18 M-STT cells, 12/12 L-STT cells, and 8/8 LM-STT cells. All neurons responsive to sympathetic stimulation receive A δ-sympathetic fiber input. 73% of M-STT cells, 50% of L-STT cells, and 38% of LM-STT cells also received C-fiber input. Injection of bradykinin (2 µg/kg) into the left atrium increased the activity of 5/9 M-STT cells, 7/9 L-STT cells and 2/4 LM-STT cells. These data indicate that T_2-T_5 cells projecting to medial thalamus receive cardiopulmonary visceral as well as somatic input. Visceral input produced similar responses of M-STT and L-STT cells. Therefore both groups of cells are well suited to mediate pain of myocardial ischemia. The bilateral, complex somatic fields for M-STT cells compared to the simple fields of L-STT cells support concepts derived from previous studies that the M-STT system is more suited to participate in the motivational-affective response to pain while the L-STT system is more important for the sensori-discriminative component. Supported by N.I.H. Grants HL22732, NS07114, HL07430, and HL00557.

143.10 SPINO-THALAMIC AND DORSAL COLUMN NUCLEI PROJECTIONS OVERLAP IN THE VENTROBASAL COMPLEX OF THE RAT THALAMUS. Wu Ma, Marc Peschanski, and Jean-Marie Besson. (SPON : Y. Lamour).INSERM U. 161, 2 rue d'Alésia, 75014 Paris, France.

In the lateral portion of the rat VB complex cells responsive to noxious and/or non-noxious stimulation were found (Guilbaud et al., 1980, Pain, 8, 303). This could be related to the existence of both afferents from the spinal cord and dorsal column nuclei (DCN) in this region (Peschanski et al., 1983, Brain Res., 278, 240). The present study was designed to analyse whether the projections of these two pathways were segregated or overlapped in the rat VB.

Male Sprague-Dawley albino rats received injections of ^{3}H-Leucine (0.5 µl, 50 µCi/µl) in DCN 5-7 days before injections of a solution of wheat-germ agglutinin conjugated to horseradish peroxidase (WGA-HRP, 0.15 µl, 10 % in water) in the cervical or lumbar enlargement of the spinal cord. After a survival time of 48 h the brain was perfused with paraformaldehyde (1 %) and glutaraldehyde (3 %) in phosphate buffer (pH 7.4, 0.1 M). Serial 30 µm frozen sections were cut and alternate sections reacted for the presence of WGA-HRP with tetramethyl benzidine. Every other sections were prepared for autoradiography using Kodak NTB$_2$ (2 month exposure). The results obtained were studied using light microscopy in bright and dark field illuminations and documented with camera lucida drawings and photographs.

The projections from spinal cord and DCN to VB are confined within the lateral portion of VB. Direct comparison of anterograde labeling in adjacent sections demonstrates that there is clear overlap of dense labeled terminals from spinal cord and DCN in this region. In addition, the rostral and dorsal portions of lateral VB receive the fiber terminals originating from gracile n. and lumbar cord and the caudal and ventral portions from cuneate n. and cervical cord.

These results show that there is overlap of the lemniscal and spinal afferents in lateral VB. These overlapping projections are somatotopically organized which proves that noxious and non-noxious inputs from the same area of the body project to the same zone of VB in the rat. Electromicroscopic experiment about the same question is in progress to establish whether a same VB neuron may receive afferents from two different pathways.

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143.11 NOCICEPTIVE NEURONS IN THE MONKEY THALAMUS. J.M. Chung, K.H. Lee*, D.J. Surmeier, L.S. Sorkin and W.D. Willis. Marine Biomed. Inst., Dept. of Anat. and Physiol. and Biophys., Univ. of Tex. Med. Branch, Galveston, TX 77550.

The role of the ventral posterior lateral nucleus (VPL) of the thalamus in pain is controversial since only a small fraction of the cells in the VPL have been reported to respond to noxious stimuli. However, the fact that the VPL is one of the major termination sites for the spinothalamic tract in monkey led us to search systematically for nociceptive neurons in the VPL thalamus.

Eighteen monkeys (Macaca fascicularis, 2.0-3.5 kg) were anesthetized with a single dose of α-chloralose (60 mg/kg) followed by a constant infusion of sodium pentobarbital (5 mg/kg/hr). Single unit activity was recorded with a stainless steel microelectrode inserted into the VPL thalamus while search stimuli were applied to the contralateral sciatic nerve. All cells which had well isolated action potentials and driven by the search stimuli were examined. The sampled cells were characterized by the use of both mechanical and thermal stimuli applied to the receptive field. The responses to peripheral nerve A and C volleys were also studied.

Of the 119 cells sampled, half responded to noxious mechanical or noxious heat stimuli applied to their receptive field. Among these, 12 cells responded exclusively to noxious stimuli, whereas 47 of them received both noxious and innocuous input. Another 32 cells responded only to innocuous stimuli. The remainder of the population consisted of cells receiving input from deep tissue or from unidentified sources. Most of the thalamic neurons had restricted receptive fields on the contralateral hindlimb. Usually, neurons receiving nociceptive input responded to C fiber volley in a peripheral nerve, whereas the majority of non-nociceptive cells did not. The C fiber responses were often very long in duration, lasting many seconds. Stimulation and lesions of different parts of the spinal cord white matter indicated that neurons in the VPL thalamus often receive parallel inputs from tracts in the dorsal and ventral white matter.

The results of the present experiments indicate that a large population of neurons in the monkey VPL thalamus respond to nociceptive stimuli applied in the periphery. Therefore, the VPL thalamus seems capable of playing an important role in pain transmission in primates. (Supported by NIH grants NS09743, NS11255 and NS18830 and grants from the Moody Foundation and the American Heart Association, Texas Affiliate.)

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS I

144.1 DEPTH PROFILES OF SOMATOSENSORY-EVOKED POTENTIALS, MUA AND CSD IN SOMATOSENSORY CORTEX OF AWAKE RHESUS MONKEYS. L.J. Cauller* and A.T. Kulics* (SPON: S. Fish). Neurobiology Program, N.E. Ohio Univ. College of Medicine, Rootstown, OH 44272

Multiple electode arrays (12 tips; 25μ dia.; 200μ fixed separation) were constructed to simultaneously sample the somatosensory-evoked potential (SEP) from six, equally spaced levels from surface-to-depth through cortex. A detachable, screw-type microdrive permitted multiple penetrations over postcentral gyrus in the awake monkey. Following each penetration, the depth of the array was marked by passing a weak current for later processing with Perl's iron reaction.

We recorded the multi-channel responses to weak contralateral hand stimuli on FM tape. Recordings were filtered and integrated to determine the multiple unit response (MUA). For penetrations that were found to be perpendicular to the cortical mantle, records were digitized and processed for current source-density (CSD). These methods enable us to histologically reconstruct, on a trial-by-trial basis, the depth profile of SEP, MUA and CSD from the awake monkey and to map those profiles over the surface of postcentral gyrus.

SEPs from awake monkeys are dominated by an early P12 peak and a late N50 peak. Both components depend upon behavioral state such that the amplitude of P12 increases when an animal falls asleep while N50 becomes delayed and attenuated to the point of extinction. The isopotential level for both peaks was found to lie 0.3-0.4mm below the surface of somatosensory cortex. The depth profile for P12 is similar to that for the hippocampal population spike: P12 covaries with the peak of integrated MUA in both amplitude and latency; the P12 MUA is coincident with a peak current sink on the CSD profile (1.0-1.2mm below the surface).

In contrast, N50 is correlated with MUA at the latency and level of a peak current source (0.8-1.2mm below the cortical surface). N50 is associated with a superficial current sink (40-70ms; 0.2-0.4mm below surface) such that N50 appears to be the field effect of a population of EPSPs. Furthermore, N50 extends over a greater region of postcentral gyrus than P12 which is concentrated on the anterior portion of the gyrus.

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144.2 CORRELATED ACTIVITY BETWEEN TWO CORTICAL NEURONS WITH OVERLAPPING RECEPTIVE FIELDS OCCURS ONLY WHEN BOTH CELLS ARE IN THE SAME SUBMODALITY BAND OF CAT SOMATOSENSORY CORTEX. R. Metherate* and R.W. Dykes, Depts. Physiology, Neurology & Neurosurgery and Surgery, McGill University, Montreal. Quebec, Canada

The multiunit activity of cortical area 3b can be divided into slowly adapting (SA) and rapidly adapting (RA) bands running mediolaterally along the forelimb representation. To examine the interactions between pairs of neurons located in the same or adjacent bands, we used two triple-barrel electrodes to record simultaneously the activity of two cells. The recording barrel contained a carbon fiber and the other two barrels contained L-sodium glutamate (0.5M, pH 8). When two cells were isolated, they were characterized by the nature of the afferent stimulus that excited them, their receptive fields were noted and each was assigned to an RA or SA band based on the surrounding multiunit activity. We recorded their spontaneous activity and responses to iontophoretically-applied glutamate, electrical stimulation of the ventrobasal thalamus and tactile stimulation of the periphery. The spike trains were analysed using cross-correlation and joint peristimulus scatter techniques to identify correlated activity and to discriminate stimulus-related correlations from neurally-related correlations.

In 20 cats we examined 94 neurons forming 53 cell pairs. In all, 30 pairs had overlapping receptive fields. Nineteen of these were located in clearly identified RA or SA bands and were fully tested. The analysis techniques demonstrated interactions ranging from strong synaptic connections to no correlation whatsoever. There were no exceptions to the observation that two cells interacted only when both were in the same submodality band (14 pairs). When one cell was in an RA band and the other in an SA band (5 pairs) there was no correlation.

The cross-correlation diagrams often implied simultaneous activation of both members by a third, unseen source. One possible source for such a shared input could be the ventroposterior lateral nucleus of the thalamus where segregated SA and RA regions have been shown to exist. These data support the hypothesis that submodality-specific thalamocortical neurons project only to cortical areas of the same submodality.

144.3 THE INTERRELATION OF CALLOSAL AND CORTICOCORTICAL NEURONS IN THE SOMATOSENSORY CORTEX OF THE RAT. Paul Herron, Brian M. Minsk*, and Lawrence D. Savoy*, Div. of Neurosci., Dept. ofPsych., University of Massachusetts, Amherst, MA 01003

Double-labelling techniques were utilized in order to determine in the primary somatosensory cortex (SI) the spatial interrelation of cell bodies of origin projecting to the primary motor cortex (MI) and the contralateral SI. We wanted to determine the following: 1) if single cells send collaterals to both target regions, and 2) if the distribution of callosal neurons was congruent with, or exclusive of, the distribution of corticortical neurons projecting to the MI. We used a combination of the retrograde tracers, nuclear yellow, fast blue, and horseradish peroxidase (HRP). Several injections of one tracer totaling 1.0 ul were made in the contralateral SI and several injections of a different tracer totaling 0.3-0.5 ul were made in the ipsilateral MI; we wanted to maximize the labelling in SI for both fiber systems. Survival times were 3-10 days for the fast blue and 18-72 hrs for the nuclear yellow and HRP. The brains were removed and sectioned tangentially at 30 or 40 um and the tissue containing HRP was reacted with tetramethyl benzidine for the HRP reaction product. All sections were examined for fluorescent labelling using an Olympus fluorescent attachment with an excitation filter for 360 nm.

The distributions of callosal and corticocortical neurons projecting to MI were virtually exclusive of one another. The callosal neurons were localized almost excusively in the agranular regions of SI whereas the corticocortical neurons were located in the barrel fields of the granular regions. However, there were more corticocortical neurons in the callosal-connected regions than vica versa. Often, several labelled neurons from a single barrel were observed. A few double-labelled neurons were observed; they were much less than one 1% of the total number of labelled neurons observed. The small number of double-labelled neurons were consistently observed in the callosal-connected regions. Consequently, we conclude that the callosal neurons and corticocortical neurons projecting to the MI form columns that are relatively exclusively of one another.

144.4 SOMATOSENSORY RECEPTIVE FIELDS OF CALLOSAL FIBRES IN THE MONKEY. F. Lepore, L. Richer*, M. Ptito and J.P. Guillemot. Dépt. de psychologie, Dépt. de kinanthropologie, Univ. de Montréal, U.Q.T.R. and U.Q.A.M., Montréal, Québec, H3C 3J7.

The corpus callosum, the principal neocortical commissure, is known to transmit various sensory information between the hemispheres. In order to determine the type of information being transmitted across the midline in the somatosensory modality, we recorded callosal axonal activity while stimulating with various "natural" stimuli the body of the animal. The subjects, macaca mulata monkeys, were deeply anesthetized (Ethrane 3%, N_2O and O_2 in a ratio of 70:30) during surgery to expose the corpus callosum and thereafter, during the electrophysiological recording, they were maintained on light anesthesia (Ethrane 0.5%, N_2O:O_2 ratio 70:30) and paralyzed with Flaxedil. The state of the animal was monitored throughout the experiment, with rectal temperature being maintained between 37° and 37.5° C and expired CO_2 at 3.5 to 4%. Tungsten microelectrodes were lowered under visual control into the corpus callosum. Electrophysiological activity was monitored in conventional manner and whenever the activity of a fibre was isolated from background, its somatosensory receptive field properties were determined. Stimuli consisted of light touch with a hand or camel's hair brushes, pressure, pinches, vibratory stimulation and joint rotation. In some cases, PSTH's were derived in ordre to evaluate a fibre's phasic and tonic properties and its ON-OFF characteristics.

Most body parts were represented in the corpus callosum. However, as we have shown in the cat, the axial and proximal parts were overrepresented with most receptive fields being situated near the body midline, which they generally touched and, in some cases, straddled. The region of the head was also well represented. The distal extremities, in accordance with anatomical studies describing patterns of callosal projections, were much underrepresented. The results are discussed in terms of the role of corpus callosum in midline fusion. Supported in part by a grant from the Conseil de Recherches en Sciences Naturelles et en Génie du Canada and from the Ministère de l'Education du Québec.

144.5 COMPARISON OF RESPONSE PROPERTIES OF NEURONS IN SOMATIC SENSORY AND MOTOR CORTEX OF RATS. G.A. Bush and M.D. Mann. Dept. of Physiology and Biophysics, Univ. Nebr. College of Medicine, Omaha, Nebraska 68105.

Single neurons located in the forepaw representation of somatosensory and motor cerebral cortices of chloralose-anesthetized albino rats were studied using extracellular recordings with glass microelectrodes. The responsiveness of each isolated unit to stimulation of each paw was tested using 0.1-msec constant current pulses through bipolar electrodes placed in the footpad. Data collected for each neuron included: responsiveness to paw stimulation, depth within the cortex, threshold, number of spikes per discharge, latencies for each spike, and frequency-following ability. Within each cortical area the neurons were grouped into sets according to the extent of their receptive fields, using the method of Towe, Patton, and Kennedy (Exp. Neurol. 10:325-344 1964). Thus, cells that responded to contralateral forepaw stimulation are referred to as sa neurons, those that responded to stimulation of both forepaws as sb neurons, and those that responded to stimulation of all four paws as m neurons. In somatosensory cortex, 6% of the studied neurons were classified as sa, 11% as sb and 83% as m neurons. All of the sa and sb neurons were isolated in the upper half of the cortex, whereas m neurons were isolated in both the upper and lower halves of the cortex. Sa neurons responded with fewer spikes per discharge, smaller thresholds, yet similar frequency-following abilities to m neurons. The sb neurons were intermediate between sa and m neurons. In motor cortex, all isolated units were m neurons. Compared to m neurons in sensory cortex, those in motor cortex had more spikes per discharge and greater thresholds, but similar distributions in depth.

Properties of sa and m neurons in rat cortex are in contrast with those in cat cortex, where sa neurons have fewer spikes per discharge, as well as higher thresholds and lesser frequency-following ability. In addition, sensory cortex (area 3B) in cats contains almost entirely sa neurons, whereas motor cortex (area 4γ) contains a mixture of sa and m neurons.

144.6 VISUALIZATION OF THE BARREL FIELD IN LIVING RAT NEOCORTICAL SLICES. A. Agmon* and B.W. Connors. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Layer IV of the primary somatosensory cortex of the rodent contains aggregates of small neurons, commonly referred to as "barrels". The barrel cortex has been well-characterized in terms of its extrinsic connectivity and sensory receptive fields, but little is known about the physiological properties of its neurons or their local synaptic connections. The brain slice preparation is highly suitable for probing such questions; however, barrels have been described thus far only in fixed and stained tissue.

We have designed, built and tested a modified interface brain slice chamber which allows visualization of individual barrels in the living preparation. Our chamber differs from those previously described in having the slices situated on a translucent surface (an agar-coated glass coverslip) and illuminated obliquely from below by a fiber optic light guide. Blocks of parietal cortex taken from Sprague-Dawley rats (10 days to adult) were embedded in agar, cooled quickly and sectioned coronally on a vibratome at 250-350 μm. When placed in the chamber and illuminated at the correct angle, the slices showed regions with a distinct dark band running parallel to the laminae. When later stained for cell bodies, this band corresponded with granular layer IV. In many places the dark band was broken into discrete patches, of dimensions similar to those of histologically defined barrels. In slices later stained for acetylcholinesterase (AChE), a marker for barrels (Kristt, Neurosci. Lett. 12:177-182, 1979), these patches corresponded precisely with patches of dense AChE staining. As a final confirmation that we were visualizing barrels, blocks of parietal cortex were flattened and then sectioned parallel to the pial surface. When observed in the chamber, slices containing layer IV showed the typical pattern of the barrel field as previously described in stained tissue. The viability of our slices was confirmed by conventional extracellular stimulating and recording techniques.

We conclude that barrels of rat somatosensory cortex can be visualized in the living tissue. This preparation is well-suited for physiological investigations of local circuitry and single cell properties in the context of a precisely defined cortical structure.

We thank Robert McGowen and Donald Kristt for doing the AChE staining. Supported by grant T32 MH17047 from the NIMH (AA) and NIH grant NS 12151 (BWC).

144.7 GLUTAMIC ACID DECARBOXYLASE IMMUNOREACTIVITY IN LAYER IV OF BARREL FIELDS IN NORMAL AND NEONATAL WHISKER MANIPULATED RATS AND MICE. S. M. Lu; D. E. Schmechel and C.-S. Lin (Spon: N. B. Cant) Departments of Anatomy and Medicine, Duke University, Durham, NC 27710

In the rodent somatosensory cortex, specialized groups of neurons located in layer IV have been identified as "barrels". The present study was designed to study the morphology and distribution of GABA-ergic neurons in the barrel fields of normal rats and mice, and, in rats that had whiskers removed neonatally. Immunohistochemistry for glutamic acid decarboxylase (GAD) was used to study the effects of whisker removal in the development of barrels.

The GAD immunoreactive neurons located in layer IV of the barrel field resemble the large smooth stellate neurons described previously in Golgi studies. Most of the GAD immunoreactive neurons are located along the walls of each barrel subfield. Usually, from 12 to 20 reactive neurons are found within the barrel walls in 20 to 30 µm thick sections. In contrast, only about 2-4 reactive neurons are found within each barrel hollow in these sections. The percentage of neurons in layer IV that are GAD reactive was estimated by labeling neurons with a second antibody, neuron specific enolase (NSE). In the double labeled sections GAD reactive neurons constituted from 13 to 15% of the NSE positive neurons in layer IV.

The densest concentration of GAD positive terminals is also in the walls of the barrels. Less dense concentrations are present in the hollows. Very few GAD positive neurons and terminls are seen in the septal regions. Similar distributions of GAD positive neurons and terminals are found in both rats and mice.

Following neonatal whisker removal in rats, the GAD reactive neurons and terminals are less confined to the walls of the deprived barrels. Instead, GAD positive neurons are distributed throughout the denervated barrel fields. The GAD reactive terminals are also more evenly distributed within the barrel fields. Thus, the results suggest that afferent activity plays an important role in the development of the cortical organization of both GABA-ergic neurons and terminals.

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144.8 THE ACTIVITY OF IDENTIFIED VIBRISSAE-DRIVEN UNITS IN RAT SI BARRELFIELD CORTEX WITH MOVEMENT AND EXPLORATORY BEHAVIOR. J.L. Uhr, M.O. West, and J.K. Chapin. Dept. of Cell Biology, U. Tex. Hlth. Sci. Ctr., Dallas, TX 75235.

The main purpose of this study was to characterize the sensory response properties of single neurons recorded in the whisker area, or "barrelfield" (BF), and the adjacent "Dysgranular Zone" (DZ) of the primary somatosensory (SI) cortex of the awake, freely moving rat. Neuroanatomical studies have shown a marked convergence of cortico-cortical projections to the DZ, and deep layers of the BF, from other parts of SI, SII, MI, and the contralateral cortices. The major question asked was: do the properties of single BF and DZ neurons reflect this convergence of cortical information?

Single units were recorded in awake, freely moving rats. Receptive fields (RF's) were determined by touching single, or groups of whiskers with a hand held probe. This was done only during periods when the rat was not spontaneously moving its whiskers. Sizes of RF's ranged from 8-30 whiskers. Many RF's were bilateral, but covered only certain whiskers on each side. Some of these bilateral RF's covered the same whisker groups on each side, while others covered different whiskers on each side.

Next, the sensory responses of the same units were studied while the animal was actively moving its whiskers, ie. "whisking". It was possible to correlate unit responses with such movements through use of a video-movement analysis system. Off-line, frame-by-frame analysis of the video-tape was used to create peri-event histograms of the unit responses to whisker movement, or to the probe movement. During active whisking, some cells followed the whisking movement. This would be expected, since they possessed whisker RF's. Other cells, however, did not fire rhythmically during whisking, even though they were extremely sensitive to passive whisker displacement. Nevertheless, such cells would often respond if an object was held stationary near the face during active whisking.

These data suggest in the awake animal a possible functional role for barrelfield processing of whisker input. Specifically, bilateral RF's may detect aperature shape, and other features of the environment surrounding the face. The ability of cells to detect stationary objects during active whisking suggests either a peripheral or a central mechanism for minimizing sensory feedback from whisker mechanoreceptors caused by active whisking movements, thus allowing discrimination of objects in space. Supported by grants NS-18041 and AA-0390.

144.9 A POPULATION OF THALAMOCORTICAL NEURONS PROJECTING CONVERGENT INPUTS TO CAT PRIMARY SOMATOSENSORY CORTEX. J.N.B. Waldron* and P. Zarzecki. Department of Physiology, Queen's University, Kingston, Ontario, K7L 3N6 Canada.

Inputs from separate afferent sources may converge upon individual neurons within primary somatosensory cortex (e.g., Zarzecki et al. Exp. Brain Res. 50: 408, 1983). The interactions among these inputs did not indicate whether this convergence occurred in the cortex or earlier in the ascending pathways (MacGillis et al. Brain Res. 276: 329, 1983). Therefore, the purpose of the present study was to determine if there is a population of thalamocortical neurons projecting convergent inputs to identified subdivisions of primary somatosensory cortex.

Experiments were performed with halothane-anaesthetized cats. Extracellular recordings were made in the ventral posterior lateral nucleus of the thalamus (VPL) from thalamocortical neurons which were antidromically activated by microstimulation of cortical areas 3a or 3b. To test for inputs from the forelimb, electrical stimuli were delivered to muscle and cutaneous nerves.

In most cases, either none or only one of the tested nerves were effective in evoking discharge of thalamocortical neurons. However, for a few thalamocortical neurons, electrical stimulation of either of two forelimb nerves evoked spikes which were followed by collision-extinction of the antidromic response. These relay neurons received both muscle and cutaneous inputs and were intermingled in VPL with thalamocortical neurons with more restricted inputs. Thus, VPL contains a population of thalamocortical neurons with convergent inputs from separate forelimb nerves. Activation of thalamocortical neurons from more than one afferent source could explain not only the convergence previously found in the cerebral cortex but also the spatial facilitation and occlusion which occur among these convergent effects. (Supported by the Medical Research Council of Canada).

144.10 CORTICAL AND THALAMIC PROJECTIONS TO SII IN THE RAT. E.L. Bold and E.J. Neafsey. Dept. of Anatomy, Loyola Univ. Med. Ctr. Maywood, IL 60153.

The thalamocortical and corticocortical connections of the second somatic sensory area (SII) in the rat were studied with fluorescent retrograde tracers. The injection volume was 0.2µl of either 2% Fast Blue or 2% Diamidino Yellow in distilled H2O. SII was electrophysiologically identified by the sensory mapping technique of C. Welker (1971) under Nembutal anesthesia. Representations of specific body parts in SI, especially the mystacial vibrissae, were identified during all mapping experiments so that partial maps of SI and SII were obtained in each animal. The distribution of retrogradely labeled cells in the thalamus and cortex were reconstructed from serial coronal sections. The injection sites in SII were confirmed by cytoarchitectonic study of counterstained sections.

In the thalamus, labeled cells were localized in the ventromedial portion of VB from mid-thalamic levels to its caudal limit, forming a cluster of cells extending from just above the medial lemniscus dorsally into PO. The labeling in VB did not extend medially into the gustatory area of VB. The labeling in PO was heaviest in the ventrolateral part but did extend somewhat into the dorsal and caudal parts of PO. Retrogradely labeled cells were also found in the central medial and paracentral intralaminar nuclei, as well as caudal VL and the dorsal aspect of VM. A few labeled cells were found in the lateral hypothalamus, zona incerta, and basal forebrain.

Cortical labeling following these SII injections was bilateral. Ipsilaterally, labeled cortical cells were found in SI cortex, and these SI projections to SII appeared to be somatotopically organized. For example, injections in rostral SII (head area) received input from the SI vibrissae barrel field while more caudal SII injections (forelimb area) received input from the SI forelimb region. Contralaterally, the pattern of labeling in SI was similar to that seen ipsilaterally but considerably fewer cells were labeled. In addition, a distinct cluster of retrogradely labeled cells were found in contralateral SII.

It appears that the cortical and thalamic labeling found in the rat correlates well with inputs to SII in the cat and monkey. Finally, based on experiments where multiple fluorescent dyes have been injected in SI and SII in the same animal, thalamic cells projecting to SII form a distinct population of cells.

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144.11 IPSILATERAL FOREPAW REPRESENTATION IN CEREBRAL CORTEX OF THE FERRET. Antonio Canedo*, Felix Viana* and A. L. Towe. Dept. of Physiology and Biophysics, Univ. of Wash. Sch. of Med., Seattle, WA 98195

Topographic maps of the body surface can be constructed in mammals using the amplitude of the primary response as an indicator. Somatosensory area I is usually organized with leg medial and head lateral, and is located in granular cortex just caudal to, or overlapping with, motor cortex. Stimulation confined to the palmar surface of each paw can be used not only to locate the most efficacious point on the cortex, but also to find the extent of cortex activated by each of the paws. This was done in laboratory-reared ferrets (Mustela nigripes), using chloralose anesthesia. Somatosensory area I was found to occupy most of the cortex between the cruciate and ansate sulci, and to be disposed in the usual manner, with contralateral hindpaw (CHP) medial to contralateral forepaw (CFP) representation. Throughout this region, however, small, long-latency, high-threshold responses to stimulation of the other paws were evident. In recording progressively more rostrally in the CFP region, the response evoked by CFP stimulation gradually decreased in amplitude, increased in threshold and latency, and lost its large negative phase, becoming predominantly positive in configuration. At the same time, the small response to ispsilateral forepaw (IFP) stimulation gradually increased in amplitude, decreased in threshold and latency, but retained its generally positive configuration. At its focus on the precruciate gyrus, it was nearly as large as the CFP response at the CFP focus. Furthermore, its threshold and latency were the same as those of the CFP response at the CFP focus! The area of cortex occupied by this IFP region was as large as that occupied by the CFP region immediately caudal to it. The latency and threshold relationships were such that the IFP response could not result from input via the opposite hemisphere. The simplest hypothesis is that the input comes entirely ipsilaterally, without crossing the midline along the way. Such behavior is different from that seen in any other mammal thus far investigated.

Direct stimulation of the precruciate IFP cortex region evoked contraction in forelimb muscles bilaterally. However, bilaterally-symmetric electromyographic recordings showed the response in contralateral muscles to appear at a shorter latency and lower threshold than those in ipsilateral muscles. (Supported by a grant from the Fogarty International Center, National Institutes of Health.)

144.12 CYTOARCHITECTURE OF THE SENSORIMOTOR CORTEX OF THE BRAIN OF THE WALLABY, *MACROPUS EUGENII*. L. Mayner* (SPON: D.R. Curtis), Department of Behavioural Biology, Research School of Biological Sciences, Australian National University, Canberra, ACT, Australia, 2601.

The brain of the wallaby, although not highly gyrencephalic, has a number of sulci. A cytoarchitectonic study of the cerebral cortex of the wallaby brain was carried out using Nissl and Golgi staining methods. The sulci approximately delineate at least seven different regions of the cortex. These regions are prefrontal, parietal, cingulate, posteriorparietal, striate, temporal, insular. The parietal region is of particular interest since this is where the motor and somatosenory regions overlap. While the prefrontal region is virtually agranular, once the parietal region begins, layer 4 becomes exceedingly prominent. The beginning of the parietal cortex coincides with the beginning of the motor and somatosensory cortex, as has been defined physiologically (Lende, Science, 141: 730, 1963). Layers 5 and 6 are also very wide throughout the parietal region. Cell size and density within each layer have been measured. Several HRP injections were made within the parietal region, the results were similar to those reported for other marsupials. There is convergence of at least three different thalamic nuclei onto one area of the parietal region of the cortex.

144.13 LAMINAR TERMINATIONS OF INDIVIDUAL AFFERENT AXONS IN SI CORTEX IN MACACA. M. Conley and E.G. Jones. James L. O'Leary Div. of Experimental Neurol. & Neurological Surgery & McDonnell Center, Washington University, St. Louis, MO 63110.

Laminar and columnar distribution of afferent and efferent projections of the first somatosensory cortex (SI) in Macaca have been described in detail using degeneration and axonal transport methods, but to date most studies have only examined populations of cells projecting to or from SI. In the present report are described individual SI afferent axons following small iontophoretic injections of HRP in the white matter just beneath physiologically identified loci within SI.

A total of 27 axons were reconstructed 22 of which were identified as thalamic and 5 of which were identified as cortico-cortical. While the designation as an axon's origin as "thalamic" rested in part upon corroborative evidence from earlier studies, the size, density and laminar distributions were so distinct as to leave little doubt about their source. Axons designated as "cortico-cortical" were equally distinct, and in most cases were confirmed directly by identifying their cells of origin.

Thalamo-cortical axons in each of the cytoarchitectonic areas within SI were distinct in terms of axon caliber, density, extent and laminar distribution of the terminal plexus. Our largest sample was taken from area 3b where two general patterns of axonal arborizations were observed, both characterized by dense terminal plexuses in layers IIIB and IV. The first type terminated in a single dense focus usually between 350-700 microns in the anteroposterior dimension and 200-500 microns in the mediolateral dimension; a second type terminated in two distinct foci separated by a (250-350 micron) gap, reminiscent of lateral geniculate axons which project to more than one ocular dominance column. Approximately one-half of the axons in 3b had thin collateral branches to layer VI.

Axons in areas 1,2 and 3a each had characteristic sizes and laminar distributions though all were more sparse than those in 3b.

Cortico-cortical axons terminated either in single cytoarchitectonic areas or multiple areas. All were characterized by very widespread projections (greater than 1.5mm), sparse terminal arbors given off at regular (approximately 500 microns) intervals and distributed throughout the supragranular layers and layer IV.

Thus, individual thalamo-cortical and cortico-cortical axonal arborizations are consistent with autoradiographic studies showing the laminar and areal connections of SI cortex in monkey and point to similarities between SI and VI axonal morphologies.

144.14 RECEPTIVE FIELD PROPERTIES OF NEURONS IN AREA 1 CELL COLUMNS. O. Favorov* and B. Whitsel, Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27514

Studies of the S-I cortex have shown that the receptive fields (RFs) of adjacent neurons can vary appreciably in size and configuration. We have analyzed these variations by performing radial penetrations within the forelimb region of area 1 of unanesthetized Macaca fascicularis monkeys. RFs of all sampled units were mapped using natural stimuli. The number of units isolated in single penetrations ranged between 11 and 39; the mean was 19. Except for layer I, most penetrations sampled several units in each layer. In a given penetration the variability in RF size is large (the average value of the ratio of the largest to the smallest RF was 40:1) and the distribution of RF sizes is broad and strongly skewed, with the majority of units having small RFs. In layers II - VI RFs can vary widely in size and configuration. The average overlap between pairs of units sampled in a single penetration (computed by dividing the RF area common to both units by their total area) is small (31%). The aggregate peripheral area mapped by all neurons sampled by a radial penetration (the Aggr. RF) can be divided into an orderly sequence of zones, each zone defining the skin field mapped by a specified fraction of the units sampled. This fraction is regarded as an estimate of the relative strength of representation of that skin field within the cell column sampled. The Aggr. RF for every radial penetration includes an extremely small region (0.1-1.0% of the total Aggr. RF) which is common to all RFs in the sample. This region (the Aggr. RF center) is, in turn, surrounded by ring-like zones each of which provides input to a specified fraction of the neurons within the column. Aggr. RFs with centers on the forearm are 3 times larger than those centered on the palm, and 9 times larger than those centered on the digit tips. Reconstructions of the data provided by arrays of penetrations show that the Aggr. RF centers shift in an orderly way which reflects known topographic gradients. The Aggr. RFs of such arrays exhibit extensive overlap. Two features of S-I topographic organization are revealed by this approach: (i) the same point on the skin can be included in the Aggr. RFs of widely separated cortical columns, and (ii) the strength of representation of a given skin locus changes systematically and gradually as a continuous sequence of area 1 cell columns is transversed. Supported by NIH grant NS10865.

145.1 THE GRANULAR INSULA IN THE RHESUS MONKEY: SOMATIC SENSORY PROPERTIES. R.J.Schneider, R.J.Nelson, D.P.Friedman, J.B. O'Neill* and M. Mishkin. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20205.

Previous studies from this laboratory have suggested that the granular insula (Ig) may serve as a link in a sensory processing pathway connecting the second somatic sensory area, SII, with medial temporal limbic structures. To examine this hypothesis electrophysiologically, we recorded the response properties of neurons in the granular insula (Ig) in awake rhesus monkeys. Extracellular unit activity, elicited in response to somatic, auditory, visual, and gustatory stimuli, was recorded with microelectrodes advanced transdurally. Specific tracks were marked with electrolytic lesions and recording loci reconstructed.

Of the 237 units recorded in Ig, the majority (54%) were driven only by simple, innocuous, somatic stimuli. Of this group, 86% had bilateral receptive fields (RFs), either cutaneous (61%) or deep (39%). Convergence of deep and cutaneous inputs was not seen. An additional 14% of the total sample was driven by intraoral stimulation; of these, none examined responded to gustatory stimuli. Furthermore, the tested units were not responsive to auditory or visual stimuli per se, though 7 of the units driven by somatic stimuli responded more intensely when the animal appeared to attend visually to the stimulating object. Many units (32%) could not be driven by any of the stimuli employed.

Several unit characteristics distinguish anterior from posterior portions of Ig. Anterior Ig contained most of the units responding to intraoral stimulation (78%) and to stimulation of the face (92%). Anterior Ig also contained 95% of the undriven units, 78% of the units with small RFs, and 70% of the units with deep RFs. The posterior portion of Ig contained all of the whole-body units, i.e. those responding to stimulation of almost the entire body surface. However, more units responding to stimulation of the body exclusive of the head were found in the anterior region (64%). In addition, in both regions, units responding to different body parts other than the head were often found adjacent to each other.

These results suggest that Ig is a somatic sensory field exclusively, though with only a rough somatotopy, if any. The data are consistent with the view that Ig serves as a modality-specific link in a somatosensory-limbic pathway.

145.2 SUBMODALITY AND COLUMNAR ORGANIZATION OF THE SECOND SOMATIC SENSORY CORTICAL AREA IN CATS. H. Burton and K. D. Alloway. Dept. of Anatomy & Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

There is little physiological evidence available on the columnar organization in the second somatic sensory area (SII). We have investigated the submodalities and receptive field organization of individual neurons in SII of cats where much of this region is more readily accessible to orthogonal penetrations.

Single neuron recordings were obtained from SII in nine cats with tungsten microelectrodes through a chamber which had been chronically implanted under aseptic conditions and sodium pentobarbital induced anesthesia. During recording sessions, the animals were restrained in a modified Alice Catham sling and received 5 mg/kg of ketamine hydrochloride as needed for tranquilization.

A well-defined columnar organization was revealed in SII by the observation that receptive field positions overlapped considerably during orthogonal electrode penetrations but shifted during tangential penetrations; submodality representation also tended to remain constant throughout vertical penetrations. SII neurons primarily showed rapidly-adapting responses; slowly adapting responses were not seen. In most cases, cells discharged during movements of guard (216/611 cells) or down (155/611) hairs; in specific regions of the somatotopic map, cells activated from glabrous skin (15/611) and claw and Pacinian receptor inputs (61/611) were also found. The remaining neurons responded to taps or other stimuli that could not be attributed to particular peripheral receptor types. Receptive field borders varied from poorly- to well-defined. The latter were more prevalent in layers III and IV, whereas the former occurred most often in layers II, V and VI.

After dorsal column lesions, some neurons in SII still responded with rapidly-adapting discharges, primarily to hair movement; this was most evident >4 days postoperative. A greater proportion of the sample responded to ill-defined cutaneous stimulation. Generally, receptive field borders were more difficult to delimit. Fewer cells responded to cutaneous stimulation and many more demonstrated spontaneous activity following the lesions.

We conclude that SII has a columnar organization that is comparable to the vertical modules seen in other sensory cortices and that this is primarily based on projections conveyed through the dorsal columns.

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145.3 CHARACTERIZATION OF NEURONS IN THE SECOND SOMATIC SENSORY AND ADJACENT CORTICAL AREAS TO VIBROTACTILE STIMULI IN M. FASCICULARIS. R. Sinclair* and H. Burton (SPON: R. Bunge). Dept. of Anatomy & Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Response properties of neurons in the second (SII) and adjacent somatic sensory regions in primates have not been examined extensively with controlled vibrotactile stimulation. Studies in cats (Ferrington and Rowe, '80) emphasized the high fidelity temporal coding in the responses of some SII neurons to vibratory stimuli that selectively activate Pacinian receptors.

Recordings from >100 single neurons were obtained with glass-coated platinum microelectrodes that were advanced through an Evarts-type chamber which had been chronically implanted under aseptic conditions and sodium pentobarbitol anesthesia. Awake monkeys were restrained in a primate chair during recording sessions. Responses were sampled to vibratory stimuli, ranging in frequency from 5-300 Hz, and ramp stimuli, indenting with velocities of 5-100 microns/ms.

Various patterns of activity were noted to ramp stimuli. For example, quickly adapting discharges occurred at the top and bottom of ramp indentations in all cortical areas studied. The discharge rates for some neurons in SII directly correlated with ramp velocities; area 7b neurons did not show this relationship. In addition to phasic responses, increased, irregularly patterned firing persisted throughout indentation times in some neurons. In other cells, prolonged discharges (lasting up to several seconds) followed brief (100 ms) indentations.

Temporal coding for low frequency (10-40 Hz) vibrotactile stimuli predominated in the responses of SII neurons. Some cells discharged 1 or more times on almost every cycle of stimulation below 20 Hz, whereas very low probabilities of cyclically induced firing occurred during higher frequency vibrations. No stimulus dependent temporal coding was found in recordings from area 7b. In contrast, cyclic activity, with greater than 0.5 frequency following probability to stimuli vibrating faster than 75 Hz, appeared in neurons from area 2 of SI. One of these neurons demonstrated periodic firing to 300 Hz.

We conclude that SII neurons in primates can differentially code for the velocity of an indenting probe and can accurately signal the temporal pattern of low frequency tactile vibrations. Area 7b neurons appear only to respond to the occurrence of a tactile stimulus.

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145.4 HOMOTYPICAL IPSILATERAL CORTICAL PROJECTIONS BETWEEN SOMATOSENSORY AREAS I AND II IN THE CAT. K.D. Alloway and H. Burton. Dept. of Anatomy and Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Connections between primary (SI) and secondary (SII) somatosensory cortex are presumed to link only cortical zones with the same somatotopic representation (e.g., Jones and Powell, Brain Res., 9:71, 1968; Friedman et al., J. Comp. Neurol., 192:21, 1980). These studies, however, failed to collect physiological data from both SI and SII to determine the precise relationship between somatotopic representation and the pattern of corticocortical connections. Therefore, we re-examined the pattern of retrograde labeling (HRP-WGA) with respect to somatotopic representation as determined by physiological recordings.

In 11 cats, anesthetized with sodium pentobarbital, a portion of the cutaneous zone (area 3b) of SI was mapped using tungsten microelectrodes. Microlesions (10-20 μamps, 10sec) were placed around selected somatotopic zones. A 1% solution of HRP-WGA (10-20 nl) was injected into the identified component of SI. The next day SII was mapped and microlesions were placed circumscribing the same somatotopic zone that was injected with HRP-WGA in SI. Subsequently, the animal was perfused and the brain removed, sectioned and prepared for HRP histochemistry.

Injections of HRP-WGA in SI were confined to cortical zones representing the distal digits (n=3), distal toes (n=2), toes and digits (n=1), proximal forelimb (n=1), proximal hindlimb (n=1), trunk (n=2), and nose and face (n=1). The pattern of retrograde labeling in SII revealed dense, heavily labeled patches of cells in regions that were precisely homotypical to the SI injection site. This dense, homotypical patch of labeled cells was usually surrounded by a less densely populated fringe of labeled cells that bordered, but did not appear to enter heterotypical zones. In 2 animals, however, some retrogradely labeled cells were found in areas representing somatotopic zones adjacent to the sites injected with HRP-WGA in SI.

These results indicated that SII primarily sends homotypical projections to SI and that the few heterotypical projections which may possibly exist only connect immediately adjacent somatotopic zones.

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145.5 THE ROLE OF PRIMARY AND SECONDARY SOMATIC SENSORY CORTEX IN RECOVERY OF TACTILE FUNCTION IN M. MULATTA. M. Carlson and H. Burton. Depts. of Anatomy & Neurobiology, Psychiatry and McDonnell Center for Studies of Higher Brain Function, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Normal development of tactile discrimination function is seen following unilateral lesions of Brodmann's areas 3b, 1, and 2 in the hand area of primary somatic sensory cortex (SI) of infant macaques, whereas the same lesion in juveniles or adults produces severe and irreversible discrimination deficits. A lesion of the second somatic sensory cortex (SII) also produces serious tactile discrimination impairment in adults. In contrast, we have found normal development of tactile function after unilateral or bilateral SII lesions in two infants. These animals made slightly more errors prior to attaining 80% correct criterion on texture (but not size) discrimination tasks; their performance on threshold tasks was at normal levels (>88% correct) soon after surgery. Having found neither SI nor SII lesions alone in infants to be of serious long-term consequence for discrimination performance, we next examined the effects of combined SI-SII lesions. Significant tactile deficits resulted in an infant when, at 30 weeks of age, an SI lesion was made in the same hemisphere from which SII had been removed at 7 weeks of age. This animal was particularly impaired on the acquisition of texture tasks. One infant with unilateral and simultaneous removal of SI and SII at 8 weeks of age showed severe impairment in acquiring the easiest (320 vs. 40 grains/inch) texture discrimination and failed on the moderate (320 vs. 80 grains/inch) task; size discriminations were normal. Animals who recovered from either an SI or SII lesion during infancy demonstrated severe texture and size discrimination deficits only following unilateral lesions to the remaining SI or SII. These results suggest that in the infant macaque SI or SII alone have the capacity to mediate normal texture discriminations - a capacity not available to the surviving somatic area in older animals.

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145.6 RESPONSES OF NEURONS IN CAT SOMATOSENSORY AREA I OR II FOLLOWING REVERSIBLE MICROANESTHESIA OF THE OTHER IPSILATERAL SOMATOSENSORY AREA. C. J. Robinson and H. Burton. Rehab. R & D Center, VA Hospital, Hines, IL, 60141 and Dept. of Anatomy & Neurobiology, Washington Univ. Sch. of Medicine, St. Louis, MO, 63110.

A precise pattern of interconnectivity exists between foci in the first and second somatosensory areas (SI and SII) that subserve the same peripheral receptive field (Alloway & Burton, this vol.). We wished to determine if reversible inactivation of part of one area would change the responses of individual neurons in the corresponding homotypical representation of the other ipsilateral area.

In eight cats anesthetized with ketamine or pentobarbital, we used a chronically implanted dual chamber (Robinson, et al.,'84, J Electrophys Tech) that permits independent translational control over a metal microelectrode in SI, a similar electrode in SII and a 10ul Hamilton syringe. We recorded from individual neurons or neuronal clusters in SI and SII that had overlapping receptive fields, determined the responses of an isolated neuron in SI or SII to electrical stimulation of its receptive field, anesthetized the corresponding region of the other area by microinjection of <2 ul of 2% lidocaine (Malpeli & Schiller, '79, J. Neurosci Methods), and observed the responses over time.

We made 57 sets of comparisons between pre- and postmicroanesthetic conditions in the averaged post-stimulus time histogram and instantaneous rate histogram of 27 SII and 10 SI neurons with forepaw or limb receptive fields. In most cases, microanesthesia did not produce changes in response latency, or in the evoked or background discharge pattern of the unit under study. However, in nine cells (6 SII, 3 SI), the unitary evoked responses became enhanced within one to three min following each injection, and background activity also increased. After 20 to 30 min, these responses returned to normal, and the repeatability of effect was demonstrated during one or more subsequent runs.

We had expected that microanesthesia would cause a transient removal of whatever interaction existed between the two somatosensory areas, either directly or through a corticothalamic path. While some effects were seen, changes were not generally evident in the short latency, electrically evoked responses of isolated cortical neurons in these lightly anesthetized animals.

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145.7 CHANGES IN FUNCTIONAL ORGANIZATION OF THE SOMATOSENSORY CORTEX OF ADULT RACCOONS FOLLOWING RESTRICTED CORTICAL LESIONS. G.S. Doetsch, K.W. Johnston* and C.J. Hannan, Jr. Depts. of Surg. (Neurosurg.) and Physiol., Med. Coll. Ga., and Dept. Clin. Invest., Eisenhower Army Med. Ctr., Augusta, GA 30912.

Our recent studies have shown that peripheral nerve transection by removal of a forepaw digit in adult raccoons causes neurons within the deprived SmI cortex to become responsive to stimulation of "new" skin regions adjoining the site of injury. This finding suggested that similar physiological changes might occur in recovery from damage to central somatosensory structures. To test this idea, small lesions were made in the SmI digit 3 cortical zone of adult raccoons by subpial aspiration, after the zone boundaries had been electrophysiologically mapped. These animals were studied under chloralose anesthesia 15-17 weeks later.

In normal raccoons, the glabrous skin of the forepaw digits and pads is represented within highly topographic SmI cortical sectors, while the hairy skin and claws are represented within surrounding "heterogeneous" subdivisions with more convergent properties. In the lesioned raccoons, these basic organizational features were modified by the appearance of "new," secondary inputs (superimposed on the primary inputs) to those cortical areas bordering on the lesion--the zones for digit 2, digit 4 and the pads. Preliminary results indicate that these zones differed from normal as follows. 1) Single neurons and clusters of neurons receiving secondary inputs responded to stimulation of "novel," off-focus forepaw regions; the "new" inputs originated from digit 3 more often than from any other digit or the pads. 2) Neuronal receptive fields (RFs), mapped with a standard suprathreshold stimulus, were larger. 3) Neuronal submodality sensitivity was less specific, with mixtures of skin touch, claw touch and/or hair deflection. 4) The location of neuronal RFs and the skin type involved were more variable as a function of cortical depth. 5) The "new" inputs seemed to be less topographically organized.

The results show that lesions within the SmI cortex may cause "novel" inputs to appear in cortical tissue near the site of injury. These inputs originate not only from the skin region represented in the lesioned area, but often from neighboring regions as well. The pattern of cortical responsiveness following central (and peripheral) somatosensory damage in raccoons appears similar to that found normally in the SmI "heterogeneous" subdivisions. Thus, neurons with convergent somatosensory properties may be selectively facilitated or disinhibited during the process of recovery.

145.8 PERSISTENT TOPOGRAPHY CHANGES IN PRIMARY SOMATOSENSORY CORTEX (AREA 3b) OF MONKEYS FOLLOWING NERVE REGENERATION. J. T. Wall and J. H. Kaas. Department of Psychology, Vanderbilt University, Nashville, TN 37240.

Cortical Area 3b in primates normally contains a topographically organized representation of mechanosensory inputs from the skin. Following nerve transection, regenerating sensory axons commonly become misdirected and reinnervate erroneous skin locations. It is frequently proposed that central somatosensory connections compensate for misdirected inputs but there is little evidence to support or refute this view. The present studies assessed the topographical organization of cortical Area 3b in adult primates in which nerve cross anastomosis and regeneration was used to misdirect sensory axons. The goal was to produce predictable reinnervation errors and to determine, after long recovery periods, whether abnormalities in cortical topography were eliminated or persisted.

The left ulnar and median nerves of two owl monkeys were transected at the wrist. The proximal ulnar nerve was joined to the distal median nerve to produce reinnervation of the median nerve skin by the ulnar nerve, and loss of innervation to the ulnar nerve skin. The radial nerve was not disturbed. After recovery periods of 2.6 and 2.9 years, the hand representation in Area 3b of these monkevs was mapped with multiunit recording techniques and compared to normal monkeys.

Monkeys with regenerated nerves were abnormal in several ways. (1) Only skin areas normally innervated by the median and radial nerves were represented in cortex. (2) The cortical zone normally representing ulnar nerve skin instead represented median nerve skin. (3) Within this shifted representation, areas of cortex representing individual pads and digits normally innervated by the median nerve were topographically disordered and distributed in a patchlike fashion. (4) Within these patches, neurons sometimes had more than one cutaneous receptive field. (5) Many parts of the hand representation were unresponsive to mechanosensory inputs.

These findings indicate normal topographical organization is highly dependent on specific patterns of peripheral innervation and that connections between the skin and primary somatosensory cortex of adult primates do not re-sort or normalize large peripheral errors in reinnervation which result from nerve cross.

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145.9 CORTICAL AND THALAMIC CONNECTIONS OF THE REPRESENTATIONS OF THE DIGITS OF THE HAND IN AREAS 3b AND 1 OF SQUIRREL MONKEYS. C. G. Cusick and J. H. Kaas, Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

Separate tracers were used to simultaneously reveal the connections of matched parts of the hand representations in Areas 3b and 1 of five adult squirrel monkeys. Microelectrode recordings first identified the locations of the representations of the distal glabrous phalanges of the hand in rostral Area 3b and caudal Area 1. Horseradish peroxidase or wheat germ agglutinin (WGA) conjugated to horseradish peroxidase was then injected into one representation and tritiated WGA was injected into the other representation. After appropriate processing, brain sections were examined for both types of label. In three cases, the cortex was first flattened and cut parallel to the surface as an aid to reconstructing surface view patterns of label.

The results indicate that the Area 3a-3b border zone devoted to distal phalanges is reciprocally interconnected to the Area 1-2 border zone representing the same distal phalanges. In cases with the most restricted injections, label was not found in cortex representing the proximal phalanges and palm near the Area 3b-1 border. Thus, Area 3b and Area 1 interconnections are somatotopically matched. This anatomical evidence, as well as microelectrode mapping evidence, supports the conclusion that Area 3b and Area 1 contain separate, roughly mirror image representations of the body surface. Labeled neurons in both fields were mainly in the supragranular layers.

The somatotopically matched injections also resulted in extensively overlapping zones of labeled cells and terminations in the subnucleus of the ventroposterior nucleus that represents the hand. Thus, the present results support previous conclusions that a single representation of the body surface in the ventroposterior nucleus is somatotopically interconnected with dual representations, in Area 3b and Area 1.

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145.10 A SINGLE BODY SURFACE REPRESENTATION IN THE S-I REGION OF THE NEW WORLD MONKEY, SAGUINUS. M. F. Huerta, M. Carlson, C. G. Cusick, and J. H. Kaas, Dept. of Psychology, Vanderbilt University, Nashville, TN 37240, and Dept. of Psychiatry and McDonnell Center for Studies of Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110.

Tamarins (Saguinus) and marmosets (Callithrix) of the family Callithrictdae are small lissencephalic New World monkeys distinguished by a number of behavioral and morphological characteristics including claws rather than nails on all fingers and four of the five toes. In detailed microelectrode mapping studies in four anesthetized tamarins, only one representation responsive to low-threshold cutaneous stimulation was evident in the region of the first somatosensory cortical area, S-I. Cortex rostral and caudal to this representation was either unresponsive or responded only to high-threshold stimulation. This single topographic projection was coextensive with a parietal koniocortical field that resembles Area 3b of other monkeys, and the general somatotopic organization of the field was similar to that of Area 3b of other monkeys. Most of the cortex devoted to the digits was activated by stimulating distal phalanges and claws. Receptors in the distal digits projected to cortex rostral to that activated by the palm and hairy skin of the hand. The face, teeth, and tongue were represented lateral to the hand, and the wrist, forearm, arm, trunk, leg, and foot related to progressively more medial cortex. The chest and abdomen were represented caudal to the back.

We regard the single body surface representation in Saguinus as homologous to the single S-I representation found in some prosimians (Galago, Perodicticus) and to the Area 3b representation in those primates with two cutaneous representations in the S-I region, the New World Cebidae (Aotus, Saimiri, and Cebus), Old World Macaca, and prosimian Nycticebus. The results suggest that the overall organization of somatosensory cortex in Saguinus is more generalized and primitive than in other monkeys.

Supported by NIH Grant DE 06554.

BIOLOGICAL RHYTHMS II

146.1 IDENTIFICATION OF A NEW POPULATION OF EFFERENT NEURONS TO THE EYE OF APLYSIA WITH RETROGRADE TRANSPORT OF ^{3}H DOPA. L. Olson and J. Jacklet, Dept. of Biol., SUNY-Albany, Albany, N.Y. 12222

A new population of efferent neurons from the cerebral ganglion (CG) to the Aplysia eye has been demonstrated. These neurons do not project to the eye through the optic nerve (ON), but through 3-5 previously unknown accessory optic nerves (Acc. ON). Previously described efferents (Luborsky-Moore and Jacklet, 1976) travel in the ON and somata are located along the ipsilateral posterior tentacle nerve (PT). Newly identified efferents were found by labeling the eye with ^{3}H DOPA to identify afferent projections of secondary neurons, the output neurons of the ocular circadian pacemaker. In addition to afferent projections, a small number (6-12) of CG neurons were labeled. These neurons occur ipsilateral to the labeled eye in a cluster close to the identified Metacerebral Giant Cell (MGC). ON efferents were never labeled. Axons from the ^{3}H DOPA efferents were not in the ON, but were seen in the ipsilateral PT nerve. The results suggested that these neurons 1) labeled by selective uptake of ^{3}H DOPA by their terminals at the eye with retrograde transport to neurons in the CG; 2) did not project to the eye through the ON. Staining a partially dissected brain with methylene blue revealed 3-5 small nerves branching from the PT nerve to innervate the eye. Electrical recordings from Acc. ONs show unitary activity, originating in the CG. Glyoxylic acid induced fluorescence shows that Acc. ONs include fibers containing serotonin, as well as a few catecholamine fibers. Serotonin phase-shifts the pacemaker (Corrent et al. 1978) and mimics a phase-resetting of the pacemaker normally requiring an attached brain (Nakakavaren and Lickey, 1979). A serotonin input to the eye would account for these effects. ON efferents probably do not contain serotonin, as neurons along the PT nerve do not accumualte ^{3}H 5HTP. However, newly identified efferents could be the CG neurons, also clustered near the MGC, which we show selectively accumulate 5HTP and have a serotonin specific-induced fluorescence, and stain for serotonin antibody (Goldstein et al. 1984). A few catecholamine-specific fluorescent neurons found in a similar position do not account for the number of ^{3}H DOPA labeled neurons. We suggest that at least some of these efferents are serotonergic, but with an ability to accumulate and transport DOPA, and thus represent a serotonergic input to the eye which may phase shift the pacemaker. Supp. by NSF BNS 82-06245

146.2 IN VIVO ISOLATED PROTOCEREBRAL CIRCADIAN PACEMAKERS IN CRAYFISH. B. Barrera-Mera, Depto. de Neurociencias, C.I.F.C., & División de Investigación, Depto. de Fisiología, Facultad de Medicina, U.N.A.M.

The complete protocerebral complex, viz. the optic lobe neuropolis, the neurons and neurohemal structures in both the supraesophageal ganglion and the eyestalks, was surgically disconnected from the rest of the nervous system in crayfish. In these preparations an additional and bilateral removal of the lateral and the caudal portions of the supraesophageal ganglion (that forming the deuto and tritocerebrum, i.e. about 70-80 percent of the ganglionary mass), permitted to characterize the protocerebrum ability to sustain a well defined circadian activity in both eyestalks of crayfish Procambarus bouvieri (B. Barrera-Mera, et. all. Neurosci. Abstr. 5, 114p, 1978) and in Procambarus clarckii (B. Barrera-Mera, G.D. Block, Neurosci. Abstr. 7, 45p, 1981). In these isolated protocerebrum preparations it was possible to record: a) Habituation of visual interneurons and inhibitory interaction among sustaining response fibers activity; b) strong hormonal dependent reflex activity of the distal retinal shielding pigment effectors; c) robust and self-sustained circadian rhythm in the eye glow area (EGA) size and in the amplitude of electroretinogram (ERG). The observations reveal not only the importance of the integrity of redundant haemolymphatic supply to neural and neurohemal protocerebral structures of both sides (a,b), but the protocerebrum inherent ability to drive the visual circadian variations (c) in conditions of their drastical disconnection.

Cold (5°C) applied to either eyestalk in these preparations induced: A) Immediate ipsilateral decrease in the amplitude (32-62%) of the ERG rhythm; B) a bilateral lengthening in the ERG oscillations (32-42 hrs) and C) a transient initial desynchronization of left from right ERG oscillations. The existence of left and right protocerebral pacemakers, -both behaving as potent pacemaking structures driving visual circadian variations- is possible as judged by the bilateral tendency to keep rigidly in phase both EGA and ERG oscillations. Furthermore, a clear bilateral desynchronizing effect of unilateral sustained illumination (60 min) and cooling (2-3 weeks) upon the isolated and additionally splitted protocerebrum were also recorded. Both ipsilateral effects suggest that a neural bridge could be reciprocally connecting the neurohemal system of the sinus gland of the eyestalks to the circadian pacemaker complex of both sides.

146.3 STUDY OF PHOTORECEPTOR POTENTIAL AND SINUS GLAND ACTIVITY IN CRAYFISH ALONG THE 24 HOUR CYCLE. B. Fuentes-Pardo and J. Hernández Falcón*. Dept. of Physiol., Sch. of Med., Natl. Univ. of México, P.O. Box 70250, México, D.F., 04510.

The present work is aimed at dilucidating the photoreceptors role in the origin of the ERG's circadian rhythm in crayfish, as well as the possible modulatory action upon the photoreceptors which might be exerted by the periodic release of light adapting hormone (LAH) from the sinus gland.

The receptor potential (RP) of single visual receptors was measured in isolated eyestalks. It was found that they respond to test light pulses with a two phase depolariza-- tion: a fast, transient phase, which depends on the intensity of stimulation, and a slow, steady phase, that can be associated with the duration of the luminous signal. The amplitude of these two phases was measured during successive periods of dark adaptation and repeated at 1 hour intervals during the 24 hour cycle.

The average recovery capacity (ARC) of the photoreceptors response obtained from the curves generated by each phase was compared. The results show that ARC changed along the day and that it is different for each phase of RP, indicating that the isolated photoreceptors keep their oscillatory capacity even when they do not present circadian characteristics. The periodic release of LAH was depicted by injections of eyestalks extracts obtained at different hours of the 24 hour cycle. Eyestalk extract was always applied at 6.00 p.m. to dark adapted animals in which the pseudopupil was measured. The greatest reduction of LAH release was produced when the eyestalk extract was prepared at 12 h, Equivalent effects were observed on the ERG amplitude.

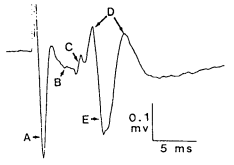
When eyestalk extract was applied directly to previously dark adapted photoreceptors no modification was found neither in the transient nor in the steady phase. This result was interpreted as an indicator that the photoreceptors' oscillations are inherent to photoreceptors themselves and that they might contribute to the circadian oscillation characteristic of ERG in crayfish.

146.4 **FIELD POTENTIALS EVOKED BY OPTIC NERVE STIMULATION IN THE MOUSE SUPRACHIASMATIC NUCLEUS IN VITRO.** **G. M. Cahill and M. Menaker. Institute of Neuroscience, University of Oregon, Eugene, OR 97403**

Photic information for entrainment of circadian rhythms is transmitted to the suprachiasmatic nuclei (SCN) of mammals via the retinohypothalamic tract (RHT). We have developed an in vitro hypothalamic brain slice preparation for electrophysiological measurement of SCN responses to RHT activation.

Single horizontal slices, 600 μm thick, containing the SCN, the optic chiasm and the optic nerves, were cut from the base of the hypothalami of male mice. Slices were submerged in a recording chamber and superfused with oxygenated, HEPES-buffered, artificial CSF maintained at 35° C. One optic nerve was drawn into a suction electrode for stimulation. Extracellular field potentials evoked by supramaximal (20V, 0.15ms) stimulation of the optic nerves were measured with low impedance carbon fiber electrodes and a low pass filtered AC preamplifier. Electrode positions were recorded and spatial maps of the fields evoked by RHT activation were reconstructed from histological sections. Substitution of Mn^{++} for Ca^{++} and high frequency stimulation were used to distinguish between directly stimulated and synaptically activated responses in the field potentials.

The figure illustrates a typical field response recorded from the ventrolateral quadrant of the SCN contralateral to the stimulated nerve. Events A and C are insensitive to lowered [Ca^{++}] and high frequency stimulation. The negative deflection at A reflects the propagated optic tract volley. The small spike potential at C probably results from lower velocity RHT fibers. Events B, D, and E are abolished by low [Ca^{++}] and high frequency stimulation, suggesting that they are postsynaptic in origin. The negativity at B is found throughout the ventral, caudal area of the nucleus. Events D and E have the form of a population EPSP (D) and spike (E). The potentials at D and E are reversed in polarity in the center of the nucleus. This suggests that there is a population of neurons in the ventrolateral SCN that receives excitatory input from the RHT on dendrites in the center of the nucleus. (Supported by PHS Training Grant GM07257 to GMC and NIH AM26972 to MM.)



Evoked Field in Ventrolateral SCN

146.5 FEEDING AND NOCTURNAL ACTIVITY IN MATERNALLY ISOLATED RAT PUPS. V.Anderson and G.K. Smith. Dep't of Psychology,McMaster University, Hamilton,ONT,Canada L8S 4K1.

Previously we demonstrated that rat pups reared without their mother on 12:12 LD from 24 to 39 days postconception(PC,day of conception =day 0) exhibited circadian locomotor activity rhythms which were less stable and of shorter duration than those seen in mother-reared pups(Neurosci.Abstr.,1983, 1075). We attributed these results in part to the absence of rhythmic maternal cues which may serve to synchronize the offspring's rhythms.

In more recent experiments maternally isolated pups were reared on feeding or temperature cycles from 25 to 39 days PC. Two groups were kept on 12:12 LD and received 65.3% of their food during the light period(08.00 to 20.00hrs,ARLD-LF) or dark period (20.00 to 08.00hrs,ARLD-DF). Two other groups were on the same feeding schedules but were kept on constant light (ARLL-LF and ARLL-DF). Results indicate that ARLD-LF and ARLL-LF animals had higher activity during the period when they were fed the least (20.00 to 08.00hrs). This was also observed in the ARLL-DF group (activity high from 08.00 to 20.00hrs) but not the ARLD-DF group. Pups on a feeding and an environmental temperature cycle (warmer during the light period) showed less nocturnal activity than the ARLD-LF group.

Rats have most of their nursing bouts during the light period. The present data indicate that maternally isolated rat pups can synchronize their locomotor activity to a cyclic feeding schedule, particularly when it is congruent with their preisolation nursing experience with the dam. The nursing cycle, therefore, may serve as a synchronizing cue for the offspring's rhythms.

146.6 THE EFFECT OF PROTEIN MALNUTRITION ON THE CIRCADIAN RHYTHM OF WAKING-SLEEP CYCLE IN RATS OF THREE AGE GROUPS. L. Cintra, S. Díaz-Cintra, W. Forbes and P.J. Morgane. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545, and Instituto de Investigaciones Biomédicas, UNAM, Dept. de Fisiología, C.P. 04510, México, D.F.

Using 25% and 8% casein diet in male rats, we studied the circadian rhythm of waking-sleep cycle in the three age groups: 60, 120 and 220 days. Animals were maintained in a schedule of LD:12 12, during four control days with recordings done on days 3 and 4 followed by eight experimental days in continuous darkness DD:24, with recordings obtained on days 5,7,8,11 and 12. We analyzed the activity-inactivity (α/ϱ) ratio, by measuring visualy a complete cycle between days 3 and 4 of LD: 12 12 and betweem days 11 and 12 of DD:24, and found only a significant difference comparing normal and malnourished rats in REM sleep, decreasing this ratio in 8% casein rats during days 11 and 12 at 220 days. Comparing α/ϱ ratio between control days 3 and 4 Vs experimental days 11 and 12, a significant increase was found in REM sleep at 60 days in both 25% and 8% rats, followed at 220 days by a significant increase in normal rats only. Slow wave sleep, presented only a significant decrease in α/ϱ ratio in normal rats at 120 days. Analyzing 1/2 hour blocks, we compared the vigilance states average of days 3 and 4 of normal and malnourished rats, and found only at 120 days a significant decrease in slow wave sleep in 8% casein rats. When we compared the day 12 and 24:DD of normals Vs malnourished animals a significant decrease in slow wave-sleep was found at 60 days, followed by a significant increase in REM sleep in 8% rats at 220 days of age. In order to know the differences between control and experimental days we compared the vigilance states average of control days 3 and 4 Vs experimental day 12 in each age and diet group, and we found that normal animals showed significant differences in all three ages in most sleep stages, while malnourished presented only significant results at 120 days. These results shows that malnutrition affects the circadian rhythm of waking-sleep cycle in the rat, and produces different effects according with animal's age, probably by affecting the structures that control these circadian rhythm. (Supported by NIH Grant HD-06364).

146.7 COUPLING BETWEEN FOOD- AND LIGHT-ENTRAINABLE CIRCADIAN SYSTEMS. F. K. Stephan. Dept. Psychology, Florida State Univ., Tallahassee, FL 32306.

It is now well established that a number of behavioral and physiological responses to periodic feeding are mediated by a circadian system. In the rat, these responses are not abolished by lesions of the suprachiasmatic nuclei which play an important role in the entrainment of circadian rhythms by light-dark cycles. The purpose of the present study was to examine interactions indicative of coupling between these two circadian systems. Specifically, the experiment tested the hypothesis that the probability of synchronization of a free-running circadian rhythm by periodic feeding should increase with increasing similarity of their respective periods.

Sixteen blind rats were maintained in ad lib. conditions for 116 days. During the first 50 days the period (τ) of the free-running activity rhythms lengthened but τ was extremely stable between days 51-116. Fifteen rats were then exposed to restricted feeding (4 h/cycle) for 53 days. The period of food access (T) was adjusted to be longer than (N=6) or shorter than (N=7) τ and the range of differences in periods (T-τ) tested was between 3 and 18 min. Following restricted feeding, all rats were placed in ad lib. for 40 days.

During restricted feeding, considerable changes in τ, displacing the phase of the free-running rhythm by as much as 8 h, were observed in 11 of 15 rats. However, the magnitude and direction of these changes appeared to depend only partially on T-τ. In three of seven rats where T-τ was < 6 min, the free-running activity rhythm was synchronized by periodic food access. Synchronization did not occur in rats with larger differences. Further changes in τ were observed following return to ad lib. conditions, demonstrating an aftereffect of periodic feeding on τ. Only 2 rats exposed to restricted feeding and the ad lib. rat showed no measurable changes in τ throughout the experiment.

These results indicate that similarity of period is not the sole factor determining synchronization. The phase angle difference between the two rhythms also influenced the results but this parameter requires further systematic study. Since periodic feeding induced changes in τ in 87% of the cases, it is clear that some coupling exists between these two circadian systems. On the other hand, the observed interactions suggest that this coupling is weak.

146.8 PERFORMANCE OF RATS IN A RADIAL ARM MAZE VARIES WITH TIME OF DAY. J. R. Leu . Department of Medical Neurosciences, Division of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, D.C. 20307.

Testing in a radial arm maze task has become a common technique for assessing memory in small laboratory animals. The effects of many experimental manipulations on the performance of this task have been reported. In view of the many known circadian differences in behavior and interactions between time of day and various treatments (both in type and degree of effects), it seemed prudent to determine whether time of day affects radial maze performance.

Method: Twenty-nine adult, male Sprague-Dawley rats were used as subjects. Rats were individually housed in a temperature-controlled room having a modified light cycle with onset of a 12 hour dark period at 1100 hours. Food and water were freely available. Animals were trained to obtain "rewards" of semi-sweet chocolate chips (92 mg) from 4 baited arms on an eight-arm radial maze. Each rat was trained and tested with a unique pattern of baited arms that remained constant during all trials. The maze was elevated 76 cm from the floor with an octagonal center area, 33 cm across, and eight radiating arms. Each arm was 10.2 cm wide and 60 cm long with sides 7 cm high. Rewards were placed in shallow (1.0 cm) depressions located approximately 2.5 cm from the distal ends of the arms. Small barriers (1.0 cm) high placed near the reward cups prevented the rat from seeing the chips until very near the end of the arm. The maze was located in one corner of the room where the rats were housed. Dim illumination for nocturnal testing was supplied by a 15 watt bulb which remained on at all times in order to minimize disruptions. Animals were tested at four times during the day: lights out, mid-dark, lights on, mid-light. Rats remained on the maze until all four pellets were eaten or until four minutes had elapsed.

Results: Statistically significant differences were noted between groups tested at mid-dark and mid-light. The pattern of results seems to reflect differences in overall activity with no apparent difference in accuracy. Important differences for mid-light group compared to mid-dark group: a) Total time in maze increased by 26% b) Total number of arm visits decreased by 18% c) Number of different arms visited decreased by 20% d) Number of pellets eaten decreased by 17% e) Time spent in the center of the maze increased by 56% f) Percent of time spent in center increased by 25% g) Percent of animals completing the maze decreased by 47% .

146.9 THE TEMPORAL LIMITS OF THE NONPARAMETRIC MODEL OF ENTRAINMENT IN RATS. C.E. McCormack* and J.S. Ferraro (SPON; P.C. Tang). Dept. of Physiol. Biophys., The Chicago Medical School, N. Chicago, IL 60064.

Previously we have shown that the positive correlation between light intensity and the length of the freerunning period (tau) of the circadian oscillator (i.e. the Aschoff effect) can be produced by 4 h. of light per circadian cycle provided the light is given exclusively during the photosensitive portion of the cycle. This was accomplished through electronic feedback lighting (LD_{FB}) wherein a preset rate of wheel running, if attained by the rat, turned the cage light on for a preset interval. In our present experiment, a running rate of 8 revolutions/2 min turned on the light (100 lux) for 1-2 min (long pulses) or 0.4-7.0 sec (short pulses). The mean taus of the locomotor activity rhythm of 4 female Charles River CD rats exposed sequentially to continuous darkness (DD), continuous light (LL), LD_{FB} long pulse and LD_{FB} short pulse were respectively 24.14h, 24.69h, 24.72h, and 24.36h. Statistical comparisons of these taus with a paired t test revealed that exposure to long pulses of LD_{FB} or to LL produced a similar tau, however exposure to short pulses of LD_{FB} produced a significantly ($P<0.05$) shorter tau than LD_{FB} long pulses or LL. Within the short pulse group, a positive correlation was seen between length of pulse and tau; e.g. 0.4 sec pulse yielded tau of 24.13h, 7.0 sec yielded 24.56h. We conclude that very short pulses and longer pulses do not act as identical zeitgebers, and that the total duration of light per circadian cycle (1-3 min. for the shorter pulses) may be inadequate to elicit the full period-lengthening effects of light. These results also suggest that temporal limits must be placed on the nonparametric model of entrainment (Pittendrigh & Dann, J. Comp. Physiol A 106:291 1976), and that at least in the rat, a phase-response-curve generated with 1 sec. pulses will differ substantially from that generated by the standard 15 or 60 min pulses. (Supported by 1-R01-HD-13131.)

146.10 CIRCADIAN RHYTHM IN VASOPRESSIN RELEASE FROM RAT SUPRACHIASMATIC EXPLANTS IN VITRO. David J. Earnest and Celia D. Sladek. Depts. of Anatomy and Neurology, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

Numerous ablation/isolation studies have established that the suprachiasmatic nucleus (SCN) of the hypothalamus plays a central role in the circadian organization of mammals. Yet, such investigations have provided little insight into the precise physiological mechanisms by which the SCN generates circadian rhythms. An experimental approach utilizing organ culture methods to study oscillations that are intrinsic to the SCN could serve as a valuable model for elucidating the cellular and subcellular basis for the rhythmicity in a mammalian circadian pacemaker. In view of the substantial number of SCN neurons containing vasopressin (VP) and the role of the SCN in the generation of a circadian rhythm in CSF concentrations of this peptide, we sought to develop a system for studying the daily pattern of VP release from the SCN in vitro.

Suprachiasmatic explants were obtained from decapitated male Sprague-Dawley rats (125-150 g) that had been maintained on LD 12:12. The explants included only the paired suprachiasmatic nuclei, their rostral projections to the organum vasculosum of the lamina terminalis and the underlying optic chiasm. These preparations were maintained in individual culture wells and serial samples of the medium were collected at regular intervals (i.e., once every 6 or 12 hr) for two days. VP concentration in the medium was determined by radioimmunoassay.

The total amount of VP released averaged 25 pg/24 hr. Importantly, this daily VP output from the SCN explants appeared to remain constant over the two-day sampling period. However, rhythmic fluctuations in VP concentrations were observed during the course of a day; VP release during the subjective day was significantly greater ($p < 0.025$) than that observed during the subjective night. Although there were differences in the amplitude of rhythm among individual SCN explants (i.e., the increase in VP concentrations during the subjective day ranged from 2- to 16-fold), the daily pattern of VP release was quite uniform. These results suggest that explanted SCN neurons may release VP in a circadian fashion and thus may provide a basis for further examination of the biochemistry and physiology of circadian oscillators.

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146.11 CIRCADIAN SLEEP RHYTHMS IN VASOPRESSIN DEFICIENT BRATTLEBORO RATS. M. H. Brown and A. A. Nunez, Department of Psychology and Neuroscience Program, Michigan State University, East Lansing, Michigan 48824-1117

Previous studies have shown that the suprachiasmatic nucleus of the hypothalamus (SCN) plays an important role in the generation of circadian rhythms. Immunohistochemical investigations have shown that the SCN of the rat can be divided into at least two subnuclei based on the distribution of peptides and putative neurotransmitters within the nucleus (Moore & Card, Neurosci. Abstr., 9:1069, 1983). Neuronal cell bodies in the dorsomedial aspect of the SCN show vasopressin (VP) immunoreactivity and send VP immunoreactive efferent projections to several intra-hypothalamic and extra-hypothalamic sites (Sofroniew & Weindl, Am. J. Anat., 153:391, 1978; Hoorneman & Buijs, Br. Res., 243:235, 1982). Brattleboro rats homozygous for diabetes insipidus (DI) lack VP. DI rats have however been shown to exhibit circadian rhythms of drinking and locomotor activity (Groblewski et al., Br. Res. Bull., 6:125, 1981) as well as pineal serotonin N-acetyltransferase activity (Peterson et al., Behav. and Neural Biol., 29:236, 1980). In the present study, cortical EEG and nuchal muscle EMG recordings were used to determine whether the VP deficiency of DI rats results in deficits in circadian sleep rhythms. Sleep rhythms of DI rats and control animals were monitored under a light/dark cycle and in constant light. DI rats showed circadian rhythms of arousal (A), slow-wave sleep (SWS), and paradoxical sleep (PS) although there was a trend toward lower amplitude of SWS and PS rhythms in DI rats. Preliminary results suggest that peripheral infusion of VP at a constant rate of 0.7 I.U./24 hours reverses the polydipsia characteristic of DI rats but fails to reverse the decreased amplitude of sleep rhythms of these animals.

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146.12 CHOLINOCEPTIVE NEURONS OF THE SCN. A.H. Lauber, J.D. Miller, D.M. Murakami* and C.A. Fuller, Division of Biomedical Sciences, University of California, Riverside, CA 92521.

The suprachiasmatic nucleus (SCN) of the hypothalamus is critically involved in the circadian regulation of a wide variety of behavioral and physiological rhythms. Over 80% of SCN neurons are excited by iontophoretic administration of acetylcholine. Radioactively labelled α bungarotoxin, an irreversible nicotinic ligand, is strongly taken up by SCN neurons. Additionally, photically responsive SCN neurons typically show an increase in firing rate to systemic nicotine. Both the photic and nicotinic responses are greatly attenuated by administration of the nicotinic antagonist, mecamylamine. Finally, intraventricular administration of the cholinergic agonist, carbachol, mimics the zeitgeber effect of light on wheel running and on pineal serotonin N-acetyltransferase; the latter effect can be blocked by peri-SCN injections of α bungarotoxin. Such results imply the importance of cholinergic modulation of neuronal activity in the SCN. To examine such phenomena further, we made stereotaxic intra-SCN injections of horseradish peroxidase (30% HRP in 2% DMSO in .1 M tris buffer) via micropipette in barbiturate anesthetized male Wistar rats. Following a 3-5 day survival time, subjects were sacrificed, perfused with Karnovsky's fixative, and brain tissue was processed for combined AchE-HRP histochemistry. HRP transport was observed in the retina, numerous hypothalamic nuclei, and the lateral septum. Cholinergic nuclei of the basal forebrain (medial septum, diagonal band, nucleus basalis of Meynert) were well labelled for AchE. Lightly AchE staining neurons were observed in the peri-SCN region. Since no choline acetyltransferase labelled SCN neurons have been reported, AchE labelled neurons are presumably cholinoceptive rather than cholinergic. The distribution of the presumed cholinoceptive neurons was compared with the distribution of retino-hypothalamic terminals in the SCN. Thus far, double labelling for HRP and AchE has not been observed in any of the cholinergic nuclei of the basal forebrain; these preliminary data suggest that the cholinergic projection to the SCN does not arise in the prosencephalon. In any event the presumed cholinoceptive neurons may be an anatomical substrate involved in the mediation of previously described photic-cholinergic interaction in the SCN.

146.13 FINE STRUCTURAL ANALYSIS OF THE NEUROPIL OF THE RAT DORSOMEDIAL SUPRACHIASMATIC NUCLEUS. G.C. Newman and R.Y. Moore. Departments of Neurology and Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, New York, 11794.

The rat suprachiasmatic nucleus (SCN) may be divided into dorsomedial (dmSCN) and ventrolateral (vlSCN) components based upon differences in cytoarchitecture, neuronal morphology and the selective termination of retinohypothalamic afferents in vlSCN. There is also a dense secondary visual input restricted to vlSCN that arises from neurons in the lateral geniculate nucleus and exhibits neuropeptide Y-like immunoreactivity (-LI). Immunohistochemical studies have revealed vasopressin-LI and somatostatin-LI in dmSCN neurons, vasoactive intestinal peptide-LI in vlSCN neurons and GAD-LI in neurons of both divisions. All of these neurons project throughout the SCN. Previous ultrastructural investigations have focused on the organization of neuropil in the vlSCN. In the present study we have analyzed the synaptic organization of neuropil in the dmSCN.

Neuropil of dmSCN differs from that of vlSCN in several respects. Large numbers of unmyelinated axon bundles course throughout dmSCN. Compact dendritic bundles and dendro-dendritic synapses, which are prominent in vlSCN, are seen only rarely in dmSCN. The frequency of axo-axonic synapses also is less in dmSCN. Several differences in axo-dendritic synapses are apparent. Synapses with electron-lucent mitochondria, thought to represent retinohypothalamic terminals, are absent from dmSCN. Similarly, terminals with light background and characteristic oval synaptic vesicles are lacking in dmSCN. Conversely, terminals with hexagonally packed round synaptic vesicles occur with greater frequency in dmSCN than in vlSCN. Terminals with many dense core vesicles and axo-somatic synapses occur with equal frequency in both SCN subdivisions. Other ultrastructural features which appear similar in frequency include coated vesicles, multivesicular bodies and multilamellar glial profiles.

Analysis of the dmSCN neuropil provides the opportunity for studying the intrinsic SCN interneuronal relationships independent of visual afferents and contributes to the elucidation of mechanisms by which the SCN entrains to environmental cues and synchronizes neuronal activity in the control of circadian rhythmicity. Comparison of dmSCN and vlSCN neuropil is a useful preliminary to the ultimate correlation of neurotransmitters and neuropeptides with synapse morphology. It may also contribute to our understanding of the topography of dendritic fields within the SCN.

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146.14 FAILURE OF RETROGRADE TRANSPORT INTO SUPRACHIASMATIC NUCLEUS FOLLOWING INTRAVENOUS OR INTRAVENTRICULAR TRACER INJECTION. J.D. Miller, A.H. Lauber, and C.A. Fuller. Division of Biomedical Sciences, University of California, Riverside, CA 92521.

The suprachiasmatic nucleus of the hypothalamus is considered to be the critical locus for timing a wide variety of behavioral and physiological rhythms, including feeding, drinking, wheel running, sleep and pineal N-acetyltransferase. However, in spite of this ubiquitous involvement of the SCN in circadian timekeeping, the efferent projections of the nucleus are rather limited, with major extrahypothalamic projections innervating only the paraventricular thalamic nucleus, lateral septum and central gray. A possible resolution of this paradox would result if SCN neurons communicated with CSF in the adjacent third ventricle or with the bloodstream via the infrahypothalamic capillary bed. The recent observation of insulin (m.w. = 6000) transport into the SCN following intraventricular injection provides some support for this hypothesis. Neurohumoral communication would allow the SCN to affect a wide variety of central and peripheral sites. To investigate these possibilities we made stereotaxic injections of both horseradish peroxidase (m.w. = 40,000, 7 μl @ 30% in 2% DMSO in .1 M tris buffer) and microperoxidase (m.w. = 2000, 7 μl @ 30% in 2% DMSO in .1 M tris buffer) into the lateral ventricles of barbiturate anesthetized male Wistar rats. In addition, intravenous injections of microperoxidase (30% in .5 ml saline) into the femoral vein of similarly anesthetized rats were made. Following a survival time of 12-24 hrs, the subjects were sacrificed and tissue was processed for HRP histochemistry. In all cases sparse labelling of cells in the arcuate nucleus-median eminence area was observed, in agreement with the work of other authors. However, no peroxidase transport was observed in the SCN under any condition. These results appear to seriously constrain the possibility of a neuroendocrine role for the SCN and eliminate a circumventricular organ-like function for it. However, the possibility of active transport into the SCN via specific carrier mechanisms (e.g. insulin carrier) remains open.

146.15 NPY-LIKE IMMUNOREACTIVITY IN THE GENICULO-SUPRACHIASMATIC TRACT. M.E. Harrington, D.M. Nance, B. Rusak. Depts. of Psychology and Anatomy, Dalhousie University, Halifax, Nova Scotia B3H 4J1.

The suprachiasmatic nuclei (SCN) play an important role in the generation of circadian rhythms in mammals. The rat SCN receive a projection from cells in the intergeniculate leaflet (IGL) (Pickard, G.E., J. Comp. Neurol., 211: 65-83, 1982) which show avian pancreatic polypeptide (APP)-like immunoreactivity (Card, P.J., & Moore, R.Y., J. Comp. Neurol., 206: 390-396, 1982). Since recent evidence indicates that APP-like immunoreactivity may be attributed to cross-reactivity with neuropeptide Y (NPY), we have examined the distribution of NPY-like immunoreactivity in the hamster brain.

Male hamster brains were processed for NPY-like immunoreactivity; 40μm sections were agitated overnight at room temperature with NPY antibody (1:3000; courtesy of J.M. Polak). NPY antibody was then developed using the Vectastain kit (Vector Labs). In some cases, colchicine (200μg/20μl) was injected intraventricularly prior to sacrifice. NPY-like immunoreactive fibers were observed throughout the SCN, but were most concentrated ventromedially. Similar to results reported for the rat, bilateral lesions of the IGL and the VLGN ventral to the IGL resulted in a reduction of NPY-like immunoreactivity in the SCN. NPY-like immunoreactive cells were observed in the IGL throughout its rostro-caudal extent. In the most caudal sections, this cell group was observed lying directly ventral to the medial geniculate body, extending into the zona incerta. Rostrally, NPY-like immunoreactive cells were also observed in the external lamina of the anterior portion of the ventral lateral geniculate nucleus (VLGN). In some brains, alternate sections were processed for retinal ganglion cell terminal fields using anterograde tracing techniques (WGA-HRP or concanavalin A). Most NPY-like immunoreactive cells in the anterior VLGN were in an area of termination of the contralateral eye; however, on the side ipsilateral to the eye injection many were outside of labeled areas. In the IGL, all NPY-like immunoreactive cells were observed to lie in areas labeled after monocular injections.

NPY immunoreactive cells projecting to the SCN appear to receive retinal innervation. This is consistent with electrophysiological evidence that some photically responsive VLGN cells project to the SCN (Groos, G.A., & Rusak, B., Neurosci. Abs., 8, 543, 1982).

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146.16 NEUROPEPTIDE Y: A POSSIBLE NEUROTRANSMITTER IN LIGHT-DARK ENTRAINMENT OF CIRCADIAN RHYTHMS H.E. Albers & C.F. Ferris. Worcester Found. for Exp. Biol., Shrewsbury, MA 01545 and Dept. Physiology, Univ. Mass. Med. Ctr., Worcester, MA 01605

The suprachiasmatic nucleus (SCN) appears to function as a circadian pacemaker in mammals. The light-dark (LD) information necessary for entrainment with the 24 hr day-night cycle could be communicated to the SCN by a primary visual projection, the retinohypothalamic tract, and a secondary projection from the ventral lateral geniculate nucleus (vLGN) that appears to contain neuropeptide Y(NPY). To investigate whether NPY could act as a transmitter in the SCN, the effects of NPY microinjected into this area were examined on the free-running circadian activity rhythm of hamsters housed in constant light (LL) or dark (DD). Hamsters were implanted stereotaxically, under Nembutal anesthesia, with chronic guide cannula aimed at the SCN and then allowed to establish a stable free-running rhythm. At 10 day intervals the unanesthetized hamsters were microinjected with NPY (200ng/200nl saline) or 200nl saline (SAL). In LL NPY (N=21) phase shifted the activity rhythm with the direction and magnitude of the phase shift depending upon the time within the circadian cycle of NPY injection. NPY advanced the phase by as much as 4.6 hr when injected during the 12 hr preceding activity onset and tended to phase delay the activity cycle during the 12 hr after activity onset. In DD NPY injected into the SCN region 3-6 hr before activity onset produced phase advances of 1.14±0.21 hr (N=4), as compared to phase advances of 2.06±0.64 hr when NPY had been injected during this same time interval in LL. The phase shifting effects of NPY appeared to be specific to the SCN region since injection of NPY into the lateral ventricle 3-7 hr before activity onset had little effect on circadian phase (-0.09±0.25 hr;N=4). No systematic alterations in circadian phase were produced by injection of SAL(N=21) into the SCN region. In summary, the pattern of phase shifts produced in LL by NPY injected throughout the circadian cycle mimicked those that have been reported by exposing hamsters housed in LL to brief pulses of darkness. However, NPY does not appear to mimic dark pulses by simply inhibiting light information communicated to the SCN by other visual pathways, since NPY is also effective in producing phase shifts in DD. The present data suggest that NPY could function as a neurotransmitter of visual information important for LD cycle entrainment from vLGN to SCN.

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146.17 GENICULATE STIMULATION PHASE SHIFTS HAMSTER CIRCADIAN RHYTHMS. J. H. Meijer*, B. Rusak, M. E. Harrington. Dept. of Psychology, Dalhousie University, Halifax, Nova Scotia B3H 4J1.

The suprachiasmatic nuclei (SCN) function as a dominant pacemaker for the mammalian circadian system. Photic entrainment of this pacemaker can be accomplished via a direct retinal projection to the SCN (retinohypothalamic tract: RHT); however, other projections may also contribute to photic effects on circadian rhythms. In particular, cells in a distinctive region of the lateral geniculate nuclei (the intergeniculate leaflet: IGL), which appear to receive a direct retinal input, also project to the SCN in rats and hamsters (Pickard, G.E., J. Comp. Neurol. 211: 65-83, 1982).

Some or all of these cells stain positively for the peptide NPY and form a heavy NPY-containing projection to the SCN (Card, P.J. & Moore, R.Y., J. Comp. Neurol. 206: 390-396, 1982). A possible entrainment role for this geniculo-suprachiasmatic tract (GST) was suggested by evidence that application of NPY to the SCN of hamsters caused phase-dependent phase shifts of free-running activity rhythms (Albers, H.E. et al., Science 223: 833-835, 1984).

We investigated the possible role of the GST in regulating behavioural rhythmicity by attempting to activate it with stimulating electrodes aimed at the IGL of hamsters. Stimulation trains (0.5 ms bipolar pulses, 150-300 μA, 20 Hz for 5 sec every 10 sec) of 1-4 hr duration were delivered via a commutator and cable to hamsters housed with access to activity wheels in constant dim illumination (~5 lux) or constant darkness. Electrical stimulation produced phase-dependent phase shifts of activity rhythms. The phase-response curve generated was consistent with those generated for NPY application to the SCN and for dark pulses delivered against a light background (Boulos, Z. & Rusak, B., J. Comp. Physiol. 146: 411-417, 1982); advances resulted from stimulation in the late subjective day and delays from stimulation in the late subjective night and early subjective day.

Activation of the GST has effects on the circadian system that are opposite to those of photic stimulation. These effects may be mediated by the stimulation-induced release of NPY in the SCN. The stimuli that normally activate this mechanism and its function in entrainment remain to be determined.

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146.18 ANATOMY OF THE RETINO-HYPOTHALAMIC TRACT IN TWO RELATED PHOTOPERIODIC SPECIES. T. G. Youngstrom and A. A. Nunez, Psychology Dept. and Neuroscience Program, Michigan State Univ., E. Lansing, MI 48824.

A retino-hypothalamic tract (RHT) with projections to the suprachiasmatic nuclei (SCN) has been described in several species. The development of sensitive tract tracing techniques, such as horseradish peroxidase (HRP), combined with tetramethylbenzidine (TMB), has permitted the description of previously unknown or ill defined neural pathways. Investigators using the HRP-TMB method have reported features of the RHT of golden hamsters (Mesocricetus auratus) that differ from those seen in non-photoperiodic rodents (J. Comp. Neurol. 211:65, 1982). Specifically, the SCN of golden hamsters receives nearly equal inputs from each eye. Recently, a similar pattern was reported for the Turkish hamster (Mesocricetus brandti) (Youngstrom et al., Neurosci. Abst. 312:2, 1983). In this study, the anatomical features of the RHT were investigated using two species of mice (Peromyscus maniculatus bairdi and Peromyscus leucopus) that have been shown to be reproductively photoperiodic, but different in their day/night distribution of activity (Baumgardner et al., Anim. Learn. & Behav. 8[2]:322-330, 1980). While there is extensive literature on the photoperiodism of these species there is no information available on the anatomy of the RHT. In order to identify the RHT, injections of 30% HRP within the vitreous of one eye were used. Brains were prepared for histology following a 24 hr. survival period using the method of Mesulam et al. (1980) as modified in this laboratory. Frozen sections (20-50 microns) were reacted with TMB and counterstained with pyronin Y or cresylecht violet. A RHT terminating in the SCN was observed in both species. Also, for both species, there appears to be an asymmetrical pattern of retinal input to the SCN comparable to the pattern observed in the house mouse (Mus musculus). Density of reaction product appears higher in the nucleus contralateral to the side of HRP injection. In both species the concentration of reaction product is highest in dorsolateral, lateral and ventral SCN and tends to be least dense in the dorsomedial region. The present data indicate that symmetry of monocular input to the SCN previously described for two species of hamsters is not a general feature of the photoperiodic rodent. The features of the RHT of the species used in this study were similar to those observed in house mice, a non-photoperiodic species. (Supported by NIMH grant MH37877 to A. A. N.)

146.19 LITHIUM CARBONATE: EFFECTS ON SPLIT ACTIVITY RHYTHMS OF HAMSTERS. J.D. Hallonquist and J.S. Brandes. Dept. of Psychiatry, Mount Sinai Hospital, Toronto, Ontario M5G 1X5, Canada.

Abnormal circadian organization has been reported in patients with major affective illness; e.g., bipolar disorder may be accompanied by a fast circadian oscillator and/or weakened coupling between at least 2 oscillators. Because lithium (Li^+) is effective in treating many bipolar patients, evidence that it slows circadian oscillators and strengthens their normal coupling in animals would suggest that its therapeutic effect reflects similar mechanisms and would support a contributory role for circadian dysfunction in affective illness. Although in many species there is evidence that Li^+ slows circadian oscillators, effects on their coupling are unclear. This study examined effects of Li^+ on abnormal coupling between 2 oscillators that is sometimes observed in hamsters housed in constant light (LL).

Aged 7 mon, 8 male hamsters had been individually housed in cages with activity wheels, in LL of constant intensity (30-100 lux) for 5 mon. All had demonstrated a stable 180° split of the normal free-running activity rhythm for at least 2 wk. Hamsters were then randomly assigned to 2 groups of 4. One group received 4.5g lithium carbonate/kg food ad lib for 5 wk; the other group received the same food ad lib without Li^+ for the same period. Groups' diets were reversed for a 2nd 5 wk period. With addition of a NaCl lick, previous housing conditions were maintained.

Li^+ failed to affect coupling between the 2 oscillators responsible for the split activity components, but did lengthen the period (τ) of both components in each hamster (16/16 components). Comparing the last 3 wk on each diet, Li^+ resulted in a significant ($p<.01$, 2-tailed, Wilcoxon) increase in τ of 0.31h (means = 23.65, 23.96h). Within hamsters, the 2 components were not affected differentially (mean difference in Li^+ effect = 0.07h). Immediate decrease in τ on withdrawal of Li^+ indicated that weight loss (mean = 23.9%) on that diet was not responsible for increased τ.

Slowing by Li^+ of abnormally coupled oscillators in this study is similar to that reported for normally coupled oscillators, suggesting that its therapeutic effect in some bipolar patients reflects the same mechanism. Inconsistent with strengthening of weak coupling in patients is the failure of Li^+ in our hamsters to normalize or even modify coupling, although development of splitting and less stable coupling between more dissimilar oscillators might be affected (Supported by the Ontario Mental Health Foundation).

146.20 ROLE OF THE PARAVENTRICULAR NUCLEUS (PVN) IN CIRCANNUAL ORGANIZATION OF GROUND SQUIRRELS. John Dark and Irving Zucker, Department of Psychology, University of California, Berkeley, CA 94720.

The neuroendocrine substrate underlying endogenous circannual rhythms remains to be specified. Ablation of the ventromedial nucleus affects amplitude of the body weight rhythm but leaves basic circannual organization intact (Mrosovsky, N., Brain Res., 99: 97, 1975); destruction of the suprachiasmatic nuclei (SCN) disrupts the body mass cycle in a small percentage of squirrels but the majority of animals manifest normal circannual organization (Zucker, I. et al, Am.J.Physiol., 244: R472, 1983).

The role of the PVN was investigated because this nucleus is important in neuroendocrine-autonomic integration, they receive efferent projections from the SCN and are involved in regulation of energy balance.

Female squirrels received histologically verified lesions of the PVN (n=8) or were sham-operated (n=6). Body weight and reproductive condition were recorded weekly for 1 cycle pre- and post-surgically.

Lesions of the PVN did not eliminate circannual rhythms of body mass or reproduction. The period length of the body mass but not of the reproductive cycle was longer in PVN than in sham-lesioned animals. Absolute levels of body mass were unaffected by PVN lesions; there were no significant intra- or inter-group differences in peak body mass pre- or post-surgically. These findings are in contrast to the marked obesity observed in rats with PVN lesions (Leibowitz, S.F., Physiol.Behav., 27: 1031, 1981) and in squirrels with VMH lesions (Mrosovsky, N., loc. cit.).

We conclude that the PVN are not an essential component of the oscillatory system that generates circannual cycles in the golden-mantled ground squirrel.

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146.21 RETINAL GANGLION CELL PROJECTIONS AND CYTOCHROME OXIDASE ACTIVITY WITHIN THE HYPOTHALAMUS OF THE RAT AND CAT. C.A. Fuller, D.M. Murakami*, and J.D. Miller (SPON: M. Nachman). Division of Biomedical Sciences, University of California, Riverside, CA 92521

The retinohypothalamic tract (RHT) has been shown to be important for the photic entrainment of circadian rhythms. In order to understand the possible underlying mechanisms for the differences in circadian rhythmicity between rats and cats, the RHT pattern to the suprachiasmatic nucleus (SCN) and other hypothalamic structures was examined with the anterograde transport of intraocularly injected horseradish peroxidase (HRP). In addition, the metabolic capacity of neurons in the SCN and other hypothalamic structures was examined using the cytochrome oxidase technique (CyOX).

Rat & Cat RHT: Both the rat and cat exhibit sparse HRP labelling in the ventral region of the anterior SCN that becomes progressively dense in more posterior sections. Middle sections of the SCN contain heavy label in the ventral region with sparse labelling in the dorso-lateral region in the rat, and in the entire dorsal region in the cat. HRP label becomes sparse within the posterior SCN and is absent from the caudal pole. In the rat there is a relatively dense projection to the anterior hypothalamus and medial preoptic nucleus, with lighter HRP label in the lateral hypothalamus immediately dorsal to the supraoptic nucleus. Sparse HRP label is found dorsal, but is broadly distributed lateral to the SCN. In the cat, sparse HRP label can be seen dorsal and lateral to the SCN, although it is not as extensive as it is in the rat. Posterior to the SCN there is a significant projection that forms a dorso-ventral column.

Rat & Cat CyOX. In the rat and cat there is a band of dark CyOX staining along the hypothalamic-optic chiasm border that is broad in the rat, but very thin in the cat. In the rat, the entire ventral portion of the SCN is darkly stained for CyOX which is coincident with the RHT projection. At the posterior pole where the SCN no longer receives a retinal projection, the SCN no longer stains significantly for CyOX. The ventral lateral SCN in the cat is darkly stained for CyOX only in the middle sections where the retinal projection is more dense. However, much of the dorsal and medial portions of the SCN in cats stain significantly less than background for CyOX, indicating a low metabolic capacity. Dark CyOX cell bodies in the rat and cat SCN can be seen with dendritic processes invading the chiasm. Other hypothalamic structures that receive retinal projections do not stain differently from background. The supraoptic nucleus and paraventricular nucleus of the rat and cat stain significantly less than background.

146.22 RECONSTRUCTION OF SINGLE RETINAL GANGLION CELL AXON TERMINALS THAT PROJECT TO THE HYPOTHALAMUS. D.M. Murakami*, J.D. Miller,* and C.A. Fuller. (SPON: M.A. Baker). Division of Biomedical Sciences, University of California, Riverside, CA 92521.

Previous investigations in this laboratory have examined the retinohypothalamic (RHT) input in the cat. It was shown that the retinohypothalamic input is not confined to the SCN. Instead there is retinal input dorsal, lateral, and posterior to the SCN in the hypothalamus. The retinohypothalamic input to SCN projects from ganglion cells with small to medium size cell bodies and gamma cell dendritic morphology. To further characterize the morphology of the RHT, individual axonal terminals have been examined in this study. Following electrophysiological identification of the optic chiasm and nerve, a Hamilton syringe filled with a 50% HRP solution (Boehringer Grade I HRP and 2% DMSO in 0.1 M tris buffer, pH 8.6) was stereotaxically placed in the nerve or chiasm. 0.5 µl to 1.0 µl of HRP was injected over a 30-minute period. After a 24-hour survival time the cat was sacrificed by barbiturate overdose and transcardially perfused with normal saline, then 1% paraformaldehyde-2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.6. Coronal and saggittal 120 µm sections were reacted with DAB intensified with nickel ammonium sulfate and cobalt chloride. Drawings of individual RHT ganglion cell terminals in the SCN were made at 1250 X.

Of the completed retinal terminal reconstructions observed in each of three cats, two basic types have been observed. One type of axon terminal invades the SCN perpendicularly from the optic chiasm. The terminal arborizes into several branches in the ventral portion of the SCN. The branches exhibit many terminal swellings as they arborize into a sparse, but confined terminal field of approximately 20 µm in diameter. These have been found only within the SCN. Another type of retinal axon enters the SCN and travels dorso-laterally or dorso-medially through the SCN. A few fibers continue dorsal to the SCN. This primary axon contains a few swellings; however, as it courses through the hypothalamus very short branches occur with several terminal endings near the primary axon. Therefore, the terminal boutons of this type of axon can influence a large portion of the hypothalamus. In contrast, the other type of terminal appears more specific and confined to the SCN.

146.23 DIURNAL RHYTHMS OF SLEEP AND BRAIN TEMPERATURE IN THE SQUIRREL MONKEY. Dale M. Edgar and Charles A. Fuller. Division of Biomedical Sciences, University of California, Riverside, CA 92521

It is generally agreed that sleep stages are correlated with circadian variations of brain/body temperature, yet the mechanisms which underly these relationships are unclear. The squirrel monkey (Saimiri sciureus) is particularly well suited for circadian studies. However, continuous recording of sleep and brain temperature from these animals has not yet been reported. Thus, we have developed an animal model to monitor sleep states and brain temperature simultaneously in unrestrained squirrel monkeys. Four animals were chronically implanted with EEG, EMG, EOG recording electrodes, and a deep brain thermistor. Stainless steel screws served as EEG electrodes, which were positioned epidural to the frontal, motor, and occipital cortical regions. EMG activity was obtained from the neck musculature. All leads were soldered to a miniature connector which was affixed to the skull. Each animal was highly trained to accept a lightweight cable attached to the implant connector. A 12 channel gold commutator provided the animal with complete freedom of movement within a temperature controlled and sound attenuated environmental chamber. Light-dark cycles were maintained 12:12, and ambient temperature was regulated at 27°C. Brain temperature was sampled every two minutes by an Apple computer system. Sleep parameters were recorded continuously on an 8 channel tape recorder. Sleep records were scored in 30 second epochs. Stages were scored as wake (W), light slow wave sleep (SWS1), deep slow wave sleep (SWS2), and REM. In these scoring criteria, drowsiness is not considered sleep, and an absence of EMG activity is required for stage REM. Other criteria for sleep stage scoring were patterned on those used for scoring human sleep. Monkeys well adapted to the recording environment show consolidated sleep patterns much like humans. Total sleep time was approximately 77%. SWS1 occupied 60% of the epochs scored, whereas SWS2 and REM occurred during 10% and 7% of the night, respectively. SWS2 was most abundant during the descending portion of the circadian temperature rhythm. REM accumulation was greatest late in the monkeys' sleep. Preliminary analyses show REM episodes of up to 10 minutes in duration which occurred at approximately 1 hour intervals. Notably, in each animal there was an increase in brain temperature of approximately 0.2°C following the initiation of REM. Larger increases in brain temperature were correlated with longer REM episodes. These data show that sleep and temperature patterns in this species are similar to those in other mammals, including humans, yet may differ with respect to some primates (Supported in part by NASA Grant NAGW-309).

146.24 RESTRICTED BUT UNSIGNALED DAILY FOOD AVAILABILITY SYNCHRONIZES ANTICIPATORY BEHAVIOR BUT FAILS TO ENTRAIN OTHER CIRCADIAN RHYTHMS IN THE SQUIRREL MONKEY. D.M. Frim*, Z. Boulos and M.C. Moore-Ede. Department of Physiology and Biophysics, Harvard Medical School, Boston, MA 02115

The ability of restricted daily feeding schedules to entrain circadian rhythms has been found to differ in rats and squirrel monkeys. In rats, such schedules generally fail to entrain free-running behavioral rhythms. However, rats show anticipatory locomotor and lever-pressing activity immediately preceding food delivery. In contrast, feeding schedules have been reported to entrain several circadian rhythms in squirrel monkeys maintained under constant illumination (LL). In these latter experiments food was presented daily to the animals by the experimenter, thus providing additional time cues. In the present study, restricted but unsignaled food availability was used to reassess the synchronizing effects of daily feeding schedules with no other external time cues. Squirrel monkeys were maintained under LL (600 lux) in individual isolation chambers containing a perch attached to a microswitch, a lever and food cup, and a drinkometer. Initially, each lever-press produced a 190mg food pellet, and all animals showed clear free-running rhythms of perch activity, feeding and drinking. Food availability was then restricted to 3h daily (1200-1500h EST), and lever-pressing at all other times was recorded but did not provide food. This schedule was maintained for at least 30 days and was followed by a return to free-feeding conditions. The feeding schedule failed to synchronize the perch-activity rhythms, which continued to free-run with periods longer than 24h. Drinking was almost completely synchronized by the schedule, with maximal intake taking place in the first few hours after feeding onset. Non-reinforced lever-pressing showed both a 24h component which anticipated the time of feeding, and a free-running component similar in phase and period to the activity rhythm. At the termination of the feeding schedule drinking and lever-pressing behavior gradually regained coherence with the free-running activity rhythm. These results are consistent with those of previous restricted feeding studies in the rat and suggest that food anticipatory behavior in both monkeys and rats is mediated by a circadian pacemaker separate from that which times the free-running rhythms.

This research was supported by NIH Grant No. GM 29327-03.

SLEEP

147.1 RELATION OF BLOOD PRESSURE TO REM SLEEP ATONIA AND ATONIA PRODUCED BY STIMULATION OF THE MEDIAL MEDULLA. J.M. Siegel, K.S. Tomaszewski*, W.J.Wilson, R. Nienhuis* and A.R. Morrison. V.A.M.C. Sepulveda, CA 91343; Dept. of Psychiatry School of Medicine, University of California, Los Angeles, CA 90024; University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA 19104

Electrical stimulation of the gigantocellularis, paramedianus and magnocellularis nuclei can produce nonreciprocal postsynaptic inhibition of motoneurons innervating neck and limb muscles. Identical stimulation can at other times produce muscle excitation. We have previously reported that transection level is correlated with the polarity of the stimulation effect, with transections at the midbrain level significantly more likely to produce inhibition than transections at mid or caudal pontine levels (Brain Res., 1983, 268:344-348). Since blood pressure varies with transection location, we hypothesized that changes in the activity of circuits receiving baroreceptor input might contribute to the regulation of muscle atonia. Accordingly, we reduced blood pressure by administration of sodium nitroprusside or acetylcholine, or increased blood pressure by administration of epinephrine, in midbrain decerebrate cats. Blood pressure increases of as much as 45 mm hg did not alter atonia elicited by medullary stimulation. However, blood pressure reductions of a mean of 33 mm hg rapidly reversed the effect of medullary stimulation from inhibition to excitation in 29 of 30 sites in 7 cats. We hypothesized that alterations in the baroreceptive feedback loop might also contribute to the syndrome of REM sleep without atonia. Therefore, after taking baseline blood pressure data we placed lesions in the dorsolateral pons in 3 chronic cats. After the lesions, two of the cats exhibited the syndrome of REM sleep without atonia. Both of these cats had tonically reduced blood pressure levels in waking and in nonREM sleep. During REM sleep, blood pressure did not show the further reduction seen in baseline conditions. One of the cats recovered normal REM sleep atonia by 2 weeks post-lesion. This recovery was correlated with a return towards baseline blood pressure levels. The third cat, which did not develop the REM without atonia syndrome had no blood pressure changes after the lesion.

We conclude that changes in the activity of neuronal systems regulating blood pressure may gate the occurrence of muscle atonia in REM sleep.

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147.2 PONTINE CARBACHOL MICROINFUSIONS PRODUCE REM RELATED DECREASE IN BLOOD PRESSURE. P. Shiromani, J.M. Siegel and D.J. McGinty. V.A. Medical Center, Sepulveda, CA 91343, and Departments of Psychiatry and Psychology, University of California, Los Angeles, 90024.

In anesthetized rats and cats, medullary carbachol infusions or systemic physostigmine infusions decrease blood pressure (BP). Brainstem and systemic cholinomimetic infusions also trigger REM sleep. In cats, there is a tonic decrease in BP throughout the REM episode. The purpose of this study was to examine the relationship of carbachol induced blood pressure changes to REM sleep.

Four cats were chronically implanted with standard sleep recording electrodes and 2-6 24 gauge stainless-steel canulae placed at (A) P=3 to 5, L=2.5 to 1.5, H=-2 to -4, and (B) P=8.0, L=2.0, H=-7. BP was chronically monitored through a catheter in the abdominal aorta. After recovery from surgery, 0.5 ul of Ringers was infused into the brainstem and BP monitored during at least one episode each of non-REM and REM. Subsequently, 0.5 ul of carbachol (8 ug/1 ul) was infused and BP was monitored for at least one hour. A total of 9 infusions were made.

During control infusions the usual decrease in BP occurred during spontaneously occurring REM sleep periods compared to non-REM. Carbachol microinfusion into the pons (cannula location A) evoked REM sleep and decreased BP, but the BP decrement was always linked to REM sleep. In two experiments, medullary (location B) or rostral PRF (P=3, L=2.5, H=-2.0) infusions produced only PGO waves or atonia, respectively: BP did not then show the customary decrease which occurs when all components of REM occur concurrently.

We conclude that in chronic animals, BP decreases elicited by carbachol micro-injections into the pontine region occur in conjunction with REM.

147.3 **INTERLEUKIN-1 (IL-1) INDUCED SLOW WAVE SLEEP (SWS) AND FEVER: SEPARATION OF RESPONSES.** Walter, J., J. Krueger, P. Meyers, and C. Dinarello. University of Health Sciences/The Chicago Medical School, Tufts University School of Medicine

Central actions of IL-1 include induction of fever (1) and promotion of SWS (2) following intracerebroventricular or intravenous injection. The primary site for pyrogenic actions of IL-1 is thought to be the preoptic area of the anterior hypothalamus (3). We investigated, therefore, the effects of microinjections of IL-1 into various diencephalic locations (on sleep and rectal temperatures) Rabbits, provided with chronically implanted guide tubes and EEG electrodes, received injections of 2 ul of solutions containing either purified human IL-1 (4) or heat inactivated IL-1 (control) in random order. EEGs were recorded for the next 6 hr and rectal temperatures were taken 1 hr after injection. Locations of injection sites were confirmed by histological examination of brains. Five rabbits received a total of 12 injections into the anterior hypothalamus and the preoptic area. Injections were approximately 1.5 mm lateral from the midline. All of these animals developed fever (mean 0.6 ± 0.2^{o}C above control). However, SWS was unaffected by these injections; %SWS for the 6 hr post injection period was 42+2% following IL-1 compared to control values of 44+2%. Injections into other diencephalic areas failed to affect either sleep or rectal temperatures. Although it is possible that higher doses at these sites might also affect sleep, present results suggest that the anatomical sites for IL-1 induction of fever and sleep are spacially separate. This reinforces our previous observation that these responses could be separated: an antipyretic blocked IL-1 induced fever but not excess SWS (2). The location of active site(s) responsible for induction of sleep by IL-1 remains unknown.

(1) Atkins, E. and W.B. Wood Jr. J. Exp. Med. 101:519, 1955.
(2) Krueger J.M. et.al. Am. J. Physiol. (1984) in press
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147.4 **PERSISTENCE OF SLEEP-RELATED THERMOREGULATION DURING FEVER AND HYPOTHERMIA.** Krueger, J. and J. Walter* (SPON: S. Ehrenpreis) The Chicago Medical School, North Chicago, IL

The transition, between wakefulness (W) and slow-wave sleep (SWS) is usually accompanied by a regulated decrease in brain temperature (Tb) whereas between SWS and rapid eye movement sleep (REM) increases in Tb are observed (1). Interleukin-1 (IL1) can induce excess SWS (2) as well as fever (3). Although the fever and SWS effects elicited by IL1 were separated (2), Tb changes associated with sleep were not examined. We now report that sleep-related Tb changes persist in febrile and hypothermic animals.

IL1 (2-5ul) or equal volumes of heat inactivated IL1 (cont) was injected into rabbit anterior hypothalamus. EEG and Tb were recorded for the next 6 h. Rabbits were kept at 23 ± 2^{o}. IL1 induced fevers which reached maximums (40.7 ± 0.1^{o} vs 39.3 ± 0.1^{o} cont) within about 1 h and persisted for the 6 h assay period. Tb decreased during the transition between W and SWS (Table). In contrast, Tb increased during the transition between SWS and REM. These changes were not significantly different from corresponding control values.

Tb changes during MSH (5ug,ICV) induced hypothermia were also determined. Hypothermic responses typically reached minimums (37.8 ± 0.5^{o} vs 39.3 ± 0.1^{o} cont) in about 1 h and continued for about 1 more h. Tb decreased during the transition between W and SWS but these decreases were less than those observed in control animals. This dose of MSH also inhibited SWS and REM during the 2 h hypothermic period. Insufficient REM periods were observed to allow analysis of Tb changes during REM.

We conclude that those thermoregulatory mechanisms associated with sleep states remain intack during periods of IL1 induced fever or MSH induced hypothermia.

Tb CHANGES ASSOCIATED WITH W-SWS AND SWS-REM TRANSITIONS

injectant	changes in Tb* W-SWS	SWS-REM
inactivated IL1	-0.05+0.01	+0.08+0.01
IL1	-0.08+0.02	+0.12+0.02
saline (ICV)	-0.11+0.02	+0.24+0.03
MSH (ICV)	-0.03+0.01	-

*Temperatures reported are differences between those determined 2 min before and 2 min after state changes.

(1) Int. Rev. Physiol. 15:147, 1977. (2) Fed. Proc. 42:356, 1983. (3) Rev. Infect. Disease 6:51, 1984.

147.5 MEDIATION OF SLEEP RELATED ACTIVITIES AND POST-EXCITATORY INHIBITION IN THE RAT DORSAL LATERAL GENICULATE NUCLEUS BY THE THALAMIC RETICULAR NUCLEUS. G.A. Marks, A. Stabrowski* and H.P. Roffwarg, Dept. of Psychiatry, Univ. of Texas Health Science Center, Dallas, TX 75235.

The excitability of relay cells in the rat dorsal lateral geniculate nucleus (dLGN) is depressed following a shock to the optic tract (OT). A body of evidence strongly implicates the visually responsive cells of the thalamic reticular nucleus (VTRN) as the responsible mechanism acting through feed-back inhibition of dLGN relay cells.

In addition to the functional significance of this mechanism on the processing of visual information, it has been suggested that the VTRN may be responsible for the alterations in discharge rate and pattern observed in dLGN relay cells over the sleep-wake cycle. If the VTRN controls state related activities in dLGN, then removal of VTRN influences should lead to state specific changes in dLGN activity. We have performed discrete electrolytic lesions of the VTRN producing a disinhibition in the dLGN. We now report the influence of these lesions on dLGN chronic spontaneous activity across the sleep-wake cycle.

Long-Evans hooded rats were surgically prepared for chronic sleep recording. Additionally, the dLGNs and caudal TRNs were bilaterally implanted with 122.5μ nichrome wire. A bipolar stimulating electrode was placed near the OT at the level of the chiasm. Spontaneous activity was monitored from the dLGNs in the form of multiple unit activity (MUA). This same electrode is used to record OT shock elicited field potentials (FP). Samples of sleep recording and FP are obtained on different days pre- and post-lesion.

All the lesions producing a diminution in dLGN post-excitatory inhibition involve destruction of the dorso-caudal TRN. Concomitant with the significant relative increase in amplitude of second OT shock elicited r_1 potentials, the absolute amplitude of all r_1 potentials increased. Further evidence of disinhibition was also realized in the spontaneous rates of MUA in the dLGN with up to a four-fold increase from pre-lesion rates. Rate increases, however, were not uniform across the sleep-wake cycle. The discharge rates in slow wave sleep underwent the greatest increase altering the rate ratios across states. State related discharges were significantly more similar after the lesion than before.

These data support the role of the visually responsive cells of the TRN in mediating both feedback inhibition and state specific firing rates of dLGN relay cells.

147.6 SLEEP-SUPPRESSION AFTER KAINIC ACID-INDUCED LESIONS OF THE BASAL FOREBRAIN IN CATS. R. Szymusiak and D. McGinty. Neurophysiol. Res. (151A3), *V.A. Med. Ctr., Sepulveda, CA 91343.

The basal forebrain (BF) is hypothesized to be the site of a major sleep-promoting mechanism. Electrical stimulation of the BF evokes sleep with cortical slow-waves (nonrapid-eye-movement, or NREM sleep), and electrolytic lesions of this area suppress sleep. Alzheimer's disease is associated with both degeneration of BF cholinergic neurons and sleep loss. Recently, we have located neurons in the ventral BF which have a sleep-selective discharge pattern. These neurons are most active during NREM sleep and drowsiness, and inactive during waking. If these neurons play an active role in sleep onset and maintenance, then sleep should be suppressed following BF cell loss produced by microinjections of neurotoxins.

Four adult cats were chronically implanted for standard sleep recordings. In addition, bilateral guide cannula were aimed at the horizontal limb of the diagonal band of Broca (A 15.5, L 3.0, H -4.5), and the lateral preoptic/substantia innominata area (A 13.5, L 4.5, H -4.5). After a 12-hour baseline sleep recording, kainic acid (2μg in 1μl) was injected at each of 4 sites. Postlesion recordings were made at weekly intervals for one month.

The major effect of neurotoxin-induced lesions was a suppression of NREM sleep. At 2 weeks postlesion, for example, only 17.1 + 3.0% (s.e.) of the recording time was spent in NREM sleep, compared to 41.2 + 3.3% for prelesion sessions. This reduction in NREM sleep was the result of both a decline in the number of NREM bouts (31.0 + 8.6 vs 52.8 + 3.0), and decreases in NREM bout duration (3.84 + 0.8 vs 5.75 + 0.7 min). The amount of time spent in REM sleep at 2 weeks was reduced by 61%. However, the ratio of REM to NREM sleep time was only moderately below prelesion values (.54 + .08 vs .59 + .09). Since adult REM sleep normally follows NREM sleep, the majority of REM sleep loss may be secondary to the decrement in NREM sleep. The time spent in drowsiness, or light NREM sleep, was unchanged.

These results support the hypothesis that neurons intrinsic to the BF play an active role in sleep onset and maintenance. Recently, neurotoxin lesions of the BF in rats have been reported to impair memory. Given that normal memory is disrupted by sleep deprivation, some memory disturbances associated with BF cell loss may be a secondary consequence of sleep suppression.

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147.7 REM SLEEP DEPRIVATION ALTERS DOPAMINE RECEPTORS IN LIMBIC STRUCTURES IN RATS. R.S. Miletich and M. Radulovacki. (SPON:G. Livezey) Dept. Pharmacology, University of Illinois College of Medicine, Chicago, IL 60612

REM sleep deprivation (REMD) has been shown to alter responsiveness to dopamine (DA) agonists in behavioral assays (Serra, G. et al. Eur. J. Pharm. 72: 131, 1981; Tufik, S. J. Pharm. Pharmacol. 33: 732, 1981) and to increase DOPAC and HVA levels of whole rat brains 6 hours following REMD termination (Wojcik, W. and Radulovacki, M., Physiol. Behav. 27: 305, 1981). However, REMD did not alter DA receptor binding characteristics in c. striatum and frontal cortex in rats (Farber, J. et al. Pharmac. Biochem. Behav. 18: 509, 1983). Since changes in DA metabolism and receptors in mesolimbic systems have been implicated in affective disorders which also affect sleep in their acute phases, we measured the effect of REMD on DA receptors in two principal DA terminals areas, i.e. in limbic structures and c. striatum in rats.

Adult male Sprague-Dawley rats (300 g) were REMD for 24 h by the flower pot technique (Mendelson, E.B. et al., Pharmac. Biochem. Behav. 2: 543, 1974). Four hours after REMD termination animals were killed by decapitation and striatum and limbic structures (n. accumbens, olfactory tubercle, amygdala, septum, midline limbic structures) were dissected on ice. 1-700 pM of ^{3}H spiperone was incubated at 37°C for 30 min with tissue homogenate and a 100 µM DA was used to define specific binding. Affinity constant (Kd) and maximal binding capacity (Bmax) were derived with a weighted nonlinear, least squares curve fitting program from 45 points (Feldman, H.A., Anal. Biochem. 48: 317, 1972).

	Control		REMD		
	Limbic Str.	Striatum	Limbic Str. Site 1	Site 2	Striatum
Kd (nM)	10.2 ± 2.6	11.5 ± 3.1	58.9 ± 45.6	.9 ± 9	11.7 ± 1.7
Bmax (fmol/mg)	159.5 ± 32.7	480.3 ± 109.2	37.8 ± 25.7	652.6 ± 444	312.3 ± 359

The table shows that of the areas examined, only limbic structures of REMD animals had a significantly better fit to a two-site model than a one-site model (p 0.05). Of the remaining areas, there was no significant difference in Kd, but in control animals Bmax of limbic structures was significantly less than that of striatum (p 0.01). Thus, these results show that REMD changes the binding characteristics of DA receptors in limbic structures. In agreement with Farber et al. (Pharmac. Biochem. Behav. 18: 509, 1983), REMD did not affect striatal DA receptors. We suggest that obtained data may be related to the altered responsiveness to DA agonists and the altered DA turnover reported in REMD rats. (Supported by ONR Contract N00014-79-C0420)

147.8 DISCHARGE PROPERTIES OF FOREBRAIN AND MIDBRAIN NEURONS DURING SLEEP-WAKING STATES AND TONIC IMMOBILITY IN THE RABBIT. R.M. Harper and W. Heron. Dept. of Anatomy and the Brain Research Institute, UCLA, Los Angeles, and Dept. of Psychology, McMaster University, Hamilton, Ontario.

Tonic immobility is a state of unreactivity to external stimuli induced in certain species by inversion or sudden exposure to massive sensory stimulation. The immobility and lack of responsivity characteristic of the state bear some resemblance to aspects of sleep states. The objective of these studies was to examine discharge properties of single neurons in forebrain and midbrain areas during sleep and waking states and during tonic immobility.

Under Nembutal anesthesia, bundles of 10-13 fine wire microelectrodes were stereotaxically placed into motor cortex, thalamic, hippocampal, septal and tegmental reticular sites of 21 New Zealand white rabbits, together with electrodes to record nuchal EMG, eye movement, and frontal cortical EEG. After one week recovery, recordings of neural discharge properties, together with physiological parameters defining sleep state were obtained from freely moving animals during alert states, quiet and active (REM) sleep states and following induction of tonic immobility by inversion. Assessment of discharge rate and pattern were determined with a special purpose computer, together with polygraphic displays of spike discharge. Discharge properties during tonic immobility, as assessed by rate and interval histograms, were most similar to patterns observed during alert, motionless conditions rather than to quiet or REM sleep states. A minority of cells in the motor cortex and septum exhibited unique discharge patterns during the tonic immobility state. We conclude that the tonic immobility state represents a waking state in which specific neural mechanisms underlying movement control are inactivated.

147.9 SEDATIVE EFFECTS OF CLONIDINE ARE ANTAGONIZED BY CLONIDINE. B. Delbarre and G. Delbarre. Lab. Chir. Exp., Faculté de Médecine, 37032 TOURS, FRANCE.

Before the adoption of the alpha adrenoceptors into alpha 1 and alpha 2 subtypes, Delbarre et al. (C.R. Soc. Biol., 163 : 1922, 1969, Eur. J. Pharm., 13 : 356, 1971, Eur. J. Pharm., 22 : 355, 1973) reported that the sleep produced by clonidine and other alpha adrenoceptor agonists in young chicks was blocked by antagonists such as yohimbine, piperoxane and phentolamine but not by thymoxamine and phenoxybenzamine.

In humans, repeated doses of clonidine do not induce sleep. To attempt to explain this mechanism, we have used small doses of clonidine (to antagonize sedative effects of clonidine).

In two day old chicks, clonidine (0,250 mg/kg to 0,62 mg/kg I.M.) 30 minute before clonidine (0,750 mg/kg I.M.) antagonizes significantly duration of sleep.

These results suggest that mechanism of sedative effects of clonidine are to be reconsidered.

147.10 EFFECTS OF 1-METHYLISOGUANOSINE ON SLEEP IN RATS. M. Radulovacki, D. Rapoza*, R.M. Virus and R. Crane*. Depts. Pharmacology and Psychiatry, University of Illinois College of Medicine, Chicago, IL 60612

1-Methylisoguanosine (1-MIG) is a marine natural product with skeletal muscle relaxant, hypothermic and cardiovascular effects similar to those of adenosine (1,2). Since adenosine and the related compounds also affect sleep (3) we investigated effects of 1-MIG on sleep in rats. Sprague-Dawley rats implanted with EEG and EMG electrodes were polygraphically recorded for 6 h following intracerebroventricular administration of 1, 10 or 100 nmoles of 1-MIG, respectively. Records were analyzed as waking (W), light sleep (S_1), deep slow-wave sleep (S_2), REM and total sleep (TS).

Sleep State	Hrs	Saline	1-MIG Dose (nmol) 1	10	100
W	0-3	41.0 ± 7.3	30.5 ± 8.1	47.0 ± 5.2	56.6 ± 10.3
	3-6	22.8 ± 3.0	19.0 ± 2.8*	37.7 ± 5.8	32.3 ± 8.5
	0-6	63.8 ± 8.1	49.5 ± 8.4	84.7 ± 7.2	88.8 ± 14.3
S_1	0-3	37.2 ± 4.3	42.2 ± 4.6	33.7 ± 2.9	44.5 ± 3.3
	3-6	47.2 ± 4.9	43.5 ± 5.6	54.8 ± 7.1	41.5 ± 6.7
	0-6	84.4 ± 8.1	85.7 ± 9.6	88.5 ± 6.3	86.0 ± 7.7
S_2	0-3	87.8 ± 6.1	95.2 ± 7.5	91.0 ± 3.6	77.0 ± 13.0
	3-6	91.7 ± 3.2	102.3 ± 6.1	76.3 ± 9.4	95.3 ± 12.7
	0-6	179.5 ± 8.8	197.5 ± 11.6	167.3 ± 12.5	172.3 ± 18.7
REM	0-3	13.2 ± 2.5	12.2 ± 1.4	8.3 ± 2.7	2.0 ± 1.1*
	3-6	19.0 ± 3.1	15.2 ± 1.9	9.3 ± 2.7*	10.8 ± 2.5*
	0-6	32.2 ± 3.4	27.4 ± 1.4	17.6 ± 4.6*	12.8 ± 3.2*
TS	0-3	139.0 ± 7.3	149.5 ± 8.1	133.0 ± 5.2	123.5 ± 10.3
	3-6	157.2 ± 3.0	161.0 ± 2.8*	142.3 ± 5.8	147.7 ± 8.5
	0-6	296.2 ± 8.1	310.5 ± 8.4	275.3 ± 7.2	271.2 ± 14.3

All values reported are means ± S.E. in minutes. There were 6 rats per group. Significantly different from saline group: *P<0.050

The results show that 1 nmol of 1-MIG decreased W by 17% and increased TS by 2.5% during 3-6 h interval in comparison to control whereas doses of 10 and 100 nmoles suppressed REM. These effects follow the general pattern of adenosine's effect of sleep but with less pronounced hypnotic action. (Supported by ONR Contract N000 14-79-C-0420)

References: 1) A.F. Cook et al. J. Org. Chem. 45: 4020, 1980; 2)J. Baird- Lambert et al. Life Sci 26: 1069, 1980.;3) M. Radulovacki, et al. JPET 228: 268, 1984.

147.11 EFFECTS OF SLEEP STATES UPON REGIONAL CEREBRAL GLUCOSE UTILIZATION IN THE CAT BRAIN. P. Ramm* and B.J. Frost (Spon. D. Wahlsten). Dept. of Psychology, Queen's University, Kingston, Ont. K7L 3N6.

14C-2-deoxyglucose (2-DG) autoradiography was used to explore the effects of sleep upon regional glucose metabolism in the cat brain. Twenty cats received chronic arterial and venous cannulas and supradural and muscle electrodes. Three days later, they were placed in chambers which permitted free movement and remote access to the cannulas and electrodes. White noise was present. During sleep or wakefulness, 100 uCi/kg of 2-DG was injected via the venous cannula. During the subsequent 45 min, polygraphic recordings were made and timed samples were taken from the arterial cannula. Autoradiographs were prepared and analyzed by computerized densitometry.

From the individual plasma 14C curves and polygraphic records, the amount of 2-DG converted to 2-DG-6-phosphate during each state (wake, SWS, REM) was calculated. This state value was correlated with glucose metabolism in each of 200 brain gray matter regions, and with the mean glucose utilization value for all sampled brain regions (MGU). Relative metabolic activity (RMA) was then calculated by dividing each regional metabolism value in an animal by the MGU value for that animal. Levels of RMA show functional activity in a region, relative to the brain mean and independent of any whole-brain changes in MGU. High RMA values suggest functions carried at relatively high levels during sleep. Low levels of RMA often reflect functional deafferentation or decreased motor function.

MGU decreased during SWS ($r = -0.68$, $p < 0.01$), but was uncorrelated with REM. Cerebellar RMA was decreased during REM ($r = -0.50$, $p < 0.02$) but not during SWS. REM-specific effects were also seen in the lateral pontine gray ($r = 0.45$ $p > 0.05$), in the principal sensory trigeminal nucleus ($r = 0.45$) and in the motor trigeminal nucleus ($r = 0.50$), and in the hippocampal dentate gyrus ($r = 0.53$) and dentate granule cell region ($r = 0.62$, $p < 0.01$). The substantia nigra pars compacta showed a correlation of 0.52 with REM.

Visual regions exhibited negative correlations specific to SWS (sup. colliculus $r = -0.71$, pulvinar $r = -0.56$, lat. geniculate $r = -0.60$, visual cortex $r -0.58$), as did somatosensory cortex ($r = -0.69$). Lower auditory regions often exhibited enhanced RMA during SWS, probably because the animal could not shut out the constant white noise (cochlear nucleus $r = 0.58$, superior olive $r = 0.47$).

The functional status of the sleeping cat appears similar to that of the rat (Ramm and Frost, Sleep 6; 1983). Most obviously, visual function decreases during SWS but exhibits partial return during REM, perhaps as a result of endogenously generated stimulation. Similar effects are evident in MGU and in many non-visual regions which exhibit decreased RMA during SWS but not during REM (an exception - cerebellar REM decrease possibly associated with atonia). Functional activity in the sleeping brain reflects the fundamental differences between SWS and REM and the active nature of the REM state.

147.12 MODELS OF EXOGENOUS (HYPNOFRAGMENTARY) AND ENDOGENOUS (HYPNODYSRYTHMIC) SLEEP PERTURBATION AS BIOLOGICAL MARKERS OF DEPRESSION. N. Ilankovic* (SPON: I. Bodis-Wollner). Department of Clinical Neurophysiology, Sleep Center, Psychiatric Clinic of the Clinical Center of the Medical Faculty University, Belgrade YU-11000, Pasterova 2, Yugoslavia.

Regular noctural sleep patterns were recorded in a group of 20 normal subjects and in two groups of 20 depressed patients, the exogenously and the endogenously depressed. We statistically analyzed 130 sleep variables and developed a discriminative profile of sleep (DPS) to use in classification of new patients. Two mathematical models were also developed to differentiate the sleep patterns of the two groups of patients. The model of EXOGENOUS SLEEP PERTURBATION was characterized by an increased number of awakenings, a decrease in nonREM (NREM) time, an increase in the first period of night (CYCLE 1) and a reduced REM/NREM ratio. The dominant factor was the increased number of noctural awakenings, which expressed regression to a polyphasic infantile sleep pattern. The ENDOGENOUS SLEEP PERTURBATION model was characterized by shorter REM latency, reduction of delta sleep, increase of the first REM phase, and a significant increase in the Index of Endogenous Perturbation (I.E.P.--Ilankovic, N., 1983). Based on the structural analysis of natural (drug-free) sleep, it is suggested that the classification of the depressive disturbances be supplemented to include "hypnofragmentary" as the sleep pattern of the exogenous or reactive depression and "hypnodrysrhythmic" as the pattern of the endogenous depression.

149 SYMPOSIUM. PRINCIPLES AND MECHANISMS OF NEURONAL MIGRATION. P. Rakic, Yale University School of Medicine, New Haven (Chairman); G.M. Edelman, Rockefeller University, New York; M.E. Hatten, New York University Medical Center; J.-P. Thiery*, Institut D'Embryologie du CNRS, France.

Cell migration plays a key role in determining the final position of each neuron in the vertebrate brain and therefore influences critically the pattern of its synaptic connectivity. The cellular and molecular mechanisms responsible for displacement of neurons from the place of their origin in the proliferative zones to permanent, frequently distant, destinations are thus central to understanding development of both the structure and function of the nervous system. With few exceptions, neuronal migration in the central nervous system is initiated after the last cell division, before development of axons, dendrites, and establishment of synaptic connectivity. The movement of neurons is a directed, active process that differs in several important respects from the morphogenetic cell movements in other organs. This symposium will focus on mechanisms of normal as well as experimentally and genetically altered migration, regulation of migratory rates and determinants of patterns of movement in selected brain regions both in vivo and in vitro. Since available evidence indicates that neuronal migration involves surface mediated communication between neighboring cells and their membrane coats, most of the presentations will deal with cell-cell interaction and related topics. Rakic will summarize recent progress and changing concepts relating to neuronal migration including new findings about environmental and genetic factors that interfere with the pattern or rate of cell displacement. Edelman will discuss the possible role of three relevant cell surface adhesion molecules (N-CAM, Ng-CAM and L-CAM) that are involved in differential cell binding and may have direct implications to specificity of neuronal movement and their settling patterns. Hatten will describe migratory behavior of cerebellar granule cells in wild type and genetic mutant mice in vitro. In addition, she will show a short but dramatic movie displaying neuronal migration along glial cords in time-lapse cinematography. Thiery will describe cell movement in the peripheral nervous system and will discuss possible molecular mechanisms of neuronal crest migration. The individual presentations will be followed by a round table discussion in which new technical approaches and new concepts will be suggested and various alternatives discussed.

150 SYMPOSIUM. COMPARATIVE NEURAL MECHANISMS OF SOUND LOCALIZATION IN VERTEBRATES. G.D. Pollak, Univ. of Texas (Co-Chairperson); T.C.T. Yin, Univ. of wisconsin (Co-Chairperson); J.A. Bastian, Univ. of Oklahoma; A. Moiseff, Univ. of Connecticut.

In this symposium we consider the neural mechanisms by which the location of a sound source is encoded and represented in the vertebrate auditory system. We will focus upon the commonality of binaural mechanisms used by vertebrates, how these mechanisms operate upon interaural disparities, and the different strategies animals have adopted for the topological representation of these features in higher acoustic centers. Joseph Bastian will describe how phase and amplitude disparities are utilized by some species of weakly electric fishes for jamming avoidance responses. These animals evaluate relative disparities in amplitude and phase of two sine waves, their own electric organ discharge (EOD) and the EOD of a nearby fish, to determine whether the frequency of their own EOD is higher or lower than that of the other fish, a process strikingly similar to the interaural comparisons required of the auditory system for sound localization. Andrew Moiseff will then discuss the space map in the owl's inferior colliculus. He will describe the parallel pathways that convey phase and intensity information and how the space mapped neurons are created by particular combinations of interaural phase comparisons at specified relative intensities. George Pollak will describe the representation of sound location in the inferior colliculus of the mustache bat. Particular attention will be given to showing how directional properties of the pinna transform the spatial location of a sound into a unique ratio of interaural intensity differences at the cochlea for the frequencies of the echolocation calls. This basic transformation of frequency into relative amounts of activity along the surfaces of the two cochleae will then be evaluated within the bat's inferior colliculus. Tom Yin will be the fourth speaker, and will consider the neural mechanisms of binaural interaction in the brainstem auditory nuclei of the cat. Emphasis will be placed on studies of the responses of low frequency neurons to interaural time disparities. The evidence that the neural mechanism involves a sensitivity to interaural phase disparities by a "coincidence" model will be reviewed. Finally, the results obtained from the cat will be compared with the type of processing Bastian finds in electric fishes, Moiseff finds in barn owls and Pollak finds in bats.

151.1 PRESYNAPTIC INHIBITION DURING CRAYFISH ESCAPE BEHAVIOR: IDENTIFIED INTERNEURONS PRODUCE BOTH PAD AND PRESYNAPTIC INHIBITION. M.D. Kirk and J.J. Wine. Dept. of Pyschology, Stanford Univ., Stanford, CA 94305.

In the central nervous system of both invertebrates and vertebrates, presynaptic inhibition of primary afferent release is correlated with primary afferent depolarization (PAD). We have studied the polysynaptic pathway by which the giant, escape command axons of the crayfish inhibit transmitter release from primary mechanosensory afferents. The synapses of these afferents are depression-prone and are responsible for behavioral habituation of the escape tailflip. The pathway producing presynaptic inhibition is of special interest because it protects the afferent synapses from the depression that would result as a consequence of reafference during the tailflip. We previously reported that identified crayfish interneurons (PADIs) produce PAD (Kirk, M.D. and Wine, J.J., Neurosci. Abstr., 9:1086, 1983). The PADIs are fired by a polysynaptic pathway triggered by the giant, escape command axons. When directly stimulated, PADIs produce constant, short-latency (<1 msec), chloride-dependent unitary PAD.

In the present study, we found that directly elicited PADI impulses produced both PAD and presynaptic inhibition of primary afferent input to identified sensory interneurons. Primary afferents were stimulated by shocking a sensory root with a stimulus strength just sufficient to consistently produce a single impulse in the postsynaptic interneuron. When the afferent volley was preceded by directly elicited spikes in the PADI, the EPSP in the interneuron was reduced in amplitude to a level subthreshold for impulse production. The reduction in EPSP amplitude was maximal when the peak of the unitary PADs coincided with the afferent root shock. Therefore, the PADIs appear to be directly responsible for presynaptic inhibition of primary afferent synapses during crayfish escape behavior. In addition, the PADIs produce short-latency IPSPs in at least one identified sensory interneuron and in the lateral giant escape command cell.

We conclude that, in this system, presynaptic inhibition is effected by a population of inhibitory interneurons, each of which synapses on a great many afferent terminals. Preliminary evidence suggests that the PADIs release GABA and cause PAD by increasing the chloride conductance of the afferent terminals.

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151.2 SENSITIZATION OF CRAYFISH LATERAL GIANT ESCAPE REACTION. F. B. Krasne and D. L. Glanzman. Department of Psychology and Brain Research Institute, UCLA, L.A., CA 90024.

Most behavioral reactions that habituate can also be dishabituated; ie., a strong stimulus can cause a transient restoration of the habituated response. In the best studied cases, such as Aplysia gill withdrawal and cat spinal flexion reflex, dishabituation seems to be due to an independent "sensitization" of the behavioral reaction that compensates for habituation without necessarily abolishing it. Crayfish lateral giant neuron-mediated escape reactions are one of the most fully analyzed behavioral reactions that are prone to habituation; however, senitization/dishabituation of LG escape has never been reported.

The experiments reported here, which were done on free animals with chronically implanted stimulating and recording electrodes, examined the effect of strong 1-10 sec. AC shocks ("sensitizing stimuli") to various parts of the body on ability of a 0.1 msec. pulse to sensory roots 2-4 of the last abdominal ganglion (test shocks) to elecit an LG escape response. Following single AC shocks, test shock threshold for eleciting LG escape reliably fell 5-80% and recovered over 15 min. to 1 hour. When AC shocks and test shocks alternated at 90 sec. intervals, test shock threshold rapidly dropped to an asymptote that was maintained as long as AC shocks were given (up to two hours); following such repeated AC shocks recovery often required a number of hours but was complete within 24. Comparable sensitization is seen in the response of interneuron A, the largest of a set of sensory interneurons that links afferents to LGs. AC shocks (to either head or tail) no longer sensitize abdominal LG reflex circuitry if the nerve cord is severed between thorax and abdomen. Thus, sensitization appears to depend on a neurally conducted influence that arises in the rostral half of the animal.

Experiments are in progress to examine the effects of close pairing of test stimuli and sensitizing stimuli--ie. to look for classical conditioning in this preparation. (Supported by NIH grant NS 08108)

151.3 SEXUAL BEHAVIOUR OF THE PRAYING MANTIS REEXAMINED: NO ONE LOSES HIS HEAD. E. LISKE[1]) and W.J. DAVIS. The Thimann Laboratories, University of California, Santa Cruz, CA 95064.

It is widely believed that female mantises decapitate and eat the male as part of the courtship ritual. Of 39 successful matings of the praying mantis, Tenodera aridifolia sinensis, that were videotaped and subsequently analysed, however, decapitation of the male never preceeded copulation. Mating behaviour instead entails an elaborate courtship display by intact males, which is answered by a characteristic female posture that may signal her sexual receptivity (Liske, E. and Davis, W.J., Anim. Behav., in press, 1984). During a typical courtship a male shows at least nine recognizable behavioural components, including visual fixation, antennae oscillation in the sagittal plane, an extremly slow approach toward the female, a display of repetitive flexion of the entire abdomen, a "flying leap", mounting, antennal lashing upon the female, "s-bendings" of the terminal abdominal segments and intromission. The female answers with a rapid forward extension of the raptorial frontlegs and subsequent flattening of the body against the substrate.

We believe that our discovery of the previously unreported courtship ritual, involving male and female praying mantises, is a persuasive alternative to the "sexual cannibalism"-hypothesis, namely the existence of behavioural mechanisms designed to inhibit aggression between the courting animals so that they may mate without destroying each other in this process.

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151.4 OPTICAL MONITORING OF ACTIVITY FROM BUCCAL GANGLIA DURING PHARYNGEAL EXPANSION (FEEDING) IN A MINIMALLY DISSECTED NAVANAX. J.A. London, D. Zecevic, and L.B. Cohen. Dept. of Physiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

We are investigating the use of optical methods to measure simultaneous action potential activity from many cells in the nervous system of a minimally dissected opisthobranch mollusc, Navanax inermis. A modified whole animal preparation was employed which allowed the animal's nervous system to be optically monitored during feeding. A 1 cm slit was made in the ventral body wall and in the pharynx immediately under the buccal ganglion. One end of a 2.5 cm long clad quartz rod, used as a light pipe, was pushed through a small hole in the dorsal side of the pharynx and dorsal body wall and positioned on the stage of a microscope so that it transmitted light from the microscope condenser. The ventral musculature of the pharynx was pinned to a platform at the end of the rod in such a way that one of the buccal hemi-ganglia was positioned over the end of the rod. An enlarged image of the stained ganglion was formed on a 124 element photodiode array. While recording, in order to reduce movement of the ganglion during the vigorous pharyngeal movements, the stage and the ganglion were covered with a 1 % agar solution. This reduced the amount of movement artifact, but did not significantly reduce signal size. The ganglia were stained with a 0.5 mg/ml solution of the symmetrical pyrazo-oxonol dye RH155b, kindly provided by Dr. A. Grinvald. This dye produced the best results of 21 oxonol and merocyanine-rhodanine dyes we tested on Navanax ganglia. Action potentials with large signal to noise ratios were obtained while no significant photo-dynamic damage was observed. Both before and after staining most animals (N = 6, 7 preparations) made spontaneous pharyngeal expansions that were similar to the expansions seen during feeding. An increase in spontaneous pharyngeal expansion rate occurred when a food stimulus was placed on the animal's chemosensory apparatus. Analysis of 20 second recordings of the diode outputs indicated that spike activity in up to 8 buccal neurons was detected during expansions. Several different neurons increased their activity during specific phases of the expansion cycle. Further identification of neurons involved in the generation of feeding and their possible roles in learning will be discussed. Supported by NIH grant NS08437 to L.B.C. and NS-0716901 to J.A.L.

151.5 RAPID FOOD-AVERSION LEARNING WITH SHOCK AS UCS IN LIMAX MAXIMUS. K. Delaney* and A. Gelperin (SPON: D. W. Tank), Dept. Biology, Princeton Univ., Princeton, NJ 08544 and Dept. Molecular Biophysics, AT&T Bell Laboratories, Murray Hill, NJ 07974

We have found that electric shock is an effective unconditioned stimulus (UCS) for conditioning food aversion in the slug. We have used electric shock to train both intact slugs and an in vitro lip-CNS preparation.

Whole animal training was carried out by applying 2-3 ml of carrot juice or potato extract onto the lips so that the anterior end of the slug was in the fluid. Before training, when juice was applied to the lips, forward locomotion ceased, the head remained in the food juice and movements of the buccal mass, interpreted as feeding, were seen. A slug will reject a food extract after 3-5 pairings of 15s extract followed immediately by 5s of shock, 15V DC, < 55 mA delivered through the food extract by electrodes spanning the head. The intertrial interval was 15 min. Rejection consists of forward locomotion through the food or turning away from the food or lifting of the head and anterior end of the foot out of the food extract. Rejection was pairing specific.

Before in vitro training of a lip-CNS preparation, baseline responsiveness was established by applying the CS food extract to both lips for 30s followed 30 min later by a 30s application of a second food extract, S2. Training consisted of 1 min of CS and 1 min of shock with 30s overlap. 30 msec pulses of 30-40V at 5Hz were delivered through electrodes placed on the inside surface of the lip. A pairing specific suppression of a neural correlate of feeding, feeding motor program (FMP), detected by extracellular monitoring of buccal roots, was observed in 4 of 5 preps following 1-3 pairings. Suppression was defined as responses < 30% of baseline. One preparation showed no suppression to either CS or S2.

Pedal nerves innervating the anteriormost part of the foot can be monitored during training of in vitro preps. These pedal nerves respond to tactile stimulation of the foot and shock to the lip and en passant stimulation will elicit contraction of the foot in this region. They are being examined as a possible neural correlate of withdrawal.

These results suggest that neural correlates of both feeding and withdrawal can be studied during reliable in vitro training using the same chemosensory pathway as an in vivo paradigm. (Supported by NSERC Scholarship to KD and NIH Grant MH 39160 to AG.)

151.6 UNITARY DRIVES OR GENERAL AROUSAL? MODELS AND PHYSIOLOGICAL EVIDENCE IN APLYSIA. J.L. Leonard*, E. Colebrook* and Ken Lukowiak* (SPON: T. Audesirk). Dept. of Medical Physiology, University of Calgary, Calgary, Alberta T2N 4N1 CANADA.

Ethological models of motivation are based on the "drive" concept. That is sets of action patterns which serve a common function are hypothesized to share a specific internal variable. In contrast, some psychological models of motivation invoke a single central "arousal" variable with non-specific effects on activity and responsiveness to stimuli.

Five drives (feeding, escape, sex as male, sex as female, and egg-laying) have been hypothesized for A. californica (Leonard & Lukowiak 1985). While some behaviors are superimposable, others are mutually exclusive. For example, escape behavior and female sexual behavior are mutually exclusive since escape involves closure of the parapodia and withdrawal of the gill, siphon and mantle in response to tactile stimuli, while female sexual behavior involves opening the parapodia (Draw Down) and raising the mantle, to allow intromission (Leonard and Lukowiak 1985). The gill withdrawal response (GWR) is suppressed in in vitro preparations taken from copulating animals (Lukowiak & Freedman 1985). It is similarly suppressed in preparations taken from food satiated animals (Lukowiak 1980). Suppression can be induced in control preparations by superfusing either met-enkephalin (Lukowiak et al. 1982) or arginine vasotocin (AVT) (Thornhill et al. 1981) over the abdominal ganglion.

A general arousal model would predict that food- and sex-induced suppression share a common mechanism while a unitary drive model would predict that the mechanism of suppression is distinct in each case. To test these hypotheses we superfused 1 uM naloxone over the abdominal ganglion of suppressed preparations taken from both copulating and satiated animals. Naloxone had no effect on the GWR in most (6/9) satiated preparations. It increased the amplitude of the GWR in most (4/5) female preparations and had no effect on most (3/4) male preparations. These results are most compatible with the drive model and suggest that met-enkephalin may be involved in female sexual behavior in Aplysia.

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151.7 SENSORY CONTROL OF RESPIRATORY PUMPING IN APLYSIA CALIFORNICA. R.P. Croll. Dept. of Psychology, Dalhousie University, Halifax, N.S., Canada B3H 4J1.

It has been hypothesized that respiratory pumping in Aplysia serves to aerate the gill and flush sediment from the mantle cavity (Kandel, 1979, Behavioral Biology of Aplysia). Studies suggest that animals pump spontaneously at a slow rate (Kupfermann & Kandel, 1969, Science 164: 847) and also that tactile stimulation of the siphon or noxious stimulation of the rhinophores elicits pumping (ibid.; Kanz et al, 1979, J. Neurophysiol. 42: 1538). However, these findings do not indicate how the animals adapt to environmental demands related to the putative function of pumping.

The present set of studies involves behavioural observations on unrestrained animals. Respiratory pumping is easily recognized by strong contractions of the parapodia coupled with withdrawal of the siphon. A jet of water can usually be seen exiting the siphon. When Aplysia are placed in seawater (SW) with a high CO_2 content they pump at much higher frequencies than when placed in SW with high O_2 or N_2 contents (means of 1.2/min, 0.0/min, 0.02/min, respectively). Since pH drops with increased CO_2 concentrations, I tested whether pH might be an adequate stimulus for increased pumping. When animals (maintained in SW of pH 7.8) are totally immersed for 90s in SW adjusted with NaOH to pH 8, 9, or 10 they did not pump at rates different from normal (means of approx. 0.1/min). In SW adjusted with HCl to pH 7 pumping increased slightly and at pH 6 and below pumping was fast and vigorous (means of approx. 2.5/min). In order to locate the site of the pH receptors, a slow stream of SW adjusted to pH 3 was directed either over the head or into the opening to the mantle between the anterior edges of the parapodia. Stimulation of the mantle resulted in significantly more pumping than stimulation of the head did (means of 1.2/min vs 0.04/min). A stream of SW at pH 7.8 directed into the mantle did not cause significantly more pumping than normal (0.04/min). Preliminary lesion studies indicate that the osphradium, located in the mantle cavity, may play a role in the pH detection.

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151.8 SCP_B EFFECTS ON THE GILL AND HEART OF APLYSIA CALIFORNICA. J. Rosenberg*, D. Cawthorpe* and K. Lukowiak* (SPON: R. Miller). Dept. of Medical Physiology, University of Calgary, Calgary, Alberta T2N 4N1 CANADA.

SCP_B, small cardioactive peptide, is endogenous to the Aplysia nervous system. In order to examine the physiological role of SCP_B, we examined the effect of SCP_B on the gill withdrawal reflex (GWR) and the isolated heart. The heart was suspended in a 50 ml ASW bath that had a circulation of 10 mls per minute. A cannula was passed through the auricle so that the tip was inserted in the anterior ventricle in the auricular-ventricular junction. The other end of the cannula was attached to a switch valve arrangement to three separate lines providing access to different perfusion media. SCP_B was perfused in ASW in incremental concentrations and had a positive chronotropic and inotropic effect. The potentiation was readily reversible and dose dependant, being measurable at concentrations as low as .01 fM but having a consistent increase in amplitude and rate of contractions at concentrations of .1 pM and higher. At .1 uM, up to a 140% increase was observed in both amplitude and rate of contraction.

In the perfused gill isolated from the abdominal ganglion, synthetic SCP_B was observed to suppress the GWR to tactile stimulation of the gill (threshold dose 10 pM); that is, the amplitude of the GWR was significantly suppressed when SCP_B was perfused through the gill. Gill stimuli were delivered at 20 minute intervals to prevent habituation of the GWR. The effect of SCP_B doses greater than 10 pM and less than 1 nM were generally dose dependant and reversible. Doses of SCP_B greater than 1 nm effectively suppressed the GWR to 35% of its control response amplitude for a period of four hours before the suppressive effect diminished. Previous work in our laboratory has indicated that certain of the neural active peptides and putative neurotransmitters exerted their effect via stimulation of adenylate cyclase. We therefore examined whether SCP_B brought about its effect via stimulation of this enzyme. We found that SCP_B activated adenylate cyclase activity in particulate gill and heart homogenates at threshold concentrations of 1 nM and .1 nM respectively.

Thus, SCP_B plays an important role in modulating the behavior of important physiological functions in Aplysia.

Supported by the MRC of Canada, AHFMR and the AHF.

151.9 PEPTIDERGIC (SCP$_B$ and FMRFamide) MODULATION OF GILL REFLEX BEHAVIORS AND ASSOCIATED NEURONAL ACTIVITY IN APLYSIA. Ken Lukowiak* J. Edstrom* and W.F. Colmers (SPON: G. Mpitsos), Department of Medical Physiology, University of Calgary, Calgary, Alberta, T2N 1N4, Canada.

Neuropeptides play an important role in the mediation and modulation of physiological and behavioral functions, such as the rate and force of heart contraction, the rhythm of gut contractions, bursting activity in central neurons, and the amplitude of evoked and spontaneous gill contractions. Neuropeptides have also been shown to affect adaptive behaviors in Aplysia, and may play a role in the mediation of behavioral state.

We report here that the superfusion of the endogenous neuropeptide SCP$_B$ over the abdominal ganglion (1 uM to 1 mM) causes a significant increase of the gill withdrawal reflex (GWR) evoked by tactile stimulation of the siphon. SCP$_B$ induced a 50 - 200% increase in the siphon-evoked GWR. At the same time, a greater number of action potentials were evoked in gill motoneuron L$_7$. SCP$_B$ did not alter any of the passive membrane properties in the cell body of L$_7$. However, we found that SCP$_B$ brought about an increase of 50 - 200% in the amplitude of the evoked EPSP in motoneurons that received monosynaptic input from the sensory neurons.

The mechanism of SCP$_B$'s facilitatory effect was analysed in two ways. A technique called frequency-dependent spike broadening (FDSB) showed that SCP$_B$ did not affect the duration of the action potential. In the same preparation, we showed that serotonin had a significant effect on FDSB in the sensory neurons. Further, SCP$_B$ had no effect on spike broadening of sensory neurons when TEA (10-20 mM) was added to the constantly-perfused seawater. SCP$_B$ appears to have its facilitatory effect on the GWR by altering the synaptic efficacy, without the induction of AP broadening in the soma of the sensory neuron, as serotonin does. Similar experiments were performed using FMRFamide. We found that FMRFamide also brought about a 50 - 200% increase in the amplitude of the GWR and increased the synaptic efficacy between the sensory neurons and the central gill motomeurons, and did not bring about this effect by AP broadening. Thus, these two peptides may play a major role in determining the behavioral state of the animal, such as the facilitated behavioral state in Aplysia.

Research was supported by the MCR of Canada and AHFMR

151.10 AN ANALYSIS OF ENDOGENOUS TRANSMITTERS THAT PRODUCE PRESYNAPTIC FACILITATION IN THE DEFENSIVE WITHDRAWAL REFLEX OF APLYSIA. V.F. Castellucci, T.W. Abrams, J. Camardo,* E.R. Kandel, and P.E. Lloyd*. Center for Neurobiology & Behavior, Columbia University, College of P & S, and New York State Psychiatric Institute, New York, NY 10032.

Sensitization of the gill and siphon withdrawal reflex involves presynaptic facilitation of the excitatory synapses made by siphon sensory neurons onto gill and siphon motoneurons. Facilitation results from the activation of an adenylate cyclase in the sensory neurons and the closure of a particular K^+ channel by a cAMP-dependent protein phosphorylation. This leads to prolongation of the action potential, increased influx of Ca^{2+} and increased transmitter release. Serotonin simulates the natural presynaptic facilitation and there are serotonergic endings on the sensory neurons as revealed by immunocytochemistry. However, immunocytochemical evidence indicates that at least one set of facilitating interneurons (the L$_{29}$ cells) is not serotonergic (Kistler et al., 1983), suggesting that additional transmitters act as facilitating agents.

To search for such endogenous facilitating transmitters we fractionated abdominal ganglia extracts by reverse phase HPLC and assayed the fractions for spike broadening activity on sensory neurons in the presence of 50 mM TEA. We observed two dominant peaks and several smaller peaks of activity. One of these dominant peaks had the same retention time as serotonin and one or more of the smaller peaks eluted near serotonin. The other dominant peak had retention times characteristic of two peptides, SCP$_A$ and SCP$_B$ (Lloyd, Fed. Proc., 1982). These peptides have been shown to be present in the abdominal ganglion by biochemical means and immunoreactive SCP$_B$ has been localized to varicosities (Kupfermann et al., this volume).

We first focussed on the nonapeptide, SCP$_B$, since it had been synthesized and cloned. Synthetic SCP$_B$ facilitated the monosynaptic EPSP from the sensory neurons and increased the duration of their action potential with a threshold for spike broadening below 10^{-9} M. To see whether the mechanism of action of SCP was the same as serotonin's, we studied the effect of SCP on the K^+ channel modulated by serotonin. Single channel recordings revealed that SCP$_B$ closed this same K^+ channel (as identified by its conductance, 50 pS, and its voltage dependence). Preliminary experiments indicate that SCP$_B$ elevates the content of cAMP in the sensory neurons suggesting that the action of SCP$_B$ is also mediated by cAMP.

These results indicate that facilitation of the defensive withdrawal reflex may be produced by a number of endogenous transmitters. These transmitters may converge on the same cAMP cascade so as to lead to increased transmitter release.

151.11 EXTRACTS OF L$_{29}$ INTERNEURONS PRODUCE SPIKE-BROADENING IN SENSORY NEURONS OF APLYSIA. D.L. Glanzman, T.W. Abrams, R.D. Hawkins, and E.R. Kandel. H. Hughes Med. Inst. for Molec. Neurobiol. & Behav., Columbia Univ., P & S, and NYS Psychiat. Instit., New York, N.Y. 10032.

Sensitization of the gill and siphon withdrawal reflex in Aplysia involves presynaptic facilitation of the connections between the siphon sensory neurons (SNs) and motor neurons (MNs), resulting, at least in part, from broadening of the SN action potential (Klein and Kandel, PNAS, 75:3512, 1978). Part of this facilitation is thought to be mediated by a group of interneurons, the L29 cells. The effects of L29 are simulated by serotonin (5-HT) (Brunelli et al., Science, 194:1178, 1975), which, together with other evidence (e.g., Bailey et al., Brain Res., 272:71, 1983), suggested that 5-HT might be the transmitter of the L29 cells. However, recent immunocytochemical data (Kistler et al., Soc. Neurosci. Abstr., 9:915, 1983) indicate that the L29 cells are not serotonergic. Moreover, recent physiological evidence indicates that at least two transmitters in the Aplysia CNS other than 5-HT are capable of producing facilitation (Castellucci et al., this vol.). These findings raise two questions: 1) Does L29 produce presynaptic facilitation directly, or via an interneuron? 2) If the connection is direct, what transmitter does L29 use? As a first step toward answering these questions we have developed a sensitive bioassay for activity of the L29 transmitter.

Individual L29 cells, identified by electrophysiological criteria, were filled with the dye, Fast Green, and dissected out of Aplysia abdominal ganglia. The cells were boiled in 0.1 M acetic acid for 5 min, and centrifuged at 100,000 g for 3 hrs. The supernatant was dried and resuspended in acidic 95% ETOH and incubated for 20 hr at -70°C to precipitate salt and protein. After centrifugation, the supernatant was dried and resuspended in artificial seawater. Using a micropipette, we pressure-ejected ("puffed") this extract of L29 onto SN somata.

Puffs of L29 extract produced a 2- to 3-fold broadening of the SN spike in 50 mM TEA sea water similar to that produced by 5-HT. These responses were unaffected by high-divalent sea water suggesting the action is directly on the SNs and not through interneurons. Extracts of a control cell (R$_2$, an identified cholinergic neuron) did not produce similar broadening of the SN spike. These results suggest that 1) L29 cells contain a facilitatory transmitter, and 2) L29 produces its action directly on the sensory neurons. By combining this bioassay method with HPLC fractionation procedures, we hope to be able to characterize the transmitter of L29.

151.12 SENSITIZING STIMULI REDUCE THE EFFECTIVENESS OF THE L30 INHIBITORY INTERNEURONS IN THE SIPHON WITHDRAWAL REFLEX CIRCUIT OF APLYSIA. W. N. Frost* and E. R. Kandel (SPON: M. Chen). H. Hughes Medical Institute for Molecular Neurobiology & Behavior, and Department of Physiology, Columbia University; New York State Psychiatric Institute, New York, NY 10032.

In addition to monosynaptic pathways from sensory cells to motorneurons, the neural circuit for the gill and siphon withdrawal reflex in Aplysia utilizes parallel synaptic pathways through interneurons. We here describe an initial analysis of the changes in these interneurons during sensitization focusing on the L30 cells, a set of inhibitory interneurons. The L30 cells are excited directly by the siphon sensory cells and in turn directly inhibit several excitatory interneurons including L29, a modulatory cell that produces presynaptic facilitation of sensory neurons.

When sensitizing stimuli are applied to the tail (or the pleural-abdominal connectives are stimulated), the monosynaptic IPSP made by L30 onto L29 and onto the other follower cells is reduced by 30 to 100% for several minutes without causing a change in the input resistance of the follower cells. Reduction in the IPSP is associated with a hyperpolarization of the presynaptic neuron L30 and a reduction in its hyperpolarizing afterpotential, a reduction which is independent of the change in V_m. The hyperpolarizing afterpotential is substantially reduced when calcium is replaced by cobalt, indicating that it is largely produced by the calcium- activated potassium current. This suggests that the depression in the IPSP may be correlated with a reduction in presynaptic calcium current and may reflect a presynaptic inhibition of inhibition. Serotonin, which simulates the facilitatory transmitters, also mimics the action of the natural inhibitory modulating transmitter(s): it hyperpolarizes L30, reduces its afterpotential, and decreases its IPSP on follower cells.

Our results therefore suggest that sensitization acts on the interneurons so as to lead to a coordinated enhancement of their output: enhancing some excitatory interneurons and concomitantly inhibiting some inhibitory interneurons. Serotonin and other facilitatory transmitters activate a cyclic AMP cascade in the sensory neurons to increase transmitter release. We are therefore testing the idea that both aspects of the coordinated enhancement might occur by means of a common set of modulatory transmitters acting on a common cAMP cascade to modify different substrate proteins in the two classes of cells (the sensory neurons and the inhibitory interneurons) so as to achieve a common integrative end.

152.1 MONOCLONAL ANTIBODIES DISCRIMINATE GLIAL CELL CLASSES IN THE INSECT NERVOUS SYSTEM. M.R. Meyer and J.S. Edwards. Dept. Zoology, Univ. of Washington, Seattle, WA 98195.

In comparison with neuronal cell lineage and differentiation the origin, development and functional diversity of glial cells in the insect nervous system, as in the vertebrate, is poorly understood. Several distinct insect glial cell types or classes have been postulated on the basis of morphological and topographic criteria, but the validity of such classes has not been confirmed by alternative approaches. The identification of glial cell-specific antigens provides one potential method for characterizing the distribution of glia in the insect nervous system. Accordingly, we are using immunological probes to investigate the properties of glia in the nervous system of the cricket Acheta domesticus.

Cultured mouse splenocytes were immunized in vitro with an homogenate of paraformaldehyde-fixed adult female cricket terminal abdominal ganglia. Fusion of isolated stimulated lymphoblast cells with mouse NS-1 myeloma cells generated a population of hybridoma cells which were initially screened for antibody activity against ganglionic determinants by solid phase enzyme immunoassay. Media from positive hybridomas were subsequently tested for glial immunoreactivity by indirect fluorescent antibody staining of frozen sections of terminal ganglia. Selected hybridomas were cloned by limiting dilution and the resultant monoclonal antibodies (MAb's) were rescreened on frozen sections.

We have produced a set of MAb's which demonstrate that several classes of immunologically distinct and topographically restricted glial cell types exist in the cricket nervous system. Several MAb's discriminate between peripheral and central presumptive glial classes. Other MAb's selectively label glia which are restricted to either ganglion cortex or neuropile, and we have been able to detect, within the cortex, an antigenically distinct class of non-neuronal cells corresponding to the glial cells which comprise the perineurium. We have also produced a MAb which uniquely labels the inner and outer surface of the neural lamella.

Our initial studies with various MAb's confirm evidence from previous morphological studies which suggest that distinct glial cell classes exist in the insect nervous system. These MAb's will be useful in future studies aimed at characterizing the source, expression and differentiation of glial determinants during neural development.

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152.2 DEVELOPMENT AND INNERVATION OF THE EXTENSOR TIBIAE MUSCLE IN THE GRASSHOPPER EMBRYO. E.E. Ball and C.S. Goodman. Department of Neurobiology, Res. Sch. of Biol. Sci., Aust. National Univ., Canberra City, A.C.T. 2601, Australia and Dept. of Biol. Sci., Stanford University, Stanford, CA 94305

The extensor tibiae muscle (ETi) of the metathoracic leg, which powers the jump of the grasshopper, is divided into bundles of fibers innervated in varying combinations by only four identified motoneurons whose distribution across the muscle have been mapped (Hoyle, J. exp. Biol., 1978). Here we describe the normal development of this system which we hope later to use for experimental analysis of neuromuscular development.

The first sign of the developing ETi muscle pioneer (MP), as revealed by the I-5 monoclonal antibody (Chang, et al., Dev. Brain Res., 1983) is in two small areas adjacent to the presumptive apodeme of the muscle. These areas merge to form a horseshoe-shaped, multinucleate structure, the arms of which grow steadily longer until ca 44% of embryonic development when their outer edges begin to appear scalloped. By ca 50% the ETi MP consists of a series of bridges connecting the ectoderm of the wall of the leg with that surrounding the apodeme and linked at their medial edges by a thin layer of cytoplasm containing numerous nuclei. Each of these bridges becomes a center surrounded by other mesoderm cells. Horseradish peroxidase injected into the MP demonstrates its syncytial nature until 52.5%. From this time the syncytium begins to break up into steadily smaller units which by 58% consist of individual bridges each of which forms the core of an unstaining mass of mesoderm cells. Once the syncytium has broken up the unstaining mesoderm cells fuse with the bridge they surround and the resulting multinucleate cell mass fragments to form the muscle fibers of the hatchling. Ultrastructurally the MPs are vacuolar and rather featureless until about 50% when the first thick and thin filaments appear. During the next 20% of development bundles of these filaments become steadily more abundant and the T-tubule system begins to develop.

SETi, which establishes nerve 3, is the first motoneuron to reach the ETi MP and by 50% its axon extends along the entire length of the MP. By 55%, as the ETi MP is fragmenting, SETi's axon has begun to branch among the fragments. FETi travels out nerve 5 but then at ca 49% leaves it and turns onto SETi, which it follows to the ETi MP. By 55% it has spread across the ETi, and by 60% it has made lateral branches around each of the developing muscle units.

152.3 TRANS-SEXUALLY GRAFTED ANTENNAE INFLUENCE PHEROMONE-DIRECTED BEHAVIOR IN *MANDUCA SEXTA*. A.M. Schneiderman, J.G. Hildebrand, M.M. Brennan* and J.H. Tumlinson*. Dept. of Biol. Sci., Columbia Univ., New York, NY 10027, and USDA-ARS, Gainesville, FL 32604.

Behavioral experiments in progress in our laboratories continue our established study of the functional organization, physiology, and development of the male-specific, pheromone-processing olfactory subsystem of the sphinx moth *Manduca sexta*.

The sexually dimorphic olfactory subsystem includes receptor neurons in the male antennae sensitive to female sex pheromone and their targets in the brain, the male-specific neurons in the antennal lobes (ALs). A characteristic neuropil structure in the male AL, the macroglomerular complex (MGC), is the site of synaptic contacts between the terminals of axons of pheromone-specialized antennal receptor cells and the dendritic arborizations of their target AL neurons [Matsumoto & Hildebrand, *Proc. Roy. Soc. Lond. B213*:249, 1981]. The MGC arises during metamorphic adult development only in ALs contacted by ingrowing antennal axons from a genetically male antenna -- in normal males or in females with grafted male antennae [Schneiderman et al., *Nature 298*:844, 1982]. Sexually dimorphic AL output neurons with dendritic arborizations in the MGC project to higher centers in the protocerebrum of the brain [Montague et al. *Soc. Neurosci. Abstr. 9*: 216, 1983] and relay information about pheromone [Christensen & Hildebrand, in progress].

To examine the role of AL neurons in the neural pathways controlling male mating behavior and to probe the extent of the influence of male antennal inputs on CNS development, we are studying the behavior of adult moths with experimentally perturbed olfactory systems. The responses of these moths to female sex pheromone are observed as they fly in a wind tunnel. Female moths whose ALs are innervated by sensory fibers from grafted male antennae have exhibited male-like behaviors. These female "olfactory gynandromorphs," unlike normal females or males lacking normal male antennal innervation of the ALs, fly in a non-random, characteristically "zig-zagging" pattern in the pheromone-carrying odor plume, exhibit positive anemotaxis toward the pheromone source, and occasionally hover around and make contact with the paper leaflet bearing the pheromone sample. These observations suggest that certain behaviors, which are normally male-specific and elicited by female sex pheromone, can develop or at least be expressed in female moths that have been experimentally altered only in receiving male antennal grafts and innervation of the ALs.

152.4 **POSTEMBRYONIC NEURONAL PROLIFERATION IN THE MOTH MANDUCA SEXTA.** R. Booker* and J. Truman Univ. of Washington, Seattle, Washington 98195

Using a variety of histological techniques we have been examining postembryonic neurogenesis within the abdominal and thoracic ganglia of the moth Manduca sexta. During the larval stages all the thoracic and abdominal ganglia contain neuroblasts. The number and location of the neuroblasts are constant for a given ganglion but vary according to body segment and sex. For example, in the fourth abdominal (A4) ganglion males had 8 neuroblasts and females 6. In the terminal ganglia, males and females had 15 and 9 neuroblasts, respectively.

During larval life each neuroblast generates a discrete cluster of small (4-5 um in diameter) cells which we refer to as microcells. Late in the third instar the number of microcells within the clusters in A4 range from 8 to 22, and by the end of the fifth instar 45 to 100 microcells can be counted within a cluster. The microcells possess a thin rim of cytoplasm and a process which enters the neuropil. Microcells remain arrested in this condition until the start of metamorphosis at the wandering stage late in the fifth larval instar.

The fates of the microcells both within and between the clusters began to diverge at metamorphosis. Depending on the cluster being examined 20 to 100% of the microcells then degenerated. At pupation the surviving microcells appeared to go through terminal differentiation. There was a significant increase in the cytoplasmic volume resulting in a two to three fold increase in cell diameter. The final appearance of these cells after the completion of the differentiation was dependent upon the cluster in which they resided. Using this relatively simple system we intend to investigate the hormonal regulation of neuronal proliferation, cell death and differentiation in the abdominal and thoric ganglia of Manduca.

152.5 THE DEVELOPMENT OF SEGMENTAL DIFFERENCES IN CELL NUMBER IN THE CNS OF THE LEECH. R.R. Stewart and E.R. Macagno. Dept. of Biological Sciences, Columbia University, New York, NY

Although generally quite similar to each other, the 21 segmental ganglia (SG) of the leech do show some individual features. The most noticeable of these is the significantly greater number of neurons in SG5 and SG6, the so-called sex ganglia which innervate the male and female organs. In large adult leeches of the family Hirudinidae these ganglia have about 700 neurons each, whereas other segmental ganglia have only about 400 (Macagno, J. Comp. Neur. 190:283-302, 1980). In order to begin to understand how this particular segmental differentiation arises, we are carrying out a series of cell counts of SG5 through SG7 in leeches of the species, Haemopis marmorata, at various embryonic, juvenile and adult stages. Our results thus far are shown in the table below.

	9-10d (n=2)	12d (n=1)	20d (n=4)	28d (n=2)	180d (n=1)	Adult (n=3)
SG5	453	421	387	430	537	681
SG6	481	448	397	458	539	677
SG7	474	451	393	405	396	405

From the data presented in the table, it appears that cell number in leech ganglia rises initially to a value greater than 400 neurons which is then followed by a decrease in the number to a value slightly less than 400. After this decrease the number of neurons in SG5 and SG6 begins to increase and continues to do so during post-embryonic life.

In grasshopper embryos the mechanism for producing segmental differences is both differential production of cells by neuronal precursors and selective cell death. Cell death is thought to play the major role in adjusting cell number (Bate et al., J. Neurosci. 1:103-106, 1981). In our case we do see a decrease in cell number during embryogenesis as seen in grasshoppers, but this is followed by segment specific increases in cell number in the sex ganglia.

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152.6 NEUROBLAST MIGRATION IN LEECH EMBRYOS

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Some of the cells that form the central nervous system of glossiphoniid leeches arise near the lateral edges of the flat, early primordium of the body wall and must migrate mediad to reach the ventral midline and the developing CNS (Weisblat, Kim & Stent, Dev. Biol. in press). Here, the migration of several groups of prospective neuroblasts is described and the cellular environment through which they migrate is examined in the hope of identifying possible guidance cues.

The cells of interest are descended from two of the four ectodermal precursor cells on each side of the embryo: the Q teloblast, whose progeny lie at the lateral edge of the early body-wall primordium, and the P teloblast, whose progeny lie just medial to the Q-derived cells. Microinjection of a lineage tracer dye into a teloblast labels both its migratory and non-migratory progeny.

In each hemisegment, two groups of Q-derived cells migrate from the lateral edge of the body-wall primordium toward the CNS. The larger and earlier group moves mediad near the anterior edge of the segment. Most of these cells enter the segmental ganglion near its anterior edge to contribute an antero-ventral cluster of neurons and the glia of the ipsilateral interganglionic connective, but the trailing cells of this group remain outside the ganglion. The second, smaller group of migratory Q-derived cells move mediad along a mid-segmental path. They stop just outside the central ganglion where they join the extraganglionic cells of the first group to form a cluster of peripheral neurons. Finally, a single cell from this peripheral cluster migrates into the ganglion to become a central neuron.

A single wedge-shaped group of P-derived cells in each hemisegment precedes the smaller group of Q-derived cells into the ganglion along the same mid-segmental path.

Muscle cells may provide guidance for the prospective central component of the larger group of Q-derived cells, which migrate next to the first circular muscle cell to differentiate (Stuart et al. Soc. Neurosci. Abstr. 8:15, 1982). However, muscle cells have not been found next to the other groups of Q- and P-derived migratory cells. The various groups of migratory cells appear to contact one another as well as the overlying presumptive epidermis, and interactions within the ectoderm could also provide guidance.

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152.7 THE CERCAL AFFERENT MAP DETERMINES THE DIRECTIONALITY EXHIBITED BY CRICKET GIANT INTERNEURONS. W.W.WALTHALL NEUROBIOLOGY RESEARCH CENTER, SUNY ALBANY, ALBANY, NY 12222

The array of wind-sensitive hairs on the cricket cercus exhibits a high degree of spatial order; as exemplified by the repeated observation of uniquely identifiable hairs on the cerci of different animals. Axons emanating from this receptor array preserve the spatial order by forming an orderly projection within the CNS in an area of neuropil called the cercal glomerulus. The orderly afferent map also has a functional dimension. Sensory receptors exhibit directional selectivities and those with the same preference aroborize in the same area of the glomerulus. Afferents with other directional selectivities arborize in other areas of the glomerlus. Identifiable interneurons characteristically have dendrites in precise locations with respect to the map. Not surprisingly, these interneurons display characteristic directional selectivities, which can be predicted based upon the location of their dendrites within the map. This structural-functional correlation raises an important issue. Does the map play a causal role in determining the directional selectivity exhibited by the interneurons? Alternatively, the sensory neurons could be specified to innervate particular interneurons, and since dendrites of the interneurons are always in the same location the map is merely a by-product of the specification. One means of distinguishing between these alternatives occurs during regeneration. Initially regenerating neurons project to inappropriate regions of the cercal glomerulus (days 6-14). Later (days 15-21) they lose inappropriate arbors while consolidating arbors in correct areas of the glomerlus. Intracellular recordings from the medial giant interneuron (MGI) at the early stage of regeneration, when the afferent map is scrambled, revealed responses to inappropriately oriented stimuli. Recordings following longer periods of regeneration indicated the restoration of the interneuron's directional selectivity. The time course closely followed the anatomical restoration of the afferent map. MGI's initial lack of directional selectivity indicates that regenerating afferents with inappropriate orientation preferences are forming functional synapses on its dendrites. This result indicates that the orderly afferent projection is a causal element in determining the directional selectivity of interneurons such as MGI.

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152.8 FUNCTIONAL AND STRUCTURAL CHANGES IN A POPULATION OF SENSORY NEURONS DURING INSECT METAMORPHOSIS. R.B. Levine, Dept. of Biology, Rice University, Houston, TX 77251

During insect metamorphosis individual larval neurons are retained to participate in different behavior at later stages. Retained motorneurons must undergo developmental changes consisting of both structural and functional alterations. Sensory neurons are also retained from the larval stage for later use as the following example demonstrates. Although their behavior is restricted in scope, pupae of the hawkmoth Manduca sexta display a stage-specific defensive reflex, the "gin-trap" behavior. During the larval stage small touch-sensitive hairs cover much of the body surface. While many of the associated sensory neurons degenerate at the end of the larval stage, a discrete subset near the anterior, lateral margins of abdominal segments 5, 6 and 7 remain to innervate sensory hairs within the pupal gin-trap (Bate, M., Ph.D. Thesis, Cambridge Univ., 1972 and J.E.B. 59:121, 1973). Tactile stimulation of presumptive gin-trap sensilla during the larval stage evokes a weak contraction of intersegmental muscles (ISM) that is neither laterally nor segmentally specific. In contrast, tactile stimulation of the pupal receptors evokes a rapid contraction of the ipsilateral ISM in the next anterior segment, which draws the sharp edges of the gin-trap together. Tactile or electrical stimulation of the larval sensory neurons evokes a small, often subthreshold excitatory response in ISM motorneurons that may be augmented or blocked by information from stretch receptors in the body wall. The same sensory neurons in the pupa evoke a large depolarization and a burst of action potentials in ISM motorneurons innervating the ipsilateral half of the next anterior segment. Unlike the larval response, this depolarization is abruptly terminated by inhibition. Contralateral motorneurons, and those in other segments are inhibited by the same stimulus. Although the pathway between sensory neurons and motorneurons is not direct, a structural reorganization of the central arborizations of the sensory neurons during the final three days of larval life accompanies these physiological changes. Larval sensory neurons enter an abdominal ganglion and branch profusely before ascending to the next anterior ganglion to terminate in the ventral neuropil. The same sensory neurons maintain both areas of arborization in the pupal stage, but the extent of branching in the anterior ganglion is increased, with processes invading more dorsal and anterior regions, while branching in the posterior ganglion decreases. This descriptive information provides a basis for an analysis of the hormonal signals which direct individual neurons to assume different properties as metamorphosis proceeds. Supported by NSF grant #BNS 8308907.

152.9 BLOCKAGE OF A REFLEX RESPONSE IN GENETIC MOSAICS BY TEMPERATURE SENSITIVE MUTATIONS: EFFECTS ON SENSORY CELLS DERIVED FROM IMAGINAL DISCS OF DROSOPHILA, M. G. Burg* and C.-F. Wu (SPON: J. Denburg). Dept. of Zoology, Univ. of Iowa, Iowa City, IA 52242

Two temperature-sensitive paralytic mutations, $para^{ts}$ and nap^{ts}, are implicated in affecting sodium currents, blocking axonal conduction at high temperature in neurons derived from centrally located neuroblasts. It is not known whether these two mutations also affect epithelial sensory cells which represent a developmentally distinct set of excitable cells. These cells differentiate from imaginal discs and are derived from a common progenitor cell that gives rise to the bristle mechanosensory apparatus.

To monitor the function of these sensory cells, a fixed-wired reflex response, cleaning by a specific leg elicited by mechanical stimulation of a single bristle in decapitated flies, was used. By using genetic mosaics having a small hemizygous patch of $para^{ts}$ tissue containing only 1-2 bristles, the effects of $para^{ts}$ on the reflex response was determined. At 23°C the reflex response was blocked for the $para^{ts}$ bristle, but not for surrounding normal bristles that elicit a response from the same leg.

The mutations $para^{ts}$ and nap^{ts} are known to interact synergistically, resulting in lethality of the organism (Nature, 286:184, 1980). Our study was extended to genetic mosaics containing small patches of $para^{ts}$-nap^{ts} double mutant tissue, which are viable. The function of the double mutant sensory cell was blocked at any temperature, as evidenced by failure to initiate a reflex, while surrounding bristles did elicit a proper reflex response. An EM analysis of the double mutant sensory cell in mosaics was done. Results indicate that the sensory cell is present and is similar to the normal sensory cell ultrastructurally. This work shows that $para^{ts}$ and nap^{ts} do affect this type of excitable cell, and blockage of axonal conduction in double mutant cells does not prevent differentiation. Supported by NIH grants NS 00675, NS18500, and a grant from Searle Scholars Program to C.-F. W., and by NIH Pre-doctoral Genetics Traineeship GM 07091 to MGB.

152.10 DEVELOPMENTAL ACQUISITION AND EXPRESSION OF A PEPTIDE NEUROTRANSMITTER IN A MODEL NEUROMUSCULAR SYSTEM OF THE GRASSHOPPER EMBRYO. H. Keshishian and M. O'Shea. Committee on Neurobiology, The University of Chicago, Chicago, Il. 60637.

The embryonic acquisition and regional expression of the neurotransmitter proctolin was examined in both the CNS and developing neuromuscular junctions of the grasshopper Schistocerca nitens. The hatchling CNS has over 70 uniquely identifiable proctolinergic cells, including the metathoracic motorneuron SETi, terminal ganglion motorneurons to the intrinsic hindgut, and several identified interneurons (see also Neurosci. Abstr. 8:899; and Witten et al., this volume). Embryonic CNS expression develops through 4 periods: 1) an early phase (50%-60% stages) prior to immune staining, when levels rise slowly at ~1 fmole/% dev.; 2) a 2nd phase (60%-70% stages) when stereotyped CNS staining is established, and total levels rise at ~5 fmoles/% dev.; 3) a 3rd phase (70%-95% stage), when CNS levels stabilize at 20-40 fmoles and transmitter accumulates peripherally, peaking at 200 fmoles; and 4) a fourth phase (95%-hatching) when peripheral levels drop by ~20%.

As a model for peptide transmitter expression in developing neuromuscular junctions, the terminal ganglion motorneurons of the intrinsic hindgut muscles were studied by dye-fill, assay, and immunocytochemistry. We identify ~30 motorneurons: 3 pairs of ventral contralateral efferents and a single midline pair that are not proctolinergic; 4 pairs of ipsilateral anterior medial (AM) motorneurons clustered at the nerve 8 level, that first stain at the 70% stage; and 7-8 pairs of ipsilateral posterior medial (PM) neurons clustered at the nerve 9 level that begin to stain 24 hrs post-hatching. The 8 AM cells exist in 3 dorso/ventral cell body patterns: In 1/2 of the ganglia there is a single ventral cluster of 8, of which 6 are proctolinergic. In 1/4 of the ganglia one of the proctolin motorneurons is displaced to the dorsal side. In the remaining cases two of the proctolinergic AM cells lie dorsally. Proctolin is detected by the 70% stage in the axons along the embryonic rectal, intestinal, and pyloric muscles, with one staining axon for each of the 6 intrinsic intestinal bundles. Hindgut proctolin increases over the next 25% of development by 300%, and declines 20% during the motor bouts that precede hatching. Proctolin is released from the hindgut, upon depolarization, in a Ca^{++} dependent fashion, showing that synaptic release is functional by the end of embryogenesis. Supported by grants NS06864-01 (HK) and BNS 8202515 (MO).

BASAL GANGLIA: ANATOMY AND PHYSIOLOGY III

153.1 SEGREGATION AND INTERDIGITATION OF CORTICO-STRIATAL TERMINAL FIELDS IN RHESUS MONKEY. L.D. Selemon and P.S. Goldman-Rakic, Sec. Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

Recent studies suggest that cortical areas which are reciprocally connected via cortico-cortical projections innervate the same topographic region in the neostriatum. In the present study, we used a double labeling paradigm, involving simultaneous anterograde transport of HRP and tritiated animo acids, to examine the striatal projections from two interconnected cortical areas in the same animal. In two monkeys, HRP pellets were implanted in the dorsolateral prefrontal cortex and tritiated animo acids were injected into the orbitofrontal or anterior cingulate cortex, respectively. In a third animal, a large frontal HRP injection site, which included dorsolateral, orbital and cingulate cortices, was combined with an injection of isotope in the superior temporal gyrus.

Analysis of adjacent coronal sections, processed for HRP histochemistry and autoradiography, respectively, showed that the dorsolateral prefrontal and orbitofrontal cortices project to separate longitudinal domains of the neostriatum: dorsolateral terminals are located in central regions of the caudate and rostral putamen while the orbital terminal field is situated ventromedially. Likewise, projections from the dorsolateral prefrontal cortex and the anterior cingulate gyrus occupy topographically distinct territories as cingulate terminals are located within the ventromedial neostriatum. Analysis of the third double-labeled case revealed that the terminal field from the large frontal injection extended into the ventromedial neostriatum where it overlapped the projection from the temporal cortex. However, superimposition of drawings from adjacent sections revealed that the frontal terminal field actually was interdigitated with the temporal field throughout the zone of topographic overlap.

The present results indicate that many reciprocally connected areas of association cortex project to spatially distinct, rather than overlapping, areas of the neostriatum and thus suggest that convergence of cortical input in the neostriatum is not related to connectivity at the cortical level. (Supported by NIMH, NIH and the Hereditary Disease Foundation.)

153.2 ORGANIZATION OF SOMATOSENSORY CORTICOSTRIATAL PROJECTIONS. R.Malach* and A.M.Graybiel (SPON:F.O.Schmitt). Dept. of Psychol. and Brain Sci., Mass. Inst. Tech., Cambridge MA 02139.

We have studied the topographic transformation of two cortical maps in the cat: the cutaneous and the deep receptor body representations of primary somatosensory cortex, as they are projected upon the striatum. This information could serve as a useful guide for future electrophysiological studies of the striatum as well as add information pertinent to the still largely unsolved problem of what type of computations this structure performs.

Variably-sized injections of wheatgerm agglutenin-conjugated horseradish peroxidase or radioactively tagged amino acids or both were placed under electrophysiological guidance into cortical loci representing different body parts. Injections that were centered in the primary cutaneous representation (S1) produced patchy labeling in the ipsilateral striatum. The patches were confined to the dorsolateral part of the caudate nucleus (CN), the putamen, and the cell bridges in between. Rostrocaudally, the labeled field lay within the middle two-thirds of the CN. Single injections usually labeled several variably shaped patches and bands that were 0.1-0.6mm wide, were up to 2mm long and were separated from each other by up to 1mm. In serial section reconstructions, nearby patches sometimes fused but there were clear instances in which patches remained segregated. In frontal sections, the labeled patches appeared to form one or two arc-shaped rows that were oriented roughly parallel to the dorsolateral border of the CN. There was a tendency for the labeled patches to be located more medially as the cortical injections were placed in the representations of progressively more posterior body parts (e.g. forepaw, shoulder, back). Interestingly, although the labeled patches were located outside the regions of low acetylcholinesterase (AChE) activity (striosomes) visualized in adjoining sections, their overall appearance was strikingly similar to these histochemically defined compartments.

Injections that were centered in various parts of the anteriorly adjacent deep receptor cortical map labeled corticostriatal patches that were similar in appearance to those formed by the projections of the cutaneous field, and that were located in the same general part of the striatum. We are currently investigating the exact topographic relationship between these two sets of discontinuous projection zones.

Supported by a Bantrell Fellowship, NIH-EY02866-06 and the Seaver Institute.

153.3 FURTHER OBSERVATIONS ON THE STRIOSOMAL ORGANIZATION OF FRONTOSTRIATAL PROJECTIONS IN CATS AND MONKEYS. C.W.Ragsdale Jr. and A.M. Graybiel, E25-618, MIT, Cambridge, MA 02139.

The corticostriatal projection in higher mammals is highly ordered, as there are both topographical differences in the distribution of indiviual corticostriatal systems, and local patterning of these fiber systems within their fields of termination. We previously reported that part of the patchiness in frontostriatal projections in cats and monkeys is accounted for by a striosomal organization in which frontostriatal fibers either project to or avoid acetylcholinesterase (AChE)-poor striosomes. In the monkeys, however, larger terminal aggregates as well as striosomal patchiness appeared, and in the cats, fibers labeled by large frontal injections both filled striosomes (dorsally) and avoided striosomes (ventrally). To extend these findings, we have made single or paired injections of anterograde tracers (HRP-WGA, ^{35}S-methionine, or mixtures of ^{3}H-proline, leucine and lysine) into the cortex and amygdala of 21 cats and 4 macaque monkeys. In the cats, we found that striosomes are multiply innervated and do not all receive equivalent innervations. Dorsal striosomes are filled after injections of frontal polar cortex (gyrus proreus) and after injections of insulo-orbital cortex. Though ventral striosomes are also innervated by insulo-orbital fibers, they are avoided by proreal fibers and instead receive a dense subcortical innervation from the basolateral amygdala. There were also both regional and widespread 'avoid' patterns formed by fiber systems projecting around the AChE-poor striosomes. For example, fibers from the anterior cingulate gyrus avoid striosomes both dorsally and ventrally. Motor cortex (area 4β) projects dorsolaterally. In several instances these fibers avoided striosomes, but this was not always clear because AChE staining is murky in this sector. Pairing more anterior frontal injections with area 4 deposits may help to identify zonal boundaries there.

In the monkeys, we placed large injections in motor and premotor cortex (M-PM), including APA, and in the supplementary motor area (SMA). As in the cats, labeling was heterogeneous and some fibers avoided striosomes while others filled them. The M-PM and APA fibers innervated putamen and lateral caudate nucleus (CN), mainly avoiding AChE-poor zones but filling some, especially in the CN. The SMA fibers projected broadly to CN and putamen, avoiding striosomes throughout. Though many labeled figures respected the histochemical borders seen, the local patterning and wide (at least up to 6mm) separation of restricted patches was not predictable from the AChE patterns alone. Supported by NIH-EY02866-06.

153.4 STRIATAL CELL BODIES EXPRESSING DYNORPHIN B-LIKE (DYN) AND MET-ENKEPHALIN-LIKE (ENK) IMMUNOREACTIVITIES HAVE COMPLEMENTARY DISTRIBUTIONS IN KITTENS AND CONTRASTING DISTRIBUTIONS IN CATS. A.M.Graybiel and M.-F.Chesselet, Dept. of Psychol. and Brain Sci., Mass. Inst. Tech., Cambridge, MA 02139.

Met-enkephalin and dynorphin B are members of different families of opioid peptides but both are present in the striatum and its efferent pathways. We have compared the striatal distributions of DYN and ENK immunoreactivities in young and adolescent kittens (2-9 mo.) and in adult cats using methods not requiring colchicine to visualize cell bodies (CB). In addition, 1 adult cat pretreated with colchicine was studied.

Clusters of medium-sized DYN-positive CB (DYNCB) were found in the dorsal part of the caudate nucleus in all of the kittens and cats. The clusters were variable in shape, 0.1-0.6mm wide, and lay in crisply bounded patches and bands of neuropil more densely immunoreactive than that of the surround. There was strict alignment of these DYNCB clusters and clusters of substance P-positive CB (SPCB) visualized in adjacent sections (see Graybiel & Chesselet, Anat. Rec. 208, 64A, 1984). Where acetylcholinesterase (AChE)-poor striosomes were visible, the DYNCB clusters matched them also.

This pattern of DYNCB distribution contrasted sharply with the arrangement of neurons expressing ENK immunoreactivity (ENKCB). In the young kittens, there was a vivid pattern in which fields of ENKCB were interrupted by "holes" in which few (if any) ENKCB lay. The neuropil staining was also low in the holes. These ENK-poor zones were about the same size as, and lay in register with, the clusters of DYNCB in adjoining sections. Thus, the dominant pattern in the dorsal caudate nucleus was one of DYNCB-rich, ENKCB-poor clusters aligned with SPCB-rich clusters in an ENKCB-rich field. In the adults and oldest kittens there were still crisp patch-patterns of DYNCB dorsally, and these still matched SPCB-rich and/or AChE-poor striosomes; ENKCB-sparse (though fewer ENKCB-free) zones appeared as well, and also mediolateral ENKCB gradients.

Ventrally and medially, dense DYN and ENK neuropil staining was present in heterogeneous arrangements. Patches of DYN and ENK neuropil were not always aligned with figures formed by DYNCB or by ENKCB, and frequently were complementary to them and even to each other.

We propose on the basis of these findings (1) that the compartmental ordering of DYNCB, ENKCB and SPCB may be related to the organization of biochemically specified striatal efferent pathways, and (2) that at least ventrally, there may be opioid-containing striatal afferents with contrasting compartmental distributions. We thank the Seaver Institute.

153.5 THE INFLUENCE OF NEOSTRIATAL PATCH AND MATRIX COMPARTMENTS ON THE DENDRITIC GEOMETRY OF SPINY PROJECTION NEURONS IN THE RAT AS REVEALED BY INTRACELLULAR LABELING WITH HRP COMBINED WITH IMMUNOCYTOCHEMISTRY. G.R. Penny, C.J. Wilson and S.T. Kitai. Division of Neuroscience, Department of Anatomy, University of Tennessee Center for the Health Sciences, 875 Monroe Avenue, Memphis, TN 38163

We used intracellular injection of HRP combined with immunocytochemistry for leucine-enkephalin (Leu-enk) to demonstrate neostriatal patches and spiny neuron dendritic fields in the same sections. Cobalt intensification of the first DAB reaction prior to the immunocytochemical steps resulted in good contrast between the black reaction product in the injected cell and the brown staining for Leu-enk. We asked: (1) Can patch-matrix boundaries partially explain the wide range of dendritic field shapes of neostriatal spiny neurons (which can vary from almost spherically radiating fields to bipolar or planar arrangements of the dendrites)? (2) How might the spiny projection neurons contribute to the organization of patch and matrix neostriatum?

We find neurons with the characteristic somatodendritic morphology of spiny projection neurons in both enkephalin-rich patches and enkephalin-poor matrix regions. Dendrites of both patch and matrix spiny neurons avoid the patch boundaries; thus, the dendrites of spiny neurons in patches are mostly confined to the patch compartment while those in matrix regions are confined to matrix. These observations may provide an explanation for the unusual organization of neurons at the edges of the neostriatum. The dendritic fields of these cells are often oriented in a planar fashion extending only 50-150 um into the neostriatum while covering distances of up to 500 um parallel to its boundary. The boundaries of neostriatum are consistently lined by an enkephalin and naloxone receptor-rich, AchE-poor patch of thickness similar to that of the dendritic fields of cell within this compartment. Hence, the variation of spiny cell dendritic fields may be related to location relative to cytochemically defined patch boundaries.

These observations suggest that the intrinsic circuitry of the neostriatum preserves the segregation of the parallel afferent and efferent pathways associated with the patch and matrix compartments.

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153.6 IMMUNOCYTOCHEMICAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE IN NEURONS AND SYNAPSES OF RAT NEOSTRIATUM. P.E. Phelps, C.R. Houser, and J.E. Vaughn. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Correlated light and electron microscopic immunocytochemistry of the acetylcholine synthesizing enzyme, choline acetyltransferase (ChAT), has been conducted to characterize putative cholinergic neurons and synaptic junctions in rat neostriatum. The specificity of the monoclonal antibodies and immunocytochemical methods used in this investigation have been previously demonstrated (Crawford et al., PNAS 79:7031-7035; Houser et al., Brain Res. 266:97-119).

Neurons filled with ChAT-positive (ChAT+) immunoreaction product were scattered throughout the striatum and, based on cell counts of counterstained immunocytochemical preparations, they comprised 1.7% of the total neuronal population. ChAT+ somata were round or elongated in shape, averaged 27x13µm in size, and had 3-4 dendrites that coursed long distances through striatal neuropil. Numerous ChAT+ fibers and punctate structures were distributed relatively homogeneously throughout the striatum, but a more densely staining stripe was observed at its ventrolateral border that was continuous with the fundus striati. EM observations of the ChAT+ neurons initially studied by light microscopy revealed that these cells exhibited abundant amounts of cytoplasm containing numerous organelles and intense reaction product, as well as unstained, deeply invaginated nuclei. Contours of proximal and distal ChAT+ dendrites were usually smooth, but the latter displayed occasional spines or varicosities. Unlabeled boutons were commonly observed to form synapses with distal ChAT+ dendrites and, less frequently, with labeled proximal dendrites and somata. ChAT+ boutons contained pleomorphic vesicles, and serial section analyses revealed that those forming synapses were associated with symmetric junctions. Such ChAT+ presynaptic boutons contacted spiny and smooth dendrites, as well as unlabeled somata that ultrastructurally resembled those of medium spiny neurons. These results indicate that ChAT+ striatal neurons correspond to the largest neurons identified in previous Golgi and EM studies. They also suggest that medium spiny striatal projection neurons receive a cholinergic innervation that could be derived from ChAT+ striatal cells. Supported by the Hereditary Disease and Wills Foundations.

153.7 INTRASTRIATAL CONNECTIONS: A FLUORESCENT TRACER AND IMMUNOHISTOCHEMICAL STUDY. M.-F. Chesselet and A.M. Graybiel, Dept. Of Psychol. and Brain Sci., Mass. Inst. Tech., Cambridge, MA 02139,and Lab of Cell Biol.,NIMH, Bethesda,MD 20817.

Recent studies have shown that the caudate nucleus (CN) is composed of a mosaic of regions characterized by their distinct neurochemical composition. In addition, afferents and efferents of the CN are both topographically organized and broken up into patchworks. To determine whether different subdivisions of the CN are linked by association connections, we have carried out a retrograde tracer study with the fluorescent dye fast blue (FB). Small (50 nl) amounts of the dye were slowly injected into the CN of 4 rats, 1 kitten, 3 cats and 2 rhesus monkeys, in most cases after removal of the overlying cortex and white matter in order to avoid uptake of FB along the needle tract. Eight days or more after the initial injections, DFP was administered and the animals were perfused 6 hours later. Sections through the CN were examined for the presence of FB-labeled cell bodies (FBCB) and were processed for fluorescence immunohistochemistry using antibodies against somatostatin (SOM) or choline acetyltransferase (CAT). Some sections were stained for acetylcholinesterase (AChE) after photography of the FBCB. In the rats and the 3 week old kitten, a variety of neurons of different sizes were labeled around and at a distance from the injection site. They included SOM-and AChE-positive CB, and many medium sized neurons that were neither AChE nor SOM-positive. By contrast, in the cats and monkeys, fewer cells were labeled, but a consistent labeling of 1-10 large neurons was observed in almost every section up to at least 1mm away from the visible limit of the injection site. These large FBCB were CAT and/or AChE-positive and thus presumably were cholinergic neurons. Despite the presence of numerous SOM-positive neurons near the injection site and in the vicinity of the labeled cholinergic neurons, hardly any SOM-positive CB contained FB. (Sample numbers for FBCB in adult cat: SOM^+/FB^+=3/78; CAT^+/FB^+=107/157; $AChE^+/FB^+$=52/53). No FBCB were detected in the putamen. This study suggests that in adult higher mammals, a network of cholinergic neurons may be specifically involved in connecting separate regions of the CN with one another. Whether the regions so interconnected share common neurochemical properties and connections is an important question for further work. Funded by the Huntington's Disease Foundation of America, the Seaver Institute and NSF BNS81-12125. We thank Drs. R.P. Elde and F. Eckenstein for their generous gifts of antisera.

153.8 DOPAMINE, BICUCCULINE, AND PICROTOXIN HAVE SIMILAR EFFECTS ON CORTICAL-STRIATE TRANSMISSION IN RAT NEO-STRIATAL SLICES. John A. Wilson and Forrest F. Weight Laboratory of Preclinical Studies, National Institute on Alcohol Abuse & Alcoholism, Rockville, MD 20852

The effects of the dopamine (DA), bicucculine methiodide (BMI) and picrotoxin (PIC) on cortical-striate excitatory transmission were investigated in rat neostriatal slices using electrophysiological techniques. A 450 uM parasagital slice consisting of the cerebral cortex, corpus callosum, and corpus striatum was stimulated using bipolar electrodes. A two component potential could be recorded extracellularly. The first component, N-1, is a negative going fiber volley. The second component, N-2, has a synaptic origin, and is glutamate antagonist sensitive, (Cordingley and Weight, Soc. Neurosci Abstr. 8: 373,1982). Both N-1 and N-2 were evoked by stimulating either within the neo-striatum (3.0 - 6.0 V, 0.02 msec) or at the cortical-callosal boundary (10 - 20 V, 0.05 msec). In 15 - 20% of the animals tested, 100 uM DA decreased the amplitude and increased the latency of N-2, without changing N-1. The effect of DA was maximal 5 to 10 minutes after the start of its application. During continuous DA application N-2 returned to its original amplitude within 20 minutes . Furthermore, if one slice from an animal was sensitive to DA, all the slices from that animal responded to DA; whereas, if no response was found in one slice, a response was not found in other slices from that animal. In DA-sensitive preparations, 100 uM PIC and 100 uM BMI decreased N-2 amplitude and increased N-2 latency. PIC and BMI at these concentrations had no apparent effect in DA-insensitive preparations. Like the response to DA, these responses decreased with time. During the maximal response to BMI, DA was ineffective. However, during continuous application of BMI, when the amplitude of N-2 had fully recovered, DA elicited a full response in the presence of BMI. These observations suggest a relationship between dopaminergic and GABAergic mechanisms in the neo-striatum.

153.9 INSTRUCTION-DEPENDENT NEURONAL ACTIVITY IN PRIMATE PUTAMEN. G.E. Alexander. Dept. Neurology, Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

The putamen receives inputs from motor cortex, premotor cortex, and the supplementary motor area. Instruction-dependent neuronal activity preceding motor responses has been demonstrated in each of these cortical fields. To determine whether the putamen also participates in such processes, a rhesus monkey was trained to perform a step-tracking task which required flexion and extension movements of the elbow to align a cursor with a series of targets presented on an oscilloscope display. Loads were applied by a torquemotor to dissociate the direction of the movements from the pattern of muscles required. Trials were initiated by presentation of a center target with which the animal aligned the cursor throughout a waiting period (750-2250 ms). The target was then displaced to the right or to the left of center, following which the monkey aligned the cursor with the new target position. The target then returned to the center, and the monkey realigned the cursor accordingly and maintained the center position during a delay period (750-2250 ms). Following the delay, targets were presented simultaneously at both lateral positions. The monkey was then required to align the cursor with the correct (previously presented) target.

Single cell recordings in the putamen revealed two major types of task-related neurons. The first type (N=22) showed increased discharge during elbow movements in a preferred direction. These responses were independent of applied loads. The second type (N=15) showed selective increases in discharge following registration of the instruction to move in a particular direction. Such increases occurred either exclusively or maximally on trials in which the impending movement was in one specific direction. The increased activity began following the animal's return to the center hold position after registering the instructed direction of the next movement, and persisted throughout the delay period. Neurons showing these instruction-dependent changes did not discharge during movements and did not show a relation to the loads.

These results indicate the putamen may participate not only in the execution of, but also in the programming of or preparation for, movements in a particular direction.

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153.10 EVIDENCE THAT DOPAMINE PLAYS AN EXCITATORY ROLE IN THE STRIATUM. M. W. Warenycia and G. M. McKenzie*. Department of Pharmacology, Dalhousie University, Halifax, N. S. B3H 4H7.

Systemic dexamphetamine (DEX) in freely moving animals produces activation of striatal neurons which may depend on intact nigrostriatal dopamine (DA) neurons. To test this idea, the nigrostriatal dopaminergic nerve terminals in Long Evans rats (270-300 g) were lesioned unilaterally (N=26) with 6-hydroxydopamine (6-OHDA) applied either in the substantia nigra (SN), or directly in the striatum (ST). Seven to fourteen days later, animals from each treatment group were implanted bilaterally with bipolar recording electrodes in the anterior ST. Five to seven days later, striatal multiple-unit activity (MUA) was recorded under freely moving conditions, before and after DEX 2.5 mg/kg i.p. HPLC was then used to measure striatal DA levels in SN-lesioned animals (N=15); in ST-lesioned animals (N=8) DA levels were measured in ST as well as olfactory tubercles and piriform and cingulate cortices.

SN lesions resulted in a 50% decrease in spontaneous striatal MUA on the denervated side compared to normal animals. DEX, which in normal animals consistently produces striatal excitation, produced inhibition on the lesioned side and excitation in the contralateral striatum. The incidence of striatal inhibition following DEX was inversely related to the percent DA depletion on the lesioned side.

In striatally injected animals, 6-OHDA produced an 87% depletion of DA in treated striata accompanied by a 212% increase in DA levels on the intact side. Again, DEX failed to produce excitation on the lesioned side, whereas excitation was observed on the non-lesioned side. Unilateral striatal injections of 6-OHDA did not alter the DA content of the olfactory tubercles, piriform cortex or cingulate cortex.

It is concluded that the excitatory response of most striatal neurons following DEX is dependent upon an intact nigrostriatal dopaminergic innervation suggesting that dopamine is an excitatory transmitter in the striatum. Furthermore, unilateral degeneration of DA fibers whether evoked from the ST or the SN, induces an increase in dopamine levels in the contralateral striatum, but not in other DA-innervated areas, due presumably to a compensatory decrease in dopamine release in the contralateral ST.

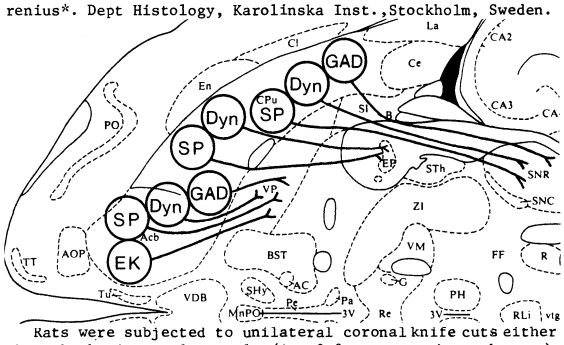
153.11 RESPONSE PROPERTIES OF VISUAL CELLS IN THE BODY OF THE CAUDATE NUCLEUS IN THE MACAQUE. J. W. McClurkin* & R. T. Marrocco. Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

Traditionally, a motor role has been assigned to the caudate nucleus (Marsden, C. D., Trends Neurosci.,3:284, 1980). More recently, investigators have reported cognitive deficits in cats, rats, and monkeys after experimental lesions of the head of the caudate (Dray, A., Prog. Neurobiol.,14:221, 1980). Furthermore, human patients with mild symptoms of Parkinson's disease, which typically affects caudate neuronal function, have been shown to have some language deficits (Damasio, A.R., Trends Neurosci., 6:442, 1983). However, previous anatomical studies have demonstrated direct projections to the body of the caudate from the striate and prestriate cortical areas (Kemp, J.M., & Powell, T.P.S., Brain,93:525, 1970), and we have observed a projection from the body to the posterior shoulder and the fundus of the lunate sulcus (unpublished), suggesting the possibility of sensory as well as cognitive and motor functions. Here we present physiological findings that further suggest that the caudate nucleus may have a sensory function in addition to motor and cognitive functions.

We recorded from single cells in the body of the caudate nucleus in paralyzed, anesthetized monkeys. Receptive fields in the rostral portion of the body tended to be several hundred deg. sq. with some extending over the entire contralateral hemifield. These cells responded vigorously only to stimulus movement, and some were directionally selective, but none were selective for shape, responding equally well to moving spots, bars, or experimenters. More caudally, the receptive fields were much smaller, being less than 100 deg. sq. in area, and the responses were less robust. However, these cells also preferred moving to stationary stimuli, and were not selective for shape. Finally, none of the cells encountered showed any habituation to repeated stimulation.

These results, together with the pattern of anatomical connections, suggest that the body of the caudate is involved in the early stages of the analysis of movement rather than in cognitive or motor functions. (Supported by NSF grant 82-07531 to RTM.

153.12 ENKEPHALIN (EK), DYNORPHIN (Dyn), SUBSTANCE P (SP) AND GLUTAMIC ACID DECARBOXYLASE (GAD) IN STRIATAL EFFERENTS. G. Paxinos, W. A. Staines*, T. Hökfelt*, W. H. Oertel*, and L. Terenius*. Dept Histology, Karolinska Inst.,Stockholm, Sweden.



Rats were subjected to unilateral coronal knife cuts either through the internal capsule (ic; 3.3 mm posterior to bregma) or through the caudal striatum immediately anterior to the globus pallidus (GP; at bregma). Following a survival period optimal for the demonstration of accumulation of immunoreactivity in interrupted fibers (2 days) or a period sufficient for depletion to occur (12 days), consecutive brain sections were stained for the demonstration of EK-, Dyn-, SP- and GAD-like immunoreactivity. Cuts of ic produced accumulation of Dyn-, SP- and GAD-immunoreactivity in descending fibers throughout the extent of the ic. In the substantia nigra (SN), the ic cuts produced a complete disappearance of the Dyn- and SP-immunoreactivity and a nearly complete depletion of GAD immunoreactivity, but no detectable depletion of the EK-containing fibers. EK-containing cell bodies were detected in the compact part of the SN. Cuts anterior to GP produced (a) accumulation of EK-, Dyn-, SP- and GAD-immunoreactivity in fibers descending in fascicles, (b) depletion of EK-, Dyn-, SP- and GAD-immunoreactivity in the ventral pallidum (VP), (c) depletion of EK- and GAD-immunoreactivity in GP and (d) depletion of Dyn- and SP- immunoreactivity in the entopeduncular nucleus (EP). The figure depicts some of the striatal projections on horizontal Figure 56 of the Paxinos and Watson (1982) atlas. The depletion of EK and GAD in both the VP and GP support the notion of a ventral continuation of the corpus striatum (Heimer and Wilson 1975).

OPIATES, ENDORPHINS, AND ENKEPHALINS: RECEPTORS I

154.1 CHARACTERIZATION OF OPIATE BINDING SITES IN THE RETINA. D.I. Gottlieb, M.M. Slaughter, and J.M. Mattler*. Dept. of Anatomy & Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110

The binding sites for enkephalin and opiate alkaloids in the retina of the chick, rabbit, and goldfish were characterized. Binding assays were done using crude retinal membrane fractions suspended in .05 M Tris HCl; bound ligand was determined by filtration and washing of the membranes. Nonspecific binding was determined by parallel incubations with excess levorphanol. Chick retinal membranes bind ^{3}H-(D-Ala^2-D-Leu^5) enkephalin in a saturable manner. Specific binding appears to be to a single class of site with K_{Diss} equal to 4.7×10^{-10} M; there are 131 fmoles/mg of membrane protein. All of the bound counts are displaceable by the highly delta selective peptide DTLET with an IC_{50} of 7×10^{-8} M. The mu selective peptide morphiceptin is ineffective as a displacer. Therefore ^{3}H-(D-Ala^2-D-Leu^5) enkephalin binds to delta sites. ^{3}H-dihydromorphine (^{3}H-DHM) binds poorly to chick retinal membranes. The binding curves for ^{3}H-DHM indicate several classes of sites. 13 fmoles/mg of the specific binding is displaceable by morphiceptin; these sites correspond to mu sites characterized in other systems. Benzomorphan sites were assayed by following the procedure of Chang et al. (PNAS 78:4141, 1981). A total of 88 fmoles/mg of benzomorphan binding sites were measured. In the chick retina all of the enkephalin is contained in a subset of amacrine cells; in spite of the limited distribution of enkephalin, the retina contains three distinguishable types of binding sites for opiate alkaloids and peptides. ^{3}H-diprenorphine (^{3}H-DPN) binds to delta, mu and benzomorphan sites with equal affinity. It was therefore used to assess the overall level of opiate binding in chick, rabbit and goldfish retinas since the last two retinas are extensively studied physiologically. The values found were 270, 60, and 44 fmoles/mg in the chick, rabbit and goldfish retina respectively. There appears to be major variation in the concentration of opiate binding sites in different vertebrate retinas. Due to low levels of total binding we did not attempt to classify rabbit and goldfish binding sites according to subtype.

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154.2 STEREOSPECIFIC OPIATE BINDING SITES OCCUR IN COATED VESICLES. C.J. Coscia, D.B. Bennett*, B.L. Roth* and M.B. Laskowski. St. Louis University School of Medicine, St. Louis, MO 63104.

Coated pits have been implicated in receptor-mediated endocytosis and exocytosis. Demonstration of receptor binding in clathrin-coated vesicle (CV) preparations that may be derived from coated pits has complemented light and electron microscopy (EM) evidence for internalization. We discovered a population of opioid binding sites in brain smooth microsomes that may be internalized receptors or intracellular precursors of receptors. CV's were prepared from bovine forebrain by the modifications of the Pearse method utilizing sucrose or deuterium oxide density gradients followed by gel permeation chromatography (Pfeffer and Kelly, J. Cell Biol. 91, 385-391, 1981). Homogeneity was monitored by EM and SDS polyacrylamide gel electrophoresis (PAGE). EM revealed that the predominant (up to 98% of the total) organelles were CV's and empty hexagonal baskets. Diameters of the CV's ranged from 50-166 nm. Upon SDS-PAGE of the CV fraction the most prominent band appeared at ~180,000 daltons. There were also three additional bands giving the overall pattern characteristic of CV's. Using the glass fiber filter binding assay it was found that both 0.5 nM naltrexone and etorphine exhibited binding to CV's. Specific naltrexone binding in CV's from gradient fractions were increased 2.5-fold over the 100,000 g pellet. An additional 7-fold enrichment in specific binding was observed after gel permeation chromatography concomittant with an increase in the volume density of CV's in electron micrographs. Naltrexone binding was stereospecific and etorphine binding was inhibited by 100 mM NaCl (40%). Both naltrexone and etorphine binding are inhibited by 50 μM Gpp(NH)p (40-50%). In summary, purified bovine brain CV's contained high affinity stereospecific opiate alkaloid binding sites with characteristic opioid binding properties. Supported by NSF Grant BNS 8114947.

154.3 IN VIVO EVIDENCE FOR INTERACTIONS AMONG MULTIPLE OPIOID BINDING SITES IN CARDIOVASCULAR RESPONSES TO ENDOTOXEMIA. J.W. Holaday, J.R. Kenner*, C.E. Glatt* and J.B. Long. Neuropharm. Branch, Dept. Med. Neurosci., Div. of NP, Walter Reed Army Institute of Research, Washington, DC 20307.

We have shown that the hypotensive effects of endotoxemia appear to be mediated in part by actions of endogenous opioids upon δ receptors in the CNS (D'Amato, R.J. and Holaday, J.W. PNAS 81, 2898-901, 1984). Additionally, μ antagonists, while without actions by themselves, prevent the usual therapeutic effects of δ antagonists in this model. Since many opioid alkaloids and peptides which are defined as κ receptor agonists [e.g. nalbuphine, nalorphine, bremazocine, β-funaltrexamine (β-FNA) and dynorphin 1-13] also function as μ antagonists, the present studies were conducted to evaluate the possible interactions among selected κ agonists (μ antagonists) and δ antagonists in the rat model of endotoxic hypotension. Specifically, cardiovascular effects of the endogenous κ agonist (μ antagonist) dynorphin 1-13 were evaluated alone and in combination with naloxone or ICI 174864 (Allyl$_2$-Tyr-Aib-Aib-Phe-Leu-OH), a novel δ antagonist, in endotoxemic rats.

ICI 174864 (3 mg/kg, i.v.) by itself increased mean arterial pressure (MAP) by about 10 mmHg, whereas naloxone by itself (5 mg/kg, i.v.) had no pressor actions. Following E. coli endotoxin injection (22.5 mg/kg, i.v.), MAP fell by 20 mmHg; subsequent injection of these doses of ICI 174864 or naloxone significantly increased MAP during the subsequent 60 minute interval. In contrast, neither pretreatment with dynorphin 1-13 (0.1 or 1.0 mg/kg, i.v., 120 min. prior to endotoxin), nor injection of dynorphin 1-13 (1.0 mg/kg, i.v.) following endotoxin, altered the usual pattern of shock hypotension. However, dynorphin 1-13 pretreatment did block the usual pressor effects obtained by naloxone, but not ICI 174864.

It has previously been shown that β-FNA, a κ agonist and μ antagonist, was without effect in reversing shock, but prevented the actions of another δ antagonist, ICI 154129 (see above ref). Since dynorphin 1-13 shares these properties with β-FNA, we suggest that these common associations between κ agonists, μ antagonists and δ antagonist blockers may indicate functional interactions among these three binding site subtypes. Unfortunately, intrinsic pressor effects of the novel "δ antagonist" ICI 174864 prevented its utility in confirming interactions with δ receptors in these studies.

Despite the apparent complexities of these observations, the many simultaneous opioid actions of these ligands form a consistent pattern of interactions among κ, μ, and δ opioid binding sites which are predictive of functional coupling within a common opioid receptor macromolecular complex.

We thank M. Rance & R. Cotton (ICI Pharm) for ICI 174864, and N. Lee, H. Loh & J-K Chang (Peninsula) for dynorphin 1-13.

154.4 A QUANTITATIVE STUDY OF [^{3}H]ENKEPHALIN BINDING TO MEMBRANES OF RAT BRAIN: EVIDENCE FOR A TWO-SITE ALLOSTERIC MODEL. R.B. Rothman, W.D. Bowen*, A.E. Jacobson*, T.R. Burke, Jr.*, K.C. Rice*, and C.B. Pert. Section on Brain Biochemistry, NSB, NIMH, Laboratory of Chemistry, NIADDK, Bethesda, Maryland 20205, and Section of Biochemistry, Division of Biology and Medicine, Brown University, Providence, Rhode Island 02912.

It is widely accepted that [^{3}H]DADL ([^{3}H]D-ala^2-D-leu^5-enkephalin) labels two binding sites on rat brain membranes in vitro. Most workers assume that mu ligands competitively displace [^{3}H]DADL from both binding sites (two-site competitive model). In this study we consider the "two-site allosteric model", which supposes that mu ligands are competitive inhibitors at the higher affinity binding site, and noncompetitive inhibitors at the lower affinity binding site. Rat brain membranes derived from whole brain and from thalamus/hypothalamus were prepared as previously described (Rothman, et al., Neuropeptides 3:493-499, 1983). Using [^{3}H]DADL, DSTLE (TYR-D-SER-GLY-PHE-LEU-THR) binding surfaces (Rothman, Neuropeptides 4:41-44, 1983) were generated. Analysis of the DSTLE binding surfaces using curve fitting techniques showed that the two-site competitive model fit significantly ($p<.01$) better than the two-site allosteric model. OXY (oxymorphone) was an apparent noncompetitive inhibitor of [^{3}H]DADL binding to thalamic/hypothalamic membranes. Analysis of the oxymorphone surface showed that the two-site allosteric model fit significantly better than the two-site competitive model ($p<.001$). Membranes devoid of detectable higher affinity binding sites were prepared using the site-directed, delta-selective, alkylating agent FIT (Rice, et al., Science 220:314-316, 1983; Rothman, et al., Neuropeptides, in press). Oxymorphone was an apparent noncompetitive inhibitor of [^{3}H]DADL binding to the lower affinity binding site. These results support the hypothesis that [^{3}H]DADL labels two delta binding sites in rat brain, mu-competitive and mu-noncompetitive binding sites, respectively. Autoradiographic studies suggest that the mu-competitive and mu-noncompetitive binding sites correspond to type-II and type-I receptors, respectively, supporting the notion that the type-I receptor is a conformationally malleable receptor complex consisting of distinct yet interacting mu and delta binding sites.

154.5 CHARACTERIZATION OF OPIATE RECEPTOR BINDING IN OFFSPRING OF ALCOHOL INGESTING RATS. W.J. Shoemaker, L. Randolph*, and F.E.Bloom. The Salk Institute, La Jolla, CA and Research Institute of Scripps Clinic, La Jolla, CA 92037.

Offspring of female rats consuming a liquid diet containing ethanol show a variety of defects including behavioral, neuroanatomical, neurotransmitter and facial abnormalities. We have used varying amounts of ethanol and protein in a liquid diet formulation to produce groups of offspring that are exposed to pre-natal alcohol, prenatal undernutrition, or both. A consistent finding with this paradigm is the altered levels of the opioid peptides, β-endorphin and enkephalin, that are measured in several brain regions at birth. β-endorphin levels in hindbrain and midbrain are greatly increased in alcohol exposed pups at birth: the levels of the peptide correlate positively with the blood alcohol level of the mother during gestation (Mongraphs in Neural Science 9:130-139, 1983). Using ^{3}H-dihydromorphine (DHM) to characterize the opiate binding sites, dissected brain regions from 5 to 20 newborn pups were pooled for Scatchard analysis over 14 concentrations of DHM (range 0.1 nM to 10 nM). Similar groups of treated offspring were fostered onto normal mothers at birth, weaned at 21 days of age and opiate binding studied at 3-6 months of age. A similar binding protocol and dissection procedure were followed except that regional brain tissues were not pooled. In chow diet and pair-fed control groups, DHM binding followed patterns seen previously; i.e. a low affinity site (K_D = 1-5 nM) and a high affinity site (K_D 15-60 nM) in all regions studied. However, in the telencephalon and midbrain of the alcohol-exposed offspring only high-affinity DHM binding was seen. When these pups are allowed to mature to adult, the binding revealed essentially the same pattern; telencephalic and midbrain DHM low-affinity binding sites were very low or absent in those animals that also have high levels of β-endorphin. Further studies are needed to specify the receptor sub-type(s) deficient, and whether these results hold implications for coordinate control of binding sites by specific neurotransmitters (Supported by NIAAA 06420).

154.6 ELECTROPHYSIOLOGICAL ASSESSMENT OF MULTIPLE OPIOID RECEPTOR TYPES IN RAT HIPPOCAMPUS USING SELECTIVE RECEPTOR INACTIVATION. C. Chavkin, S.J. Henriksen, G.R. Siggins, and F.E. Bloom. Div. Preclin. Neurosci. & Endocrin., Research Inst of Scripps Clinic, La Jolla, CA 92037.

We investigated the opioid receptor type mediating the effects of the dynorphins in the rat hippocampus. The response of rat hippocampal CA1 pyramidal cells to electrical stimulation is known to be facilitated by opioids. Using the in vitro hippocampal slice preparation and recording the amplitude of the electrically evoked population field response in the CA1 region, we found that the opioid peptides dynorphin-A(1-17) and dynorphin-B(1-13) have the same effect as normorphine, leu-enkephalin (LE) and [d-Ala, d-Leu]-enkephalin (DADLE). Opioids were tested by bath application at concentrations from 0.05 to 10 μM, and the rank order of potency was found to be DADLE > normorphine > dynA = dynB = LE. The effects of each of the five opioids at the concentrations listed above were blocked by superfusion with 1 μM naloxone. Compared to other assay systems measuring opioid action, the relative potencies of dynA and B were low, requiring 10 times more dynA than DADLE for an equal effect. Two other opioids with kappa receptor selectivity, ethylketazocine and U50,488H were both about 100 times less potent than DADLE.

Treatment of the in vitro hippocampal slice with 1.0 μM β-funaltrexamine (βFNA) for 15 min followed by 60-90 min of washing by continuous superfusion, blocked the subsequent effects of normorphine, dynA and dynB but did not reduce the effectiveness of DADLE or LE. Identical treatment of the slice with vehicle alone did not change the potency of any of the five opioids. Extensive characterization of the selectivity of βFNA by Takemori, Portoghese and coworkers has demonstrated its selectivity as an irreversible antagonist at mu-type opioid receptors. Our data suggest 1) that both mu and delta receptors are functionally represented in this region of rat hippocampal cellular field, but kappa receptors are not, 2) demonstrate that these receptor categories can be distinguished by a selective receptor inactivation paradigm in this brain region, and 3) provide the first evidence that in a region lacking kappa receptors, the dynorphins can act as agonists at the mu opioid receptor. This study supported by NIDA 03665 and NIAAA 07456.

154.7 A MONOCLONAL ANTIBODY TO A MU OPIATE BINDING SITE. J.M. Bidlack and R.R. Denton*. Center for Brain Research, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642.

A monoclonal antibody to an opiate binding site in rat neural membranes has been generated by the use of hybridoma technology. Mice were immunized with a partially purified opioid receptor complex obtained from a 14-bromoacetamidomorphine affinity column. Spleen cells from an immunized BALB/c mouse were fused with P3-X63-Ag8.653.3 myeloma cells. Supernatants from cell lines were initially screened for antibody production by radioimmunoassay using the ^{125}I-receptor complex. Cell lines producing an antibody to the complex were twice cloned by limiting dilution and bulk cultured. From 1032 cell lines tested, 32 cell lines produced an immunoglobulin to the ^{125}I-receptor complex. One cell line, OR-689.2.4, produces an IgM cryoglobulin that inhibits opiate binding to rat neural membranes. Under equilibrium binding conditions, using 0.25 mg/ml of rat neural membrane protein, 10^{-10} moles of OR-689.2.4 inhibited 45% of the binding of 0.1 nM ^{3}H-dihydromorphine (^{3}H-DHM). The maximum number of moles of ^{3}H-DHM binding sites inhibited occurred at 2 nM ^{3}H-DHM. The inhibition of the binding of ^{3}H-[D-Ala^{2},N-Me-Phe^{4},Met-(O)^{5}-ol]enkephalin and ^{3}H-naloxone by OR-689.2.4 followed a similar pattern. The immunoglobulin could inhibit the binding of ^{3}H-D-Ala^{2}-D-Leu^{5}-enkephalin (^{3}H-DADLE), a putative delta ligand, and ^{3}H-ethylketocyclazocine, a kappa ligand, but not to the degree that it inhibited the binding of mu type ligands. The antibody does not inhibit the binding of ^{3}H-DADLE to NG108-15 cells, a neuroblastoma-glioma cell line that contains only the delta type opioid receptor. The cryoglobulin will precipitate the opioid receptor from a CHAPS solubilized preparation of rat neural membranes. After incubating at 4° for 16 hr, the immunoglobulin - receptor complex was precipitated by the addition of goat anti-mouse Ig conjugated to agarose. Binding to the supernatant fraction showed a titrable reduction in the total number of ^{3}H-naloxone binding sites to a maximal reduction of 48%. OR-689.2.4 appears to be acting as a competitive inhibitor to a mu type opiate binding site.

154.8 ANALGESIC EFFECTS OF μ ANTAGONISTS AFTER NALOXONE NON-REVERSIBLE STRESS INDUCED ANALGESIA. *P.Sacerdote,L.Vicentini,*P.Mantegazza,A.E.Panerai.Dept.Pharmacol.School of Medicine, University of Milano, Milano,20129, Italy.

An analgesic effect of naloxone has been reported in the human,and the rat, and it appears to be specifically related to the occupation of the opiate receptor.In most occasions,the analgesic effect of naloxone was observed not in the normal human or rat,but in particular conditions such as post-operative pain,pain insensitive subjects,or rats with chronic arthrytis. In our experiments,two antagonists atthe μ opiate receptor site:naloxone and naltrxone,and three agonist-antagonist compounds: nalorphine,buprenorphine and diprenorphine, at doses usually not analgesic, elicited analgesia in rats when administered after non-naloxone-reversible shock induced analgesia had disappeared. Another agonist-antagonist compound:nalbuphine,the K receptor antagonists MR 2266,and MR 1452, and the δ antagonist ICI 154129 were all ineffective. None of the compounds tested elicited analgesia after the naloxone-sensitive shock induced analgesia. The analgesic effect observed after naloxone seems to be elicited in the central nervous system since it was not duplicated by the quaternary derivative naloxone methyl-bromide (MR 2453),which does not cross the blood brain barrier. These results suggest that u opiate receptor may change its conformation in particular conditions such as continous inescapable shock and μ antagonists and agonist-antagonists can behave as agonists and induce analgesia under particular conditions, such as continous,inescapable shock, but not under apparently similar conditions such as intermittent shock. The reason for this ambigous behaviour of naloxone and the other μ antagonist remains however unexplained. One might consider our results an in vivo proof of the one-receptor-multiple-conformations model proposed. If this was true, one might speculate that naloxone-reversible and non-naloxone-reversible shock-induced analgesia are the result of different aspects of a single receptor that changes consequently to the different treatments and becomes sensitive to the K or μ endogenous agonists in eliciting the two types of analgesia.

154.9 AUTORADIOGRAPHIC DEMONSTRATION OF THE UNIQUE DISTRIBUTION OF mu_1 OPIOID RECEPTORS IN RAT BRAIN. R.R.Goodman and G.W.Pasternak. Lab. of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center,N.Y.,N.Y.10021

All opioid ligands bind to a common high affinity receptor subtype, termed the mu_1 receptor. This receptor has been found to mediate the supraspinal analgesia of a variety of opioid agonists and has been distinguished from other receptor subtypes that mediate opioid lethal, respiratory depression and certain endocrine effects. Homogenate assays have demonstrated a unique regional distribution and developmental appearance of mu_1 receptors. The present study utilized quantitative in vitro autoradiography to demonstrate the unique distribution of mu_1 receptors in rat brain. Since no presently available ligand allows direct labeling of mu_1 receptors alone, several distinct indirect approaches were used. ^{3}H-Dihydromorphine (^{3}H-DHM) and ^{3}H-[D-Ala^{2}-D-Leu^{5}]Enkephalin (^{3}H-DADL) each label mu_1 receptors as well as another receptor subtype with high affinity(mu_2 and delta, respectively). Their mu_1 labeling was selectively displaced in adjacent thin brain sections, using both reversible ligands and an irreversible ligand, and autoradiograms were generated on tritium sensitive film. Computerized densitometry was then utilized to determine regional variations in the proportion of mu_1 binding for ^{3}H-DHM and ^{3}H-DADL. As an example, the percentage of ^{3}H-DHM(1.0nM) displaced by DADL(5.0nM) varied throughout many of the brain regions examined. Relatively high mu_1 proportions occur in numerous regions, including midline thalamic nuclei, periaqueductal grey, raphe nuclei, hypothalamic nuclei and medial nucleus accumbens. Somewhat lower proportions occur in certain of the examined regions, including the clusters in the caudate nucleus. Relatively low mu_1 proportions occur in the pyramidal cell layer of the hippocampus and in certain regions of the cerebral cortex. Also, computer generated subtraction images allow for a direct visualization of the unique mu_1 distribution.

154.10 COMPARISON OF μ, K, AND σ RECEPTOR DISTRIBUTION IN RAT BRAIN USING IN VITRO LIGHT MICROSCOPY AUTORADIOGRAPHY. A. Tempel and R.S. Zukin. Depts. of Biochemistry and Neuroscience, Albert Einstein Coll. of Med., Bronx, NY 10461.

A wide body of biochemical, pharmacological and behavioral evidence indicates that the actions of opiates upon nervous tissue are mediated by μ, δ, and K opioid receptors and the related σ receptor. We have used in vitro light microscopy autoradiography to determine the precise neuroanatomical distribution of the μ, K, and σ receptors. Autoradiograms were prepared using sections of frozen rat brain and the dry film method, controlling for background film density, nonspecific binding, and variations across films. Autoradiograms of sections labeled with ^{3}H-dihydromorphine revealed the classic μ distributional pattern. In the striatum, discrete patches of densely-labeled opiate receptors were observed, surrounded by areas of diffusely-organized receptors. In the region of the hippocampus dense labeling of μ receptors was seen to overlie molecular layers; diffuse labeling was observed in the surrounding areas. Other structures exhibiting a particulary high density of μ receptors included certain of the thalamic nuclei and layers I and III of the neocortex. Notably, the cerebellum was devoid of this receptor type. K receptors (labeled using ^{3}H-Ethylketocyclazocine in the presence of μ and δ blockers) were of similar distribution to μ receptors in the striatum and hippocampus but were also present in the cerebellum. Density and distribution of σ receptors in the hippocampus was found to be similar to that of μ and K receptors. In contrast, σ receptor labeling in the striatal area was nearly uniform; notably lacking were the dense patches characteristic of the μ receptors. Other areas of high σ receptor density were the central gray area, the interpeduncular nucleus, the posterior hippocampal formation and the cerebellum, motor nuclei of the 5th nerve, the locus coeruleus and the parabrachial nuclei of the brainstem. Thus, pronounced differences in the neuroanatomical distributions of the μ, K and σ receptors may account in part for the diverse pharmacological profiles of μ, K, and σ opioids, exhibited by their prototypic drugs.

(This work is supported by NIH grants DA 01843 & DA 00069.)

154.11 OCCUPATION OF BRAIN OPIATE RECEPTORS FOLLOWING IN VIVO ADMINISTRATION OF MORPHINE. J.W. Lewis, M.E. Lewis, and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

Pharmacological, behavioral, and in vitro binding data indicate the existence of multiple receptors for opioid peptides. The present studies represent our initial attempt to describe brain opiate receptor occupation following in vivo administration of morphine and to correlate these data with behavioral analgesia.

Rats were given morphine (2.5-20 mg/kg) or saline (s.c.). Pain-sensitivity was assessed prior to, and 60 min following, drug injection using the hot-plate test. Immediately following analgesia testing, rats were sacrificed, and their brains removed and homogenized. To separate endogenous peptides and morphine not associated with membrane receptors, homogenates were centrifuged and the pellet resuspended in buffer. To further dissociate bound materials some tissues were centrifuged a second time prior to binding. Mu receptor binding was defined using 3H-dihydromorphine or 3H-DAGO; delta binding using 3H-D-ala-D-leu-enkephalin, or 3H-DSTLE; and kappa binding using 3H-bremazocine in the presence of 100 nM DAGO and DSTLE.

As expected, morphine caused a dose-dependent analgesia. Compared to controls, tissues from morphine-treated rats, centrifuged 1 time prior to binding, showed primarily a dose-dependent loss of mu binding as well as a loss of delta binding, but to a lesser extent. This presumably reflects occupation of these receptors by morphine. By contrast, binding to morphine-treated tissues centrifuged 2 times revealed a marked increase (50-100% above control values) in mu binding sites with a concomittant loss of delta sites. This apparent generation of mu sites seems to require presence of drug in vivo as it is not mimicked by pre-incubation of homogenates with morphine. Administration of morphine (5 mg/kg) does not appear to affect kappa binding.

The physiological signficance of increased mu binding following acute administration of morphine remains to be determined. Data from morphine-tolerant rats suggest that a dose of morphine, which no longer elicits analgesia, still causes increased mu binding thus dissociating this effect from alterations in analgesia. (Supported by NIDA grants DA02265 & F32DA05221)

VISUAL CORTEX: STRIATE AREA II

155.1 GENESIS OF VISUAL CORTICAL NEURONS IN THE FERRET. C. A. Jackson*, J. D. Peduzzi, and T. L. Hickey. School of Optometry/The Medical Center, University of Alabama in Birmingham, Birmingham, Alabama 35294.

^{3}H-thymidine autoradiographic techniques were used to determine the time period of neuronal cell birth for the ferret (Mustela putorius furo) visual cortex and the final adult locations of visual cortical neurons that share a common birthdate. Prenatal cortical neurogenesis was studied using injection procedures described earlier (J. Neurosci. Methods, 8: 139-147, 1983). Individual ferret fetuses were exposed to a single 'pulse' injection of ^{3}H-thymidine administered on a given gestational day between embryonic day 22 and embryonic day 38 (E22-E38). Postnatal cortical neurogenesis was studied in ferret kits ranging in age from birth (E41 ± 1 day) to postnatal day 14 (P14), using intraperitoneal injections of ^{3}H-thymidine. All radioactively labeled animals were sacrificed at maturity (2-4 postnatal months) and the tissue processed according to standard autoradiographic techniques.

The earliest generated visual cortical neurons are produced on, or slightly before, E24 and are located in the white matter beneath layer VI and in the depths of layer VI. Neurogenesis of the neurons in the white matter continues until E28, while the genesis of layer VI neurons continues until E36. Layer V neurons are also produced during the latter part of this period (E30-E36). The majority of layer IV neurons in the visual cortex are generated between E36-E40, although a few are generated as early as E32. Neurogenesis for layers III and II begins in the last few days of gestation (E40-41) but occurs primarily during the first postnatal week. Visual cortex neurons generated (on P14) are located at the border between layers II and I.

These results establish the existence of an inside-out pattern of neurogenesis in the ferret visual cortex, a pattern that is qualitatively similar to that described in other species. Our findings also show that the genesis of visual cortex neurons in the ferret extends into postnatal life, making the ferret especially well suited for studies of visual cortical development.

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155.2 RETINAL CONSTRAINTS UPON ORIENTATION SENSITIVITY IN CAT VISUAL CORTEX. J.D. Schall, A.G. Leventhal and *D.J. Vitek. Dept. Anat., Univ. Utah, Sch. Med. S.L.C., Ut. 84132

Most ganglion cells in cat retina are sensitive to stimulus orientation. Outside of the area centralis, retinal ganglion cells respond best to stimuli oriented radially, i.e. oriented parallel to the line connecting their receptive fields to the area centralis (Levick and Thibos, 1982, J. Physiol. (Lond.) 329:243-261). This radial orientation bias appears to reflect the radial orientation of retinal ganglion cell dendritic fields (Leventhal and Schall, 1983, J. Comp. Neur. 220:465-475).

There is also a radial bias in the distribution of preferred orientations of cells in cat striate cortex (Leventhal, 1983, J. Comp. Neur. 220:476-483). In order to see how retinal orientation sensitivity relates to cortical orientation sensitivity, we have compared the distribution of the orientations of retinal ganglion cell dendritic fields with the distribution of the preferred orientations of cells in retinotopically corresponding parts of visual cortex.

We have analyzed the orientations of about 1200 retinal ganglion cells and determined the preferred orientations of about 1200 cells in cortical areas 17 and 18. The results indicate that, outside of the area centralis, the distributions of the dendritic field orientations of retinal ganglion cells subserving the horizontal, vertical and diagonal meridians differ significantly. Similarly, outside of the representation of the area centralis in areas 17 and 18 the preferred orientations of cells subserving different retinal meridians differ. Comparisons of the retinal and cortical distributions indicate that the distributions of the orientations of beta cells located along the horizontal, vertical and diagonal meridians do not differ significantly from the distributions of the preferred orientations of area 17 S cells subserving the horizontal, vertical and diagonal meridians, respectively. Similarly, the distributions of the orientations of alpha cells do not differ from the distributions of the preferred orientations of cells having narrow receptive fields in retinotopically corresponding regions of area 18.

These results suggest that the distribution of the preferred orientations of first order cells in visual cortex is constrained by the distribution of the orientations of ganglion cells in topographically corresponding regions of retina. Supported by PHS grant EY04951.

155.3 THE LAMINAR PROJECTIONS OF X- AND Y-CELL AXONS IN LAYER IV OF CAT AREA 17 REFLECT THE CELLS' LOCATIONS WITHIN THE DEPTHS OF THE A-LAMINAE OF THE LGN. A.L. Humphrey and D.J. Uhlrich*, Dept. of Neurobiology & Behavior, SUNY-Stony Brook, NY 11794.

The axon terminal fields of single, physiologically identified geniculocortical cells were visualized using intracellular injections of HRP. Terminal fields of X- and Y-cell axons were found to overlap substantially in layers IV and VI of area 17. The 12 X-cell axons analyzed in detail exhibited considerable heterogeneity, with some terminating mainly in layer IVb, others mainly in layer IVa, and still others throughout the depth of layer IV. The latter two groups also projected up to 100μm into lower layer III. The 7 well analyzed Y-cell axons terminated primarily in layer IVa and up to 200μm into lower layer III, but 2 also arborized throughout the depth of layer IVb. Both X- and Y-cell axons terminated throughout the depth of layer VI, although there was considerable cell-to-cell variability in the size and sub-laminar position of this layer VI input.

We also retrogradely labeled the cell bodies of 10 X- and 7 Y-cell axons injected in cortex and found that much of the variability in the laminar terminations within layer IV reflects the cells' locations within the depths of the geniculate A-laminae. X-cells located in the dorsal or ventral thirds of lamina A or A1 projected mainly to layer IVa or throughout layer IV. Those located in the central thirds projected mainly to layer IVb ($r=0.63$, $p<0.05$). Y-cells showed a similar positional relationship but they appeared to follow different rules. Y-cells in the outer thirds of the A-laminae projected mainly to layer IVa; those in the central third of either lamina, in addition, expanded their projections to include the full depth of layer IVb ($r=0.75$, $p<0.05$). Differences in cell body location apparently were not related to variations in the cells' projections within layer VI.

To date, we have observed no obvious physiological differences among the X-cells or among the Y-cells that relate to their heterogeneous projection patterns in layers IV or VI. Thus, the functional significance of this geniculocortical organization, both in the LGN and in cortex, is unknown. It is clear, however, that the result of the geniculocortical projection upon layer IV is not to segregate X- and Y-afferents into lower and upper tiers (cf. Ferster & LeVay, J. Comp. Neurol. [1978] 182:923-944). Rather, it may be to re-establish a positional organization existing within the depths of the geniculate A-laminae.

Supported by USPHS grants EY04091 and EY05688.

155.4 THE PROJECTION PATTERNS OF PHYSIOLOGICALLY-IDENTIFIED GENICULATE AXONS IN AREA 18 OF THE CAT. D.J. Uhlrich* and A.L. Humphrey (SPON: R. Weller), Dept. of Neurobiology and Behavior, SUNY at Stony Brook, NY 11794.

We studied the geniculocortical projection to area 18 by injecting HRP into single, physiologically-identified axons in the optic radiations. For many axons, we obtained both anterograde labeling of the terminal fields and retrograde labeling of the somata in the LGN.

The laminar projections of 9 axons (8 Y, 1 non-Y) in area 18 were analyzed in detail. They arose from somata in the A-laminae, lamina C and the medial interlaminar nucleus (MIN). Most arborized densely within layer IVa and the lower 200-400μm of layer III and provided little or no input to layer IVb or layer VI. Thus, except for a more pronounced input to layer III, the laminar projections of Y-cells to area 18 are similar to those of their area 17 counterparts.

Most axon terminal fields in area 18 were 2-3 times larger than those in area 17. They spread over 2.0 to 2.8 mm^2 of layer IV; one occupied up to 70% of the mediolateral extent of area 18, while others spread over similar distances anteroposteriorly. This indicates that a given region of area 18 receives converging inputs from a relatively wide retinotopic region of the LGN. Such convergence may account for the large receptive fields of cortical cells in area 18 (Hubel & Wiesel, J. Neurophysiol. [1965] 28:229-289). The terminal arbors were also highly asymmetric, generally being 2-4 times longer anteroposterially than mediolaterally; they may provide the structural basis for the anisotropic organization of the azimuth and elevation lines in area 18 (Tusa et al., J. Comp. Neurol. [1979] 185:657-678).

Some Y-cell axons bifurcated in the white matter to innervate both areas 17 and 18, but most innervated one area or the other. All bifurcations occurred at the level of the lateral sulcus or higher in cortex, within 1-5 mm of the termination zones in the grey matter. Of 12 well-labeled Y-cell axons arising from the A-laminae, 2 branched to areas 17 and 18, 9 projected to area 17 only and 1 projected to area 18 only. Of 4 lamina C Y-cells, 2 branched and 2 projected only to area 18. Thus, in each region of the LGN, there are substantial and dedicated populations of Y-cells that project either to area 17, to area 18, or to both areas via branching axons (Geisert, J. Comp. Neurol. [1980] 190:793-812). These results directly demonstrate the existence and location of branching Y-cell axons and also suggest that, at least in the A-laminae, they are a minority of the Y-cell population. (Supported by USPHS grants EY05688 and EY04091.)

155.5 EVIDENCE FOR THE SEGREGATION OF PARVO- AND MAGNOCELLULAR CHANNELS IN THE VISUAL CORTEX OF THE MACAQUE MONKEY. J.H.R. Maunsell and P.H. Schiller*. Dept. of Psychology, Massachusetts Institute of Technology, Cambridge, MA 02139.

Neurons in the magnocellular layers of the primate lateral geniculate nucleus (LGN) relay visual information with faster conductance speed and greater response transience than those in the parvocellular layers. We have collected data on latency and transience in visual cortex which suggest that the information relayed through the magnocellular and parvocellular laminae of the LGN remain segregated through several levels of processing in the cortex.

Responses to multiple presentations of optimally oriented stationary gratings were collected from single units in an alert, behaving rhesus monkey. Data were collected from V1 and two extrastriate visual areas: V4 and the middle temporal visual area (MT). In V1 both transient and sustained responses were obtained; in V4 nearly all cells were sustained while in MT the majority of cells were transient.

The latency to onset of activity in V1 for the transient population was 28 ms and for the sustained population it was 38 ms. In V4 the latency was 49 ms. The 11 ms difference between activity in the V1 sustained units and those in V4 is consistent with the expected delay in passing through V1 and V2 to reach V4, which is the shortest substantial pathway. In contrast, the 21 ms difference in latency between V4 and the V1 transient population makes it unlikely that V4 is driven by the V1 transient population. The response latency in MT was only 40 ms, suggesting that cells in MT are driven primarily through the transient V1 population.

These results suggest that the magnocellular and parvocellular channels retain their distinction within V1 and V2 and project differentially to V4 and MT.

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155.6 DISTRIBUTION AND PATTERNS OF SYNAPTIC INPUT OF NEURONS IN STRIATE CORTEX THAT CONTAIN A CYTOPLASMIC LAMINATED BODY. G. Einstein, J. Hamos, T. Davis and P. Sterling. Dept. of Anatomy, Univ. of PA Sch. of Med., Phila., PA 19104.

Neurons containing a cytoplasmic laminated body (CLB) have been described in layer IV of cat striate cortex (Winfield, 1979). Their proportion, distribution, and patterns of synaptic input however, have not been studied in detail. We investigated these characteristics to determine whether the presence of a CLB might mark a specific category of cell. Two cats provided 8 and 2 um consecutive sections for light microscopy and two, the thin sections for serial electron microscopy (EM). One cat used for EM investigation was injected intracortically with radioactively labeled gamma-aminobutyric acid (GABA) and the other had lateral geniculate nucleus (LGN) axons labeled by transport of radioactive proteins. CLB-cells represented a significant fraction of all the neurons in layer IV; 11% in IVab and 8% in IVc. In both sublayers they were clumped in groups of 4-8. We observed no CLB-cells that accumulated exogeneous GABA above background levels. CLB-cells were of medium size (10-15 um maximum diameter) and were round or slightly oval. Their nuclei occupied from 43-70% of their cytoplasm. In IVab synaptic contacts were sparsely distributed to the cell bodies (7-13 contacts/100 um^2) and most of these had pleomorphic vesicles with symmetrical synaptic specializations (P/S). LGN axons did not contact the somas or proximal dendrites of IVab CLB-cells. In layer IVc CLB-cells had different patterns of synaptic input. Contacts were densely distributed to somas (16-33 contacts/100 um^2) and in this layer they did receive somatic contacts from the LGN. Labeled and unlabeled contacts were of both the P/S and the R/A form. In summary, CLB-cells in layers IVab and IVc were relatively similar in numbers and distribution. None accumulated GABA, all had large nuclei with respect to their cytoplasm, and all were rich in P/S somatic contacts. These data suggest that they might be spiny stellate cells (LeVay, 1973; Mates and Lund, 1983) and that they do not belong to the category of small, intrinsic neurons thought to mediate intracortical inhibition. There are differences in synaptic input between the sublayers. Those in IVab receive sparse somatic input with none from the LGN while those in IVc receive dense somatic input some of which is from the LGN. Whether CLB-cells form a homogeneous group within each sublayer is as yet to be determined. Supported by USPHS grants EY00828, EY07035, and EY01583.

155.7 **RECEPTIVE FIELD PROPERTIES OF EPSPs AND IPSPs IN CAT VISUAL CORTEX** David Ferster, Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois 60201.

How do the connections of the visual cortex, particularly geniculocortical and intracortical connections, interact to produce the receptive field properties of cortical neurons? The function of inputs to complex cells was examined directly by recording visually evoked synaptic potentials intracellularly and mapping their receptive fields.

Neurons were impaled with 50 Mohm micropipettes filled with 2M K^+ methylsulfate. Visually evoked EPSPs were recorded in the absence of IPSPs by suppressing the IPSPs with hyperpolarizing current. Since it was important to choose levels of current that would avoid contaminating the EPSPs with reversed IPSPs, the effects of the current were monitored by observing the change in potentials evoked by electrical stimulation of the LGN. Electrically evoked EPSPs and IPSPs could be distinguished easily by their different latencies whether they were reversed or not. In a similar way, depolarizing current was used to study visually evoked IPSPs.

Synaptic potentials were recorded from complex cells of layers 2, 3 and 5 in area 17. In all cases, IPSPs and EPSPs showed identical orientation preferences. There was no evidence that mutual inhibition between cells of different orientation contributed to orientation selectivity, even in layer 3 where all cells receive monosynaptic excitation from the LGN. The EPSPs themselves were already well enough oriented to give each neuron the response plotted from extracellular recordings.

Both the EPSPs and IPSPs exhibited a single receptive field. The centers of the two fields were usually superimposed, the maximum responses from each coming at the same point in the stimulus sweep. The IPSP field was often larger than the EPSP field, however. IPSP fields larger in the direction perpendicular to the preferred orientation probably contributed to end-stopping, while elongation of the field in the perpendicular direction was probably important for producing inhibitory flanks. Thus, an IPSP field resembles the surround of a geniculate neuron in that it extends through the entire receptive field but is obvious in extracellular experiments only at its edges where it is not overlapped by the more powerful center mechanism. The "surround" in a complex cell, however, is generated by orientation selective neurons, which in most cases have the same orientation as the cell itself.

155.8 EFFECTS OF AN EXCITATORY AMINO ACIDS ANTAGONIST ON VISUAL RESPONSES OF STRIATE CORTICAL NEURONS IN THE CAT. T. Tsumoto, H. Masui*, and H. Sato*. Dept. of Neurophysiol., Inst. of Higher Nerv. Activ., Osaka Univ. Med. Sch., Osaka, 530 Japan

Glutamate and aspartate are supposed to be excitatory transmitters in the cat visual cortex. To test this possibility and to locate their operating sites in neuronal circuits in the cortex, we studied effects of ionophoretic application of antagonists of the amino acids on visual responses of striate cortical neurons. The animals were anesthetized with a mixture of 70 % N_2O-30 % O_2 with the addition of 0.5-1.0 % Halothane when necessary, and paralysed with Flaxedil. Multibarrelled micropipettes contained kynurenic acid (KYNA) which was reported to antagonize actions of excitatory amino acids (Perkins & Stone, Brain Res, 247, 184, 1982), other antagonists, glutamate, and acetylcholine as a control excitant. Among the antagonists so far tested, KYNA was most effective to abolish visual responses of cortical cells. Therefore, the subsequent analysis was done with KYNA.

In almost all the cells tested, KYNA antagonized excitations induced by application of glutamate but did not block those by acetylcholine. KYNA clearly suppressed visual responses of about 80 % of the cells tested. Suppression was judged as complete, incomplete, or absent when the total number of spikes in the responses was less than 10, 11-49, or more than 50 % of that in the control responses, respectively. Effectiveness of KYNA was related to types of receptive fields and to laminar locations. In 24 of the 36 simple cells tested, KYNA completely suppressed their visual responses while such a complete block was seen in only 6 of the 31 complex cells. The great majority of the cells in layers IVab, IVc and the upper part of layer VI were completely suppressed by KYNA whereas most of the cells in the other layers were incompletely or not suppressed at all.

These findings suggest that glutamate/aspartate may be excitatory transmitters from X- and Y-type geniculate afferents to first-order neurons in the visual cortex.

155.9 ORIENTATION SPECIFICITY IN LAYER 6 OF CAT AREA 17 REMAINS DURING REVERSIBLE INACTIVATION OF SUPRAGRANULAR LAYERS BY COOLING. H.D. Schwark and J.G. Malpeli. Neural and Behavioral Biology Program and Dept. of Psychology, University of Illinois, Champaign, IL. 61820

One component of the vertical organization of visual cortex is the projection of cells in supragranular layers to cells in deep layers. By recording from cells in layer 6 during reversible synaptic inactivation of layers 1-3, we have examined the possibility that this projection may confer orientation specificity upon cells in the deep cortical layers. We have found that layer 6 cells which remain active in the absence of supragranular layer activity retain their specificities for stimulus orientation.

Supragranular layers were inactivated by cooling the cortical surface in the medial bank with a Peltier-controlled copper plate positioned between cortex and dura. The effects of cooling were monitored throughout the vertical extent of the cortex with a linear array of 15 lacquer-coated tungsten microelectrodes. Signals from each electrode were recorded on magnetic tape. During cooling sessions the temperature of the cooling plate (measured at the cortical surface) was initially lowered rapidly to 20-18 degrees C, then more slowly while the activity at various recording sites in the cortex was monitored. This procedure usually silenced activity in the supragranular layers, while deep layer activity continued. Cooling was continued until all visual activity was silenced, after which the cortex was rewarmed. Comparison of visual activity before and after cooling sessions indicated that the effects of cooling were reversible.

Cells in layer 6 which continued to respond to visual stimulation in the absence of supragranular layer activity retained their selectivity for stimulus orientation. These cells, which had simple or complex receptive field properties, remained orientation selective until they were finally silenced as a result of continued cooling. In no case have we recorded from a layer 6 cell which lost its orientation selectivity as a result of cooling. Thus, the supragranular cortical layers are not necessary for orientation selectivity in layer 6 cells. Since orientation specificity in layers 2, 3 and 5 survives the abolition of activity in layers 4 and 6 (Malpeli, J.G., J. Neurophysiol., 49:595-610, 1983) it is likely that this property is created in more than one lamina of the cortex.

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155.10 INTRINSIC, LATERAL GABAERGIC CONNECTION IN PRIMARY VISUAL CORTEX OF THE RAT. A.Burkhalter* and R.W.Baughman (SPON: C.E. Jahr). Dept. of Neurobiol. Harvard Med. School. Boston, MA.

In visual cortex it is uncertain whether intrinsic, lateral connections are excitatory, inhibitory or both. We studied this question by co-localizing GAD immunoreactivity in intrinsic neurons that were retrogradely labelled on the basis of their local projections to layer 4.

Small injections (100-150um in dia.) of fluorescent latex beads (Katz, Burkhalter and Dreyer, 1984) were made into layer 4 of area 17 of the rat. This resulted in a pattern of retrogradely labelled neurons in the vicinity of the injection site. Radially, a column of labelled cells extended throughout layers 2 to 6. Laterally, in layers 2/3, 5, and at the border to white matter in layer 6, the labelling was more wide spread and extended for up to 2 mm.

Sections from visual cortex labelled with fluorescent beads were stained for GAD immunoreactivity. GAD-positive neurons were uniformly distributed. Most, interestingly, in deep layer 6, lateral to the area immediately below the injection site, more than a third of the GAD-positive cells were labelled with beads, while in upper layers no laterally located cells were double labelled. In layer 6 double labelled cells were oriented horizontally and fusiform in shape.

To analyze in detail the axonal arborization of layer 6 cells projecting to layer 4, in particular those with fusiform morphology, tissue slices including the injection site and the surrounding area were prepared, and placed in an electrophysiological recording chamber. Bead labelled cells were impaled under visual control and filled with Lucifer Yellow. Three types of Lucifer/bead labelled cells were observed: Intrinsic, fusiform, sparsely spiny neurons with horizontally oriented somas, dendrites and axon collaterals that travelled in layer 6 for 0.3-0.5 mm before coursing upwards to terminate in layer 4; a morphology closely resembling that of the GAD-positive cells. The somas of two types of extrinsic neurons were oriented vertically or obliquely; both had axons leaving grey matter. In one type axon collaterals stayed within layer 6. In the other type, axon collaterals extended vertically and terminated in layer 4. Similar cells were identified in the cat as claustrum and LGN projecting neurons (L.C.Katz, pers.comm.).

These results indicate, that intrinsic GAD-positive cells in layer 6 contribute to a surround-like projection to layer 4. Such a projection could mediate the suppression of activity within the receptive field of layer 4 neurons.

155.11 INTRINSIC CONNECTIVITY OF IDENTIFIED PROJECTION NEURONS IN CAT VISUAL CORTEX BRAIN SLICES. L.C. Katz, Div. of Biology 216-76, Caltech, Pasadena CA. 91125.

Each lamina in the visual cortex contains a multitude of morphologically distinct pyramidal cells. Cells may be functionally differentiated by the different informational requirements of efferent sites to which they project. Accordingly, the relationship between cell morphology and projection site was analysed in layer 6 of cat area 17. This layer contains two distinct, yet spatially overlapping, populations of cells: those projecting to the visual claustrum, and those projecting back to the lateral geniculate nucleus (LGN). The cell bodies of origin of the two projections were identified by injecting a fluorescent retrograde tracer (latex microspheres, "beads") into the efferent targets. Brain slices of area 17 were prepared several days later. Under microscopic control, individual retrogradely labelled cell bodies were identified, intracellularly impaled, and filled with lucifer yellow. Neurons projecting to the claustrum (30 cells) had 3-5 basal dendrites, with one usually much thicker and longer than the rest, giving the arbor a highly asymmetric appearance. The apical dendrites reached layer 1, with short side branches in layers 5 and 6 only, and their horizontal extent was significantly narrower than that of the basal dendrites. Most dramatically, all claustrum projecting neurons had long (1 mm), thin, horizontally directed intrinsic axonal collaterals which remained in layer 6.

In contrast, LGN projecting neurons (50 cells) had 6-8 basal dendritic arms, all of equal thickness and length, forming a highly symmetric arbor. The apical dendrites reached only to layer 3. Frequent and extensive side branches originated in layers 4, 5 and 6, and their horizontal spread was wider than that of the basal dendrites. In marked contrast to claustrum projecting neurons, the LGN projecting cells had virtually no horizontal axonal collaterals within layer 6, possessing instead thick, recurrent collaterals that terminated within layer 4.

The different patterns of dendrites demonstrate that these two cell classes receive different information from overlying layers, as well as from other areas. The non-overlapping patterns of intrinsic axons reveal that, although they occupy the same laminar position, these two projection classes participate in fundamentally different intrinsic circuits.

HUMAN NEUROPSYCHOLOGY AND BEHAVIORAL NEUROBIOLOGY II

156.1 NMR VERIFICATION OF SURGICAL SECTION OF THE HUMAN CORPUS CALLOSUM AND PRESENCE OF THE ANTERIOR COMMISSURE. M.S. Gazzaniga, J.D. Holtzman*, J.Gates*, M.D.F. Deck*, and B.C.P. Lee*. Depts. of Neurology and Radiology, Cornell Medical College, New York, NY 10021.

There has been intense interest over the past two decades in the neuropsychological assessment of patients who have undergone surgical division of the neocortical commissures. All but one of the most studied patients are living, and all are reported by surgical record to have either complete callosal and anterior commissure section or only callosal section. In recent years a number of interhemispheric interactions have been reported in these patients that range from integration of crude spatial information to transfer of discrete patterned and colored stimuli. The variability seen in such instances does not appear to be related to the presence or absence of the anterior commissure since rich integrations have been reported in patients both with and without the anterior commissure sectioned. It therefore becomes important to verify the extent of commissure surgery in these patients. Until recently this was not possible using imaging techniques such as CT. It has recently been observed by Gates and colleagues that Nuclear Magnetic Resonance (NMR) imaging is capable of detecting callosal sparing after its presumed surgical section. We followed this observation and now report the NMR results of case J.W., a patient who has featured prominently in our neurobehavioral studies. J.W., consistent with other patients with both callosal and anterior commissural section, is capable of cross integration of crude spatial information. Most striking, however, is J. W.'s complete inability to transfer between the hemispheres visual pattern or color information. Consistent with his surgical report, both midline saggital sections using saturation and inversion recovery procedures reveal a complete total resection of the corpus callosum with the expected sparing of the unapproached anterior commissure. These results demonstrate that verification of the extent of forebrain commissurotomy can now be achieved easily and without risk. The results also suggest that, unlike subhuman primates, the intact anterior commissure need not subserve interhemispheric exchange of pattern or color information. It now appears that NMR imaging will allow for an assessment of the extent of cortical commissurotomy and thereby provide accurate physical evidence for possible commissural systems active in interhemispheric interactions. (Aided by USPHS Grant 5-P01 NS17778.)

156.2 COMPREHENSION OF VERBAL NARRATIVE AND VISUOSPATIAL ABILITIES AFTER RIGHT HEMISPHERE DAMAGE. K.L. Moya*, L.I. Benowitz, D.N. Levine* and M.D. Horner* (SPON: M.H. Teicher). Mailman Research Center, McLean Hospital; Depts. of Psychiatry and Neurology, Harvard Medical School; Spaulding Rehabilitation Hospital; Massachusetts General Hospital; Harvard College.

Forty patients with right cortical strokes, 4 neurological controls with right basal ganglia damage, and 10 age-matched normal controls were tested for visuospatial abilities and for comprehension of brief verbal narrative passages. The tests were designed to evaluate the appreciation of individual details, overall form, and relationships among elements for both the verbal and visuospatial material. Forward and reverse digit span were obtained for all subjects. Eight patients were also given an object sorting test to examine their ability to form abstract concepts. Verbal IQ scores were available for 12 of the patients. In accordance with our previous reports (Moya et al, Neurosci. Abstr. 9:918, 1983; Benowitz et al, Neurol. 34:190, 1984), the right hemisphere damaged (RHD) group was found to be significantly impaired in the comprehension of verbal narrative, the extent of which correlated highly with the degree of patients' visuospatial deficits. The appreciation of relationships among elements was the aspect of verbal comprehension that showed the greatest deficit in the RHD group, and also the one that had the highest correlation with constructional apraxia ($r=0.77$, $p<0.0001$). This association held up across individual subjects: no patient who was severely impaired on the visuospatial tasks performed in the normal range on the verbal task, or vice versa. RHD patients' performance on the visuospatial tasks also correlated with their performance on the object sorting test. On the other hand, neither verbal comprehension nor visuospatial abilities were correlated with verbal IQ or with forward or reverse digit span. Analysis of the CT scans indicated that both visuospatial and verbal comprehension deficits were associated with damage in the territory of the right middle cerebral artery (MCA), and that both were exacerbated by the existence of premorbid brain atrophy.

These results indicate that the right hemisphere plays a role in verbal reasoning, perhaps involving the same processes of evaluating the interrelationships among elements that are presumed to underlie visuospatial abilities.

156.3 GESTURE FLUENCY: THE EFFECT OF UNILATERAL CORTICAL EXCISIONS IN MAN. G.W. Jason* (SPON: I.Q. Whishaw). Montreal Neurological Institute, McGill University, Montreal, and Department of Clinical Neurosciences, University of Calgary, Health Sciences Centre, 3330 Hospital Drive N.W., Calgary, Alberta, Canada, T2N 4N1.

Patients with left frontal-lobe lesions are impaired on tests of word fluency, in which they are required under time pressure to say or write as many words as possible beginning with a specified letter (e.g., Milner, in Warren and Akert, Eds., The Frontal Granular Cortex and Behavior, 1964). Patients with right frontal lesions are impaired on "design fluency", in which they are required to produce meaningless abstract designs under a time constraint (Jones-Gotman and Milner, Neuropsychologia, 1977, 15, 653-674). The present study was an attempt to develop a manual analogue of these fluency tasks, here termed "gesture fluency".

Patients with unilateral cortical excisions from the left frontal (LF), right frontal (RF), left temporal, and right temporal lobes and normal control subjects were given two gesture-fluency tasks. Under a two-minute time limit, subjects were first asked to demonstrate as many novel finger configurations as they could. They were then asked to demonstrate as many different meaningful gestures as they could, also in two minutes.

The LF group was impaired on both tasks, with an increased rate of perseveration and decreased novel output. The RF group was impaired on the meaningful-gesture task, showing decreased novel output. The difficulty of these patients seemed to lie in the production of varied responses meeting the requirements of the tasks, rather than decreased ability to generate responses, because no group differences were seen in total output. A general male superiority was found on the gesture-fluency tasks, contrasting with a female superiority on a word-fluency task in the same patients. Neither sex difference interacted with location of the excision.

The deficit after RF lesions appeared to be associated with involvement of ventro-lateral or orbital cortex, although relatively good performance was not inconsistent with involvement of these areas. There was no evidence for localization within the left frontal lobe.

The results extend previous findings of deficits after frontal lesions on other fluency tasks.

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156.4 RECOGNITION MEMORY AND SKILL LEARNING IN HUNTINGTON'S DISEASE. M.Martone,* N. Butters, J. Wolfe*, L. Cermak* . San Diego VA Medical Center, La Jolla, CA 92037.

Two studies are reported assessing skill (procedural) learning and recognition memory in Huntington's Disease (HD). Study 1 : Recall and recognition memory were compared in 10 HD, 9 amnesic patients and 14 normal control subjects using the Rey Auditory Verbal Learning Test. For the recall test, subjects were read 15 words and asked to recall them in any order. Five presentation-recall trials were administered. For the recognition test, subjects were presented a second list of 15 words. After presentation of the 15th word, subjects were read 30 words sequentially (15 targets and 15 fillers) and asked to indicate which words were on the initial list. Five presentation-recognition trials were administered.

Both the HD and amnesic patients were impaired on the recall and recognition tests relative to normals. However, although the 2 patient groups' performance was equally impaired on the recall test, the HD patients' performance on the recognition test was superior to that of the amnesics. Study 2 : Skill learning was assessed in 12 HD patients, 5 alcoholic Korsakoff patients and 10 normal control subjects using the Tower of Hanoi puzzle. The HD patients were divided into early and advanced patients on the basis of years since diagnosis. Subjects solved the puzzle in 2 blocks of 4 trials on each of 2 consecutive days. The number of moves needed to complete the puzzle was recorded for each trial. A recognition test dealing with various aspects of the puzzle was administered at the beginning of the second day of testing.

Both normal control subjects and early HD patients showed a marked decrease in the number of moves needed to solve the puzzle by the end of day 2. In contrast, the advanced HD and Korsakoff patients showed very little improvement. On the recognition test, the HD patients' performance was superior to that of the Korsakoff patients.

The results of these 2 studies provide further evidence that HD patients, unlike amnesics, exhibit better performance on recognition rather than on recall tests of memory. The findings for the Tower puzzle were inconclusive. Although HD patients have been reported to be impaired on a test of skill-based, procedural learning (mirror-reading), amnesic patients usually perform normally on such tasks. It appears then that the Tower of Hanoi may not be a pure indicator of procedural learning. Supported by the VA Medical Research Service and by NIAAA grant AA-00187.

156.5 CEREBRAL METABOLIC RESPONSES TO COMPLEX MOTOR TASKS: NORMAL SUBJECT VERSUS PATIENTS WITH HUNTINGTON'S DISEASE. J.C. Mazziotta, J. Wapenski, M. Phelps. Department of Neurology, Division of Biophysics, Department of Radiological Sciences, UCLA School of Medicine, Los Angeles, CA 90024

Positron computed tomography with F-18 fluorodeoxyglucose was used to measure the cerebral glucose metabolic (LCMRGlc) responses to complex motor tasks in normal subjects (N=11) and patients with Huntington's Disease (HD) (N=3). Paired studies consisting of a control state (no movement) and a stimulation study (writing one's signature) were obtained in all subjects. Normal subjects demonstrated significant activations (increase in LCMRGlc versus control) of the contralateral (to the moving hand) sensory-motor cortical strip (18.6 ± 13.0% S.D.) and bilateral activations of the striatum (18.6 ± 13.0%). Despite normal or minimally atrophied appearance of the caudate on x-ray CT scanning, HD patients have in the control state gross hypometabolism of the striata bilaterally. The HD patients can, however, perform the writing task with good proficiency. During the performance of the task HD patients have activations in glucose metabolism of the sensory-motor cortical strip without metabolic changes in the striatum. In normal subjects that perform novel tasks (sequential finger movements, N=4) contralateral sensory-motor cortical metabolic activations have been observed without metabolic changes in the striatum.

These results suggest that HD patients may use neurophysiological strategies to perform overlearned tasks (writing) that are reserved for novel tasks (finger sequences) in normal subjects. This hypothesis is presently being explored in normals by the evaluation of writing with the nondominant hand. Such studies demonstrate the ability of positron computed tomography to investigate functional cerebral organization in health and disease.

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156.6 GLUCOSE METABOLISM IN VENTROMEDIAL PREFRONTAL CORTEX PREDICTS VISUAL RECOGNITION IN SUBJECTS WITH ALCOHOLIC KORSAKOFF'S SYNDROME. E.S. Parker, R.M. Kessler*, P.R. Martin*, D.T. George*, H. Weingartner*, L. Sokoloff, M.H. Ebert, and M. Mishkin. NIAAA, NIH, NIMH, Bethesda, MD 20205.

Although the neuropathology in alcoholic Korsakoff's Syndrome involves the medial diencephalon, a wider area of cerebral dysfunction may underlie the associated amnesia. To explore this possibility we examined the relation between regional cerebral glucose metabolism (GM) and memory ability in six males with a diagnosis of Korsakoff's Syndrome and eight male normal volunteers. The two groups were matched for age and education. The methods and findings of global and regional reductions in GM in the Korsakoff group are described by Kessler et al. (Soc. Neurosci. Abstr. 10: 1984). Five of the memory tests on which the Korsakoff group was significantly impaired were selected for the analysis. The tests were facial, pictorial, and word recognition, word recall, and figure copying with delay. For each subject group separately, correlations were calculated between performance on each memory test and metabolism in each of seven regions matching approximately three cerebral loci that have been implicated in memory, i.e. medial temporal, medial thalamic, and ventromedial prefrontal (VMP). The seven regions selected were left and right medial temporal, left and right thalamic, and left, middle, and right VMP.

In the Korsakoff group, impairment in facial recognition was correlated with reduction in GM in each of the three VMP regions (ps .05). Similarly, impairment on the picture recognition test was correlated with reduction in GM in the right VMP region (p .05). By contrast, on neither visual recognition test was impairment significantly correlated with GM reduction in other regions implicated in memory or with mean cerebral GM. Further, GM in the three VMP regions was not significantly correlated with impairment on the other memory tests. No significant relation between visual recognition and glucose metabolism in the VMP region was found in the control group.

In monkeys, lesions of VMP cortex produce severe visual recognition deficits (Mishkin & Bachevalier, Soc. Neurosci. Abstr. 9: 29, 1983). The results in monkeys and these in humans suggest that dysfunction of VMP cortex contributes to the amnesia in alcoholic Korsakoff's Syndrome.

156.7 ALTERED FRONTAL AND TEMPORAL LOBE METABOLISM IN MULTIPLE SCLEROSIS. W.A. Sheremata, R.Siddharthan*, P.Ziajka* and S.Sevush*. PETT, Core Facility Mt. Sinai Medical Center, Dept. Neurol., Univ. of Miami Sch. of Med., Miami, FL 33101 (Sponser: W.Weiner)

Cognitive deficit in multiple sclerosis (MS) is common, but often is clinically misinterpreted. Positron emission computed tomography (PET) of the cerebral hemispheres has allowed measurement of glucose metabolism in anatomically defined areas of the living human subject. A neuropsychological study of 30 patients (Sevush and Sheremata, Neurology 1983) led us to conclude that cognitive dysfunction of the frontal lobe type was characteristic of MS.

PET studies using 11C 2-deoxyglucose and a random word recall task were performed in 5 normal subjects and 5 MS patients.

Automated analysis of 46 regions of interest for each brain yielded the following mean values (mg glucose/minute/100 gm brain): 7.26±1.32 for frontal lobes and 7.55±1.63 for temporal lobes in normals. Values of 5.39±1.44 ($p<0.05$) for frontal lobes and 5.40±1.15 ($p<0.05$) for temporal lobes were obtained in MS patients. No significant differences for any other areas of brain were seen.

This pilot study provides evidence that cortical metabolism in frontal and temporal lobes is impaired in multiple sclerosis. Data obtained from magnetic resonance scanning in a larger number of subjects will allow better anatomical correlation. However, it is clear that metabolic alterations are associated with altered cognitive function seen in patients with MS and probably will be found in other white matter disease.

156.8 USING THE "BOOTSTRAP" TECHNIQUE TO UNDERSTAND CEREBRAL INTERREGIONAL METABOLIC RELATIONSHIPS IN CLINICAL STATES. E. J. Metter*, W. H. Riege, D. E. Kuhl, M. E. Phelps (SPON: N. P. Rosenthal). V. A. Medical Center, Sepulveda, CA 91343, and Lab. Nuclear Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

In previous studies using (F18)-fluorodeoxyglucose with positron emission tomography, we have examined region to region metabolic correlations in (1) normal subjects, (2) normal elderly versus younger individuals, and (3) Alzheimer's, Huntington's and Parkinson's diseases for 13 brain regions in each hemisphere. Differences in the correlation matrices between the groups suggested changes in brain function with age and disease. For example, frontal-parietal-occipital correlations were strongly present in normal subjects, while lost entirely in Parkinson's disease and replaced in Alzheimer's disease with frontal-frontal and frontal-temporal correlations. An alternative explanation was that the distribution of the matrices were not distinctive and each was derived from different samples from the same population. To study this issue, we examined the distribution of correlation matrices using a bootstrap simulation. Random samples were drawn with replacement from a given group of subjects and correlation matrices were calculated from each new sample. 1016 matrices were examined for each group of subjects. Correlations were considered as reliable if an r represented a $p<.01$ uncorrected for the number of possible correlations, and the number of reliable correlations was counted for each region to region pair within a matrix. Frequency distributions for each clinical group derived from the bootstrap simulation were compared to each other and to matrices derived from the original subjects. Some original correlations were found not to be as important in the frequency distribution while other correlations not observed seemed to assume greater importance. The overall structure of reliable correlations remained basically unchanged in the frequency distributions for each of the group matrices supporting the reliability of regional intercorrelations.

156.9 INVOLVEMENT OF THE HIPPOCAMPUS AND AMYGDALA IN CLASSICAL AUTISM: A COMPARATIVE NEUROPSYCHOLOGICAL STUDY. P. M. Merjanian, L. Nadel*, D. D. Jans*, D. A. Granger*, I. T. Lott* and M-L. Kean*. Cognitive Sciences Program, University of California, Irvine, CA 92717.

Considerable converging evidence suggests that damage in the hippocampus and/or amygdala is involved in classical autism. Classically autistic and Downs syndrome controls were matched for age, sex, and nonverbal ability and tested on two behavioral tasks adapted from work with brain-damaged animals and sensitive to damage in the amygdala or hippocampus. It seems reasonable to assume that comparable cognitive behaviors in animals and humans would be associated with comparable brain damage.

A behavioral task recently developed for use with brain-damaged monkeys and highly sensitive to damage in the amygdala but not to damage in the hippocampus, was modified and applied to these subjects. The cross-modal delayed nonmatching-to-sample task (Murray & Mishkin, Society for Neuroscience Abstracts, 8, 1982) involves presentation of a baited sample object, followed by a 6-second delay and subsequent presentation of the sample and a baited, novel object. Food rewards or plastic tokens were used to bait the objects. Percent correct and average latency of response were calculated over 200 trials per subject. Each subject was tested for 45 minutes per day until the task was completed. A fixed set of 40 objects were used.

A task sensitive to dysfunction of the hippocampus was adapted from animal work (O'Keefe & Nadel, The Hippocampus as a Cognitive Map, 1978). A food reward was hidden under one of 8 identical objects located on the floor of a room with visually distinctive walls. The subjects were required to walk through the room, looking under the objects until the reward was found. The number of attempts before correct choice was recorded over 50 trials. Solution of the task required the learning of a particular location in the room and hence, was considered to involve the hippocampus.

Results suggest that the present method of adapting tasks from animal work for use with an autistic population is practical and capable of producing results that contribute to our knowledge of brain and behavior relationships in this population.

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156.10 CHRONIC PERSONALITY DISTURBANCE FOLLOWING BILATERAL ORBITOFRONTAL LOBE ABLATION. THE CASE OF PATIENT EVR. P. J. Eslinger and A. R. Damasio. Dept. of Neurology, Univ. of Iowa College of Medicine, Iowa City, IA 52242.

Patient EVR underwent extensive ablation of orbital and lower mesial frontal lobe cortices for the treatment of a meningioma in 1975. Since then he has exhibited profound changes in affective and social behavior. Although he was previously a successful businessman and head of a family, EVR has since been unable to meet personal and professional responsibilities. He cannot arrive at routine daily decisions, plan and follow a coherent course of social or professional action, make sound judgments, or realize the implications of his acts. Yet, his "measurable intelligence" is in the very superior range (98th percentile), a fact responsible for his being considered a malingerer and being denied disability benefits. Comprehensive neuropsychological assessment failed to identify deficits in abstract reasoning, verbal encoding, memory, speech and language, visual perception, spatial processing and praxic abilities. The Wisconsin Card Sorting test was completed in a near-perfect number of trials. Personality inventory (MMPI) reveals only a K+ normal profile. Clinical neurological examination is intact but for anosmia.

Neuroimaging procedures (computerized tomography and single photon emission tomography) demonstrate a well-localized, bilateral frontal lobe lesion which includes most of Brodmann's area 8, 9, 10, 11, 12, 25, 32, 47 and the anterior portion of area 24, as well as areas 45, 46 and 47 in the right hemisphere. There is normal structure and blood flow levels elsewhere including the dorsolateral and the superior mesial sectors of both frontal lobes (cingulate gyri, motor and supplementary motor regions). Subcortical gray and white matter structures are intact.

The findings constitute evidence for a unique and rarely encountered orbitofrontal lobe syndrome. It is characterized by extreme dissociations in behavior, and is quite unlike the syndromes caused by damage to the dorsolateral and mesial-superior aspects of the frontal lobe. Supported by NINCDS Grant PO1 NS 19632-01.

156.11 A POSITIVE CORRELATION BETWEEN FRONTAL CORTICAL BLOOD FLOW AND PERFORMANCE DURING COGNITIVE TESTING IN CHRONIC SCHIZOPHRENIC PATIENTS. Karen Faith Berman*, Ronald F. Zec, and Daniel R. Weinberger* (SPON: R.J. Wyatt). Adult Psychiatry Branch, NIMH, St. Elizabeths Hospital, Washington, D.C. 20032

Regional cerebral blood flow is correlated with regional brain metabolism and neuronal activity, and so it can provide an indicator of brain activity under different conditions. Distinctive regional patterns of brain activity have been found for different types of behavioral activation. What has not been previously reported is a correlation between regional patterns of brain activity and levels of performance on a cognitive task performed during rCBF measurement.

Seventeen patients with chronic schizophrenia ($\bar{x}$ age 30 ± 8) medication-free for at least four weeks and 23 normal volunteers ($\bar{x}$ age 29 ± 8) underwent three consecutive Xe133 inhalation rCBF procedures: first at rest, then in counterbalanced sequence, while performing a simple numbers matching task and while taking an automated version of the Wisconsin Card Sort (WCS)--a frontal cortical task. The patients while doing the WCS decreased their frontal lobe metabolism while normals showed an increase. The relative degree of frontal flow, i.e., the frontal index, was found to be highly correlated with a variety of performance measures on the WCS in the group of schizophrenic patients. These included number of categories completed ($p < .03$), percent conceptual level ($p < .01$), and number of items completed ($p < .006$). No correlations were found in the control group.

These findings suggest that within a group of patients with schizophrenia, level of performance on this cognitive task is related to the degree of relative frontal cortical metabolism. Thus, it appears that the "hypofrontality" that has been described in schizophrenia has functional implications and may be linked to neuropsychological function mediated by the dorsolateral region of the frontal lobes.

156.12 STEREOLOGICAL ANALYSIS OF CELLULAR ARRANGEMENTS IN CINGULATE CORTEX OF SCHIZOPHRENICS. F.M. Benes, S. Matthysse, J. Davidson* and E.D. Bird. Depts. of Psychiatry and Neurology, Harvard Medical School and McLean Hospital, Belmont, MA. 02178.

Speculation that structural variations might accompany schizophrenia has been prompted by CT-scan evidence of brain volume loss. Although various brain regions could be involved in psychosis, the anterior congulate cortex (ACC) is of particular interest to schizophrenia research since it a) is dopaminergically innervated, b) occupies a strategic position in limbically related circuits, and c) probably is important to the integration of thought and affect. Although data from a recent study have not shown evidence of neuronal degeneration, other more subtle variations in cytoarchitecture could theoretically occur in this disorder. This present study has considered whether the spatial distribution of neurons of the ACC may be different in relation to schizophrenia. To test this hypothesis, analyses of both "nearest neighbor" distances between cells (NND), as well as the entire distribution of distances from every neuron to every other neuron in a field (EDD), have been performed using computer-assisted methods. The glial NND which showed a peak distribution at 20 μm were identical throughout layers I-VI and were the same for both control (N=9) and schizophrenic (N=10) ACC brains. The neuronal NND showed a peak distribution at 20-30 μm for both groups. The schizophrenic group, however, showed some neurons in layers II, III, and V to be more widely separated (40-50 μm) than in the controls, suggesting that some differences in neuronal arrangements might exist between the two groups. In support of this possbility, the neuronal EDD showed a reduced occurrence of cells at distances beyond 60 μm. When the EDD data were expressed as percent reduction in frequency at various distances for schizophrenics relative to controls, there was a marked drop-off in frequency beyond 240-300 μm for these same layers ($p=2.5 \times 10^{-9}$), and this was most marked for layer II. Neuronal EDD measurements for prefrontal cortex (n=177,873) did not show the drop-off in frequency in schizophrenics which was observed in ACC (n=85,975). Future studies will seek to determine whether these pattern analysis findings in ACC are of primary, secondary or even epiphenomenal significance to our understanding of chronic psychosis. This work has been supported by NIH grants MH00423A and MH/NS31862.

156.13 NEW AND UNKNOWN METABOLIC PATHWAYS ELICITED BY ACUTE BEHAVIORAL STATES OF DECREASED ACTIVATION. R. Jevning, A.F. Wilson,* and S. Guich*. Departments of Medicine and Physiology, University of California, Irvine, CA 92717

Present mechanisms of "resting" intermediary metabolism in human tissue have been formulated with almost no attention to level of activation, especially as it may be modulated by the behavioral state of the individual. In particular there have been few studies of tissue metabolism and mechanisms of metabolic control in states of behavioral hypometabolism. We now describe profound acute departure from "resting" metabolic mechanisms during the "transcendental meditation technique" (TM), a state of acute decreased activation of behavioral origin elicited regularly by many individuals. Relative forearm pulsatile blood flow, oxygen consumption, and carbon dioxide elimination changes were measured at 15 minute intervals during, and for 30 minutes after, 45 minutes of practice (TM for the TM group (n = 40) and normal, unstylized rest for a separate group (n = 23) of individuals studied prior to learning TM).

O_2 consumption and CO_2 elimination were calculated using the Fick principle: O_2 consumption = $(CaO_2 - CvO_2)$ x blood flow; CO_2 elimination = $(CvCO_2 - CaCO_2)$ x blood flow, where the quantities in parentheses are arteriovenous differences of oxygen and carbon dioxide contents, respectively, across forearm. From these quantities, RQ could also be ascertained: RQ = $(CvCO_2 - CaCO_2) \div (CaO_2 - CnO_2)$.

During TM forearm O_2 consumption declined 28%; CO_2 elimination ceased and arteriovenous differences of CO_2 content reflected net forearm uptake of CO_2 and therefore a negative RQ (-0.36) during this state. Smaller, but marked decline of CO_2 elimination (85%) also occurred during rest. No change of forearm blood flow accompanied either behavior. Initial RQ for TM and rest groups were 0.42 and 0.68, respectively. Since forearm blood flow did not change, net CO_2 uptake during TM is metabolic in origin, although not easily explained in terms of current understanding of intermediary metabolism. We believe these findings of net CO_2 uptake by tissue during TM and marked decreases of RQ for both of these rest states imply major importance of behavior in the control of metabolism and need for expansion of the repertoire of metabolic pathways currently understood in human physiology.

156.14 CEREBROCORTICAL HYPOMETABOLISM IS A NECESSARY BUT NOT SUFFICIENT CONDITION FOR SPONTANEOUS MENTAL IMAGERY: A HYPOTHESIS. S. Warach. Harvard Medical School, Boston, MA 02115.

Mental imagery is a quasi sensory awareness that is not temporally contiguous with the sensory stimulation that normally elicits such an awareness. Spontaneous mental imagery (SMI; e.g., hallucinations, dreams, auras) is imagery that is not volitional. Hughlings Jackson described epileptic auras and ictal hallucinations as release phenomena, a concept commonly interpreted as a kind of disinhibition, and neurologists have long accepted an association between cerebral hypoxia and hallucinations. The validity and generality of these claims can be examined in the contemporary literature, which contains considerable data about cerebral activity (cerebral oxygen and glucose metabolism and measures that are correlated with metabolism: cerebral blood flow and electrical activity) in conditions that are associated with SMI.

From the literature a general hypothesis of SMI generation can be inferred: Cerebrocortical hypometabolism is a necessary but not sufficient condition for the occurrence of SMI. The imagery state itself is subsequent to the hypometabolism and is characterized by a relative hypermetabolism in cortex of appropriate sensory modality.

Among the conditions reveiwed 3 have been studied separately during the pre-SMI and SMI states: alcohol withdrawal, epilepsy, sleep; the results are consistent with the hypothesis. Other SMI conditions reveiwed include cerebrovascular disease, cerebral neoplasm, hypoxia, near death experience (with cardiac arrest), sensory deprivation, classical migraine, dementia, intoxication with hallucinogenic drugs, and schizophrenia. All these conditions are associated with cortical hypometabolism. Hypometabolism is considered insufficient for SMI generation since many subjects in these conditions do not report SMI. At least 7 conditions that are associated with SMI (not all of which are listed above) also have been associated with seizure activity, suggesting a similarity between SMI generation and epileptogenesis.

None of the studies reveiwed had been designed to test this hypothesis; therefore none represent an adequate test of it. Most of the studies do not consider SMI directly, but are studies of conditions that happen to be associated with SMI. Nonetheless, the data reveiwed are generally consistent with the hypothesis presented. The hypothesis is potentially testable for any condition that produces SMI and is potentially refutable.

157.1 TTX DISPLACEMENT OF ^{3}H-NITRENDIPINE BINDING IN DEVELOPING SPINAL CORD NEURONS. M.J. Litzinger and D.E. Brenneman. Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, Maryland 20205.

The developmental specificity of ^{3}H-nitrendipine (NTP) binding was investigated in dissociated spinal cord cultures. Nitrendipine is reputed to bind to voltage-dependent calcium channels. The specificity of this binding was studied with tetrodotoxin, a blocker of voltage-dependent sodium channels. These binding studies were conducted on intact cells at 37°C. Spinal cord-dorsal root ganglia (SC-DRG) cultures were prepared from 12-14 day old fetal mice.

Kinetic studies of ^{3}H-NTP binding indicated non-linear Scatchard plots throughout development in culture. Specific binding was determined with 50μM nifedipine. Apparent dissociation constants increased from 0.34 nM on day 3 to 1.1 nM on day 21 for the high affinity site and from 22 nM to 50 nM for the low affinity site. The B_{max} increased two-fold from day 3 to day 21 for the high affinity site and almost six-fold for the low affinity site.

In immature cultures (day 5 in vitro), tetrodotoxin (TTX) displaced 1 nM ^{3}H-NTP. Significant displacement of 1 nM ^{3}H-NTP was observed with 1 nM TTX (10% of total binding). A dose-dependent increase in binding displacement was observed with TTX. The addition of TTX at a concentration sufficient to block all spontaneous action potentials (10^{-6} M) reduced ^{3}H-NTP binding by 55% whereas 1 μM nifedipine decreased binding to 80% of the total. At 10^{-5} M TTX, 70% of total ^{3}H-NTP binding was inhibited. In day 5 cultures, TTX (1 μM) displaced ^{3}H-NTP from both the high (.5 nM) and low (50 nM) affinity sites. Displacement was shown on days 5, 8, and 23, but not in cultures 27 days or older. Maximum displacement was observed on day 5. Thus the interaction between these two channel ligands is a developmental phenomena. These studies suggest that there may be binding site similarities for the sodium and calcium channels during the ontogeny of neurons in culture.

157.2 DOES NITRENDIPINE BLOCK CALCIUM CHANNELS IN NEURONAL PREPARATIONS? R.Y.K. Pun and M.J. Litzinger, Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, Md. 20205.

A paucity of direct physiological data on the effect of 1,4 dihydropyridines (a class of calcium antagonists) on neuronal preparations exists.In cardiac muscle, nitrendipine (.5-5 μM) was shown to block Ca currents (Lee and Tsien, Nature 302:28,1983). On the other hand in cultured rat pars intermedia cells, 100 μM concentration of nifedipine (a 1,4 dihydropyridine analogue) reportedly inhibited Na^+ spikes with little effects on Ca spikes (Douglas and Taraskevick,J. Physiol.326,201-211,1982).In young (day 5) spinal cord-dorsal root ganglia cell preparations, binding data shows that TTX (>10^{-9} M), a selective Na^+ channel blocker, displaces H3-nitrendipine binding (see Litzinger and Brenneman, this volume, 1984).

Intracellular recordings were made from dissociated fetal mouse spinal cord dorsal root ganglion (DRG) cells greater than 3 weeks in culture. Experiments were performed in the presence of 1 mM $MgCl_2$, 5 mM $CaCl_2$, 25 mM TEA and 1 μM TTX using CS Cl or CS_2SO_4 microelectrodes. Drug delivery was based on diffusion from a large bore pipet directly over the cell. Drug action was accessed by the change in duration of Ca^{++} spike in DRG cells.

100 μM concentration of nitrendipine reversibly blocked Ca spikes (75%) in 5 different cells. This antagonism was unlikely related to enhancement of K^+ currents, since the rate of repolarization and the after hyperpolarization following the spike was not affected. Both onset and recovery from antagonism were rapid. Interestingly, 5 μM concentration of nitrendipine potentiated Ca spikes (80%) in 4 different cells. Onset of potentiation was rapid, while recovery was delayed depending on the amount of exposure to the drug - the longer the exposure the slower the recovery. Prolonged exposure to the drug vehicle, ethyl alcohol (2.8%), also led to a reduction in duration of the Ca^{++} spike. No difference in response was noted in DRG's cultured in the presence or absence of NGF.

If the effect of nitredipine is concentration-dependent, then caution should be taken in the dosage for clinical applications, e.g. seizure control.

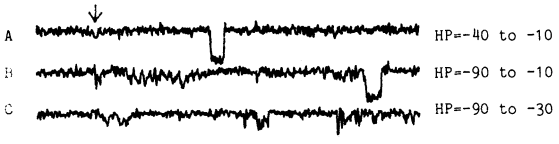
157.3 MULTIPLE TYPES OF CALCIUM CHANNEL IN DORSAL ROOT GANGLION CELLS DISTINGUISHED BY SENSITIVITY TO CADMIUM AND SINGLE CHANNEL PROPERTIES. M.C. Nowycky~, A.P. Fox*, R.W. Tsien*, Section of Neuroanatomy~ and Department of Physiology, Yale University School of Medicine, New Haven CT 06510.

Llinas & Yarom, Fishman & Spector and others have suggested that two components of Ca current may coexist in the same neuron. Our whole-cell patch clamp recordings from tissue- cultured chick DRG cells supported this idea (Biophys. J. 45, 36a). The overall Ca current can be divided into two components, L ("long- lasting") and T ("transient"). During depolarizing pulses, component T declined with an average $t_{1/2}$ of 25 ms, while component L showed little inactivation during pulses lasting >0.5 s. The time-dependence of both components remained unchanged when Ba_o replaced Ca_o. Voltage-dependence was very different: in 10 Ca_o, component T was fully inactivated at holding potentials (HP) more positive than -60 mV and became activated positive to -80 mV; component L inactivation began only above -50 mV and its activation began only above -30 mV.

The components respond differently to Cd. At 20-50 uM Cd, component L was almost fully blocked while component T was reduced by less than 30%.

We recorded from cell-attached patches on DRG somata with pipettes containing 110 mM Ba to look for differences in kinetics or conductance at the single channel level. Traces A-C are from a patch showing two types of unitary inward current. A large unitary current (~25 pS slope conductance) appeared with strong test steps from depolarized (A) or negative HPs (B). A small unitary current was evoked by strong or weak test steps but only from a negative HP (B,C); it gave rise to an average current with a prominent decay.

These large and small unitary events correspond rather well to components L and T. However, in other patches, we have also seen additional unitary events that are not so easily classified.

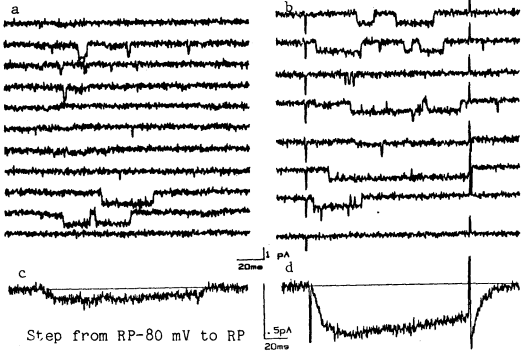


140 mM K-aspartate external solution with 5 uM Bay K 8644.

157.4 SINGLE Ca CHANNELS IN DORSAL ROOT GANGLION CELLS ARE SHIFTED BETWEEN MODES OF GATING BY THE CALCIUM AGONIST BAY K 8644. A.P. Fox*, M.C. Nowycky~ and R.W. Tsien* (SPON: M.Schwartz), Department of Physiology and Section of Neuroanatomy~, Yale University School of Medicine, New Haven CT 06510.

Dihydropyridine (DHP) compounds such as nitrendipine are widely assumed to be specific ligands for Ca channels in nervous tissue. However, little is known about DHP effects on Ca currents across neuronal membranes. We find that the DHP Ca agonist Bay K 8644 increases Ca channel activity in chick DRG neurons. The drug acts by favoring a mode of gating in which channel openings are greatly prolonged.

Cell-attached patch recordings were made with gigaseal pipettes filled with 110 mM Ba, and Ca channel activity was evoked by depolarizing pulses. The Figure shows results from a patch containing at least two Ca channels with ~25 pS slope conductance. Before drug (a), unitary Ca channel activity was seen as sweeps with typically brief openings ("mode 1"), interspersed with sweeps contained no openings ("mode 0"), as previously reported by others. In addition, a number of sweeps were dominated by long (>20 ms) opening events ("mode 2"), and these tended to occur in groups of consecutive sweeps. After exposure of the cell to Bay K 8644 (b), the percentage of mode 2 sweeps greatly increased, and the reconstructed macroscopic current was strongly enhanced (c,d). Similar results have been found for single heart cells (P. Hess, J. B. Lansman & R.W.T., Nature, in press).



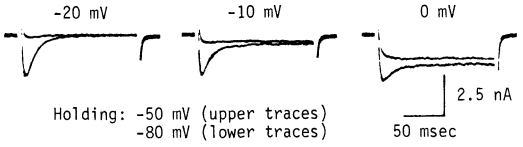
157.5 TWO TYPES OF CALCIUM CHANNELS IN NEUROBLASTOMA CELLS AND THEIR SENSITIVITIES TO CYCLIC AMP. A. Tsunoo*, M. Yoshii* and T. Narahashi. Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Cultured neuroblastoma cells are known to have voltage-gated Ca channels. It has recently been proposed that two types of Ca channels are present in nerve cell bodies. We have found that the neuroblastoma cells(N1E-115) are endowed with two types of Ca channels which exhibit differential sensitivity to the intracellular cyclic AMP.

Ca channel currents were recorded using the whole cell variation of the patch clamp technique. The external solution contained (mM): $BaCl_2$(50), NaCl(30) and CsCl(5). Tetrodotoxin(0.5 μM) and tetraethylammonium(25 mM) were also added. The internal solution was composed of 145 mM Cs-glutamate. The pH of both solutions was adjusted to 7.4 by 5 mM HEPES-Cs.

Step depolarizations from a holding potential of -80 mV to potentials more positive than -50 mV evoked transient inward Ba^{2+} currents which reached a maximum amplitude at -20 mV. A second component of the inward current appeared around -20 mV and reached its maximum at 0 to +10 mV. This component did not inactivate during prolonged depolarizing steps lasting more than 200 msec. When the holding potential was changed to -50 mV, step depolarizations failed to evoke the fast, transient component due to inactivation; however, they induced the slow, non-inactivating component in the isolated form. Both components of the inward current were abolished by 1 mM La^{3+}, indicating that the non-inactivating component of the current also flowed through Ca channels. Dibutyryl cyclic AMP (1 mM) caused an increase in the amplitude of the non-inactivating component by 30-50 %, but failed to alter the transient component significantly.

The present results indicate that there are two distinct types of Ca channels in the neuroblastoma cells, each differing in channel gating properties and in cyclic AMP sensitivity. Supported by NIH Grant NS14144 and Muscular Dystrophy Association Fellowship (A.T.).



157.6 ^{3}H-NITRENDIPINE AND ^{3}H-VERAPAMIL BINDING IN MOUSE FOREBRAIN:A COMPARISON. Paul Sumner and Ron Kochman Dept. of Biol. Res., G.D. Searle & Co., Skokie, IL 60077.

At least two calcium (Ca) channels have been identified in in vitro preparations of rodent brain, smooth muscle, and heart. We sought to study two of these sites in mouse forebrain, one labeled with ^{3}H-nitrendipine(^{3}H-NT) and one labeled with ^{3}H-verapamil (^{3}H-VP) and to examine the relationship between them.

For ^{3}H-NT binding adult male mice were decapitated, the brains removed and the forebrains (.12-.16 gm.) separated. The tissue was homogenized in 150 vol. 50 mM Tris-HCl buffer, pH 7.4, centrifuged twice at 30,000 x g and rehomogenized for use. The homogenate was incubated with ^{3}H-NT for 60 min. in the dark and the assay terminated by vacuum filtration. Non-specific binding was determined in the presence of 1 uM nifedipine. Specific binding was 80% of the total. Membrane preparation and assay for ^{3}H-VP binding were similar except that we varied combinations of EDTA-EGTA washes and Ca concentrations in order to optimize binding.

The K_D for ^{3}H-NT binding was 0.3 nM and the B_{max} 370 fmoles/mg protein. EDTA treatment of the membranes reduced binding, which was partially restored by the addition of Ca to the media. Nifedipine and nimodipine potently inhibited 0.3 nM ^{3}H-NT binding with IC_{50}'s of 1.3 nM and 1.7 pM, respectively. Displacement by VP and its methoxy derivative D-600 was complex with VP able to displace only 60% and D-600 only 30% of the specifically bound ligand. Specific binding of ^{3}H-VP was about 50% of total binding, with greater variability than for ^{3}H-NT. After EDTA and/or EGTA treatment 10^{-7} M Ca slightly stimulated binding and higher concentrations (10^{-4}-10^{-2} M) inhibited binding somewhat.

Our data support the conclusion that there are at least two Ca channel sites in mouse forebrain. 1.The membrane preparation required for binding and regulation by Ca is different for the two ligands. 2.The dihydropyridines displace ^{3}H-NT completely from its site whereas VP and D-600 displace only some of this ligand.

157.7 VOLTAGE-DEPENDENT CALCIUM CURRENT IN PC-12 CELLS. G. G. Schofield and F. F. Weight, Laboratory of Preclinical Studies, National Institute on Alcoholism and Alcohol Abuse. Rockville, MD 20852.

Rat pheochromocytoma cells (PC-12) grown in the presence of nerve growth factor (NGF) develop tetrodotoxin sensitive action potentials (Dichter et al, Nature 268: 501, 1977). Voltage-clamp investigations have shown that the underlying current responsible for these action potentials is similar to sodium currents in neuronal cells (Schofield and Weight, Neurosci. Abs. 9: 504, 1983). A study of large chemically fused PC-12 cells showed calcium action potentials in these cells in the presence of tetraethylammonium and elevated calcium (O'Lague et al, P.N.A.S., 77: 1701, 1980). We have studied a slow TTX-insensitive inward current in NGF-treated PC-12 cells using the gigaohm seal technique in the whole cell voltage-clamp configuration. Recordings were made using an electrode solution containing 145 mM CsCl, which rapidly blocked outward currents normally recorded at membrane potentials positive to -40 mV. Depolarizing command pulses positive to -40 mV induced a transient inward current followed by a slow inward current. This slow inward current was invesigated in a bathing solution containing 1 uM TTX or in which TRIS was substituted for NaCl. These conditions eliminated the transient inward current, allowing the slow inward current to be studied in isolation. The slow inward current was activated by depolarization positive to -40 mV from a holding potential of -70 mV. The inward current was maximal near 0 mV and reversed near +60 mV. The rising phase of the slow inward current was voltage sensitive, the time constant decreasing with increasing depolarization. The current was sensitive to external Ca^{2+} and was completely abolished in Ca^{2+}-free medium. Varying external Ca^{2+} concentration shifted the reversal potential of the slow inward current : increasing external Ca^{2+} shifted the reversal potential to more positive membrane potentials and decreasing external Ca^{2+} shifted the reversal potential to more negative potentials. The current was insensitive to 1 uM TTX, but was completely abolished by superfusion with 2 mM $MnCl_2$. The data suggest that PC-12 cells treated with NGF possess a slow inward calcium-sensitive current similar to calcium currents observed in other excitable cells.

157.8 STIMULATION OF $^{45}Ca^{2+}$ FLUX INTO PC12 CELLS BY THE DIHYDROPYRIDINE ANALOG BAY K 8644. D.A. Greenberg, E.C. Cooper*, and C.L. Carpenter*. Department of Neurology, University of California, San Francisco, CA 94143.

Dihydropyridine (DHP) calcium entry blockers inhibit ion flux through voltage-dependent calcium channels (VDC) and label VDC in excitable tissues. However whether DHP binding sites participate in physiologic regulation of VDC, or act solely as targets for exogenous drugs, is uncertain. The DHP analog BAY K 8644 has recently been shown to stimulate calcium-dependent smooth muscle contraction, an effect opposite to that of, and antagonized by, calcium entry blockers. If BAY K 8644 acts at the DHP binding site to stimulate voltage-dependent calcium flux, then DHP sites could mediate VDC activation by endogenous factors.

We have shown that BAY K 8644 competitively inhibits binding of the DHP calcium entry blocker, [^{3}H]nitrendipine, in brain (K_i=4.5 nM) and PC12 pheochromocytoma cell membranes (K_i=3.1 nM). In contrast, [^{3}H]nitrendipine binding is unaffected by much higher concentrations of drugs that influence calcium disposition by different mechanisms, including the calcium ionophore A23187, aminopyridines, dantrolene, ethanol, and barbiturates. Thus BAY K 8644 interacts in a specific fashion with the same membrane sites that recognize DHP calcium entry blockers.

We now provide correlation of this binding interaction with physiologic function, demonstrating that BAY K 8644 stimulates $^{45}Ca^{2+}$ flux into PC12 cells in culture. Depolarization by 50 mM K^+ enhances $^{45}Ca^{2+}$ uptake 4-6 fold compared with uptake under non-depolarizing (5 mM K^+) conditions. Depolarization-stimulated uptake is rapid, reaching equilibrium between 2 and 5 min. BAY K 8644 has no effect on $^{45}Ca^{2+}$ flux at 5 mM K^+, but markedly augments flux when membranes are partially depolarized by K^+ concentrations above 20-30 mM. At 30 mM K^+, the EC_{50} for BAY K 8644 stimulation of $^{45}Ca^{2+}$ flux is approximately 30 nM. Nitrendipine (100 nM) shifts the concentration-response curve for BAY K 8644 to the right, which is consistent with a common site of BAY K 8644 and nitrendipine action on calcium flux.

The ability of BAY K 8644 to inhibit [^{3}H]nitrendipine binding and enhance voltage-dependent calcium flux suggests that DHP binding sites can regulate VDC function. Since its effect on $^{45}Ca^{2+}$ uptake requires partial membrane depolarization , BAY K 8644 may act by stabilizing VDC in the "open" state, rather than increasing the probability of channel opening.

157.9 MAITOTOXIN INDUCES A STEADY CURRENT WHICH IS INHIBITED BY CALCIUM CHANNEL BLOCKERS. M. Yoshii*, A. Tsunoo*, Y. Kuroda, C. H. Wu and T. Narahashi. Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Maitotoxin (MTX), a non-proteinaceous toxin isolated from the dinoflagellate Gambierdiscus toxicus, has recently been suggested to activate Ca channels. However, it remained to be seen whether voltage-gated Ca channels were sensitive to this toxin. To clarify this point, experiments were performed with Ca channel currents of neuroblastoma cells (N1E-115) using the whole cell variation of the patch clamp technique.

Ca channel currents as carried by Ba^{2+} ions (50 mM Ba^{2+}) were isolated from the currents flowing through Na and K channels using 0.5 μM tetrodotoxin (TTX) and 25 mM tetraethylammonium. The patch electrode contained 145 mM Cs-glutamate. Under these conditions, an isolated Ba^{2+} current was evoked by a step depolarization to -20 mV from a holding potential of -80 mV.

MTX (10^{-9}g/ml) did not cause a significant change in either amplitude or time course of the Ba^{2+} current. Instead, an inward holding current (at -80 mV) began to increase 10-20 sec after application of MTX and developed progressively at a rate of -0.4 nA/10 sec from the control value of -0.1 nA. This MTX-induced current was completely blocked by 1 mM La^{3+}. Verapamil (0.1 mM) also prevented the development of the steady current.

In order to characterize the ionic mechanism underlying the MTX-induced steady current, the resting membrane potential of neuroblastoma cells was measured with a microelectrode. In divalent cation-free medium, MTX did not depolarize the membrane by more than 5 mV. However, upon addition of 1.8 mM Ca^{2+}, the membrane was quickly depolarized toward 0 mV. Further experiments were performed with crayfish giant axons. Much higher concentrations of MTX (5-10 x 10^{-8}g/ml) were required to cause a depolarization of 15 mV. The depolarization was antagonized by 1 mM La^{3+} but not by 0.3 μM TTX. Pretreatment of the axon with TTX did not protect the membrane against the depolarizing action of MTX.

The results suggest that MTX activates Ca channels at large negative potentials at which the channels do not normally open. Alternatively, MTX creates a Ca ionophore with pharmacological characteristics similar to those of Ca channel. Supported by NIH Grants NS14144 and RR-05370.

157.10 Ca^{2+}-INDUCED INACTIVATION OF Ca^{2+} CONDUCTANCE IN PROLACTIN-SECRETING CLONAL PITUITARY CELLS. B. Dufy, B. Dupuy*, D. Georgescauld* and J.L. Barker. Lab. Neurophysiologie, Bordeaux and Centre Paul Pascal CNRS, Talence, France and Lab. Neurophysiology, NINCDS, Bethesda, MD, U.S.A.

Although prolactin (PRL) secreting pituitary cells derived from the GH_3 clonal line generate Ca^{2+}-dependent action potentials that are thought to play a role in PRL secretion, the stoichiometry between Ca^{2+} entry during action potential generation and PRL release has not been established and there is convincing evidence that intracellular Ca^{2+} stores may contribute to the release process as well. In this study we have examined the role of intracellular divalent cation accumulation in the regulation of divalent cation entry in GH_3/6 cells. Ca^{2+} current (I_{Ca}) inactivation was studied at room temperature using a single microelectrode voltage-clamp technique. The microelectrode was filled with either 3 M KCl, 3 M KCl-45 mM EGTA, 3 M KCl-10 mM $CaCl_2$, 3 M CsCl, 3 M CsCl-45 mM EGTA, or 1.5-3.0 M K-citrate (tip resistances ranged from 50-90 megohms). I_{Ca} inactivation was measured with a double-pulse voltage step protocol: a test command sufficient to activate I_{Ca} was preceded by a conditioning step of variable amplitude and duration. I_{Ca} inactivation was inversely and linearly related to Q_{Ca} evoked during the conditioning step, where Q_{Ca} is the product of I_{Ca} and time, or the charge carried by Ca^{2+} ions. Inactivation required 10 sec for complete recovery. Inactivation was virtually absent when Ba^{2+}, rather than Ca^{2+} was the external divalent cation. Inactivation was also markedly attenuated during experiments in which EGTA or citrate were included in the microelectrode solution, while including Ca^{2+} ions completely eliminated I_{Ca}. Thyrotropin releasing hormone, which momentarily increases both K^+ conductance and intracellular Ca^{2+} concentration, also transiently inactivated I_{Ca}.

The results strongly suggest that intracellular Ca^{2+} accumulation, arising from extra- and/or intracellular stores directly regulates extracellular Ca^{2+} entry, presumably to limit the intracellular concentration of Ca^{2+} ions. The importance of Ca^{2+}-limited Ca^{2+} entry in clonal and primary pituitary cell physiology remains to be elucidated.

157.11 CALCIUM-DEPENDENT POTASSIUM CONDUCTANCE OF CLONAL PITUITARY CELLS: COMPARISON OF MACROSCOPIC AND SINGLE-CHANNEL CURRENTS. J.L. Barker, M.A. Rogawski and B. Dufy. Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, MD 20205 and Universite de Bordeaux II, Bordeaux, France 33706.

Voltage-clamp recordings from prolactin secreting GH_3 cells have demonstrated the existence of two major potassium conductances with distinct kinetic and pharmacological properties. One conductance is associated with a slowly rising, non-inactivating outward current, $I_{K(Ca)}$; the other inactivates rapidly in a time dependent fashion. $I_{K(Ca)}$ is active at rest and provides a major contribution to the generation of the resting potential. It is dependent upon external Ca^{2+} as it is absent in Ca^{2+}-free solution and in the presence of the Ca^{2+} channel blockers Co^{2+} or Cd^{2+}. The current is also blocked by TEA and by Ba^{2+}.

To gain further insight into the conductance mechanisms underlying these currents, we carried out gigaseal patch clamp recordings in the cell-attached configuration. Early passage GH_3/6 cells were bathed in buffered medium containing (in mM) 5.5 K^+, 143 Na^+, 5 Ca^{2+}, 1 Mg^{2+}. Large conductance outward single-channel currents were readily apparent. The amplitude of these unitary currents increased linearly with membrane potential in the range 0 to 60 mV depolarized from rest. The extrapolated reversal potential was approximately 20 mV below rest, near the K^+ equilibrium potential. The channels opened with low frequency at rest but the total time in the open state increased markedly upon depolarization. Even at highly depolarized potentials the frequency of channel opening remained constant for up to minutes, indicating that there is minimal time dependent inactivation. Externally applied Ba^{2+} (in the bathing medium but not in the patch electrode) caused a rapid and pronounced decrease in the channel opening frequency, suggesting that the ion enters the cell (presumably through Ca^{2+} channels) and blocks $I_{K(Ca)}$ from the internal membrane face. In contrast, Cd^{2+} had no effect on the channel when added to the bathing medium. These results suggest that a voltage- and Ca^{2+}-dependent K^+ channel underlies the delayed outward current in GH_3 cells.

158.1 **DEVELOPMENTAL CHANGES IN THE DISTRIBUTION OF THE CALLOSAL PROJECTION NEURONS OF THE FETAL RHESUS MONKEY SOMATOSENSORY CORTEX.** H.P.Killackey* and L.M.Chalupa. (SPON:J.Conway) Dept. of Psychobio., Univ. of Calif., Irvine, CA 92717 and Dept. of Psychol., Univ. of Calif., Davis, CA 95616.

The distribution of callosal projection neurons was determined in fetal rhesus monkeys between embryonic days 108 (E108) and 133 (E133). Large injections of 50% horseradish peroxidase (HRP) were made into the pre- and postcentral gyri. The fetuses were sacrificed 24 to 28 hours later, brains sectioned in the parasagital plane and the tissue reacted for HRP histochemistry.

At E108 there was a continuous band of HRP labelled neurons in the superficial layers of the pre- and postcentral gyri. At this age individual labelled cells were relatively immature and the density of labelled neurons in the superficial layers was comparable to that at E119. However, there appeared to be more labelled cells in the deep layers. Further, a group of non-neuronal cells in the white matter between the corpus callosum and the cortex was also labelled at this and later ages. At E119 there is still a dense continuous band of labelled cells in the superficial layers as well as an occasional labelled cell in the deeper layers of the postcentral gyrus. The density and distribution of the deep labelled cells is similar to that of the mature animal. In the precentral gyrus the superficial band of labelled cells is both wider and less dense. There is also a second less dense band of labelled cells in the deeper layers. At this age there was no indication of anterograde HRP label above the white matter.

By E133 the distribution of labelled neurons in the postcentral gyrus was clearly discontinuous and resembled the adult. Anterograde HRP label could also be seen within the cortical layers. This was most obvious in caudal portions of the postcentral gyrus where discrete arrays of anterograde label were seen in layer IV beneath the superficial band of labelled neurons.

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158.2 INTERHEMISPHERIC CONNECTIONS OF THE POSTERIOR NEOCORTEX IN NORMAL-EYED, CONGENITALLY ANOPHTHALMIC AND NEONATALLY ENUCLEATED MICE J. Olavarria and R.C. Van Sluyters Neurobiology Group and School of Optometry, University of California, Berkeley, CA 94720.

After injecting horseradish peroxidase in the posterior neocortex of one hemisphere, we examined the distribution of retrogradely labeled cells and anterogradely labeled terminations in tangential and coronal sections through contralateral areas 17 and 18. The distribution of label in HRP-tested sections was related to the borders of area 17 in adjacent myelin- or Nissl-stained sections in three groups of adult mice: normal-eyed (ZRDCT-n and C57Bl/6J strains), congenitally anophthalmic (ZRDCT-an strain), and neonatally enucleated (ZRDCT-n strain). In agreement with previous studies, we observe that the pattern of callosal connections in areas 17 and 18 of normal-eyed mice contains the following features: (1) a dense band of callosal cells and terminations separating the bodies of areas 17 and 18 which have few callosal connections, (2) a ring-like configuration anterolateral to area 17, (3) a region of dense labeling lateral to area 18, (4) a narrow band of labeling bridging the posterior portion of area 18, and (5) a labeled region anteromedial to area 17.

We find that all these features of the normal callosal pattern are recognizable in congenitally anophthalmic mice. Their presence, in mice that never had eyes, supports the hypothesis that central visual pathways can develop many aspects of their connectivity in the absence of input from the periphery. However, we also find that the details of a given feature of the callosal pattern in congenitally eyeless mice often differ from those of the same feature in normal-eyed mice, and that the between-animal variability in the appearance of these features is much higher in eyeless mice. These latter findings indicate the eyes are needed during normal development to fine-tune the pattern of callosal connections.

This study also reveals that the callosal pattern in neonatally enucleated mice does not differ significantly from that in congenitally anophthalmic mice, indicating that the sensitive period for callosal development extends into postnatal life. While the present data do not delineate the time course of this sensitive period, the finding of similarly abnormal callosal patterns in anophthalmic and neonatally enucleated mice suggests that the eyes exert little if any influence prenatally. Finally, examination of coronal sections indicates the laminar distribution of callosal connections develops normally in both these groups of eyeless mice.

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158.3 DEVELOPMENTAL CHANGES IN AUDITORY EVOKED BRAINSTEM RESPONSES (ABRs) IN CHICKENS. A. Katayama* (SPON: C. Zomzely-Neurath). Dept. of Zoology and Bekesy Lab. of Neurobiology, University of Hawaii, Honolulu, HI 96822.

Auditory evoked brainstem responses (ABRs) were recorded from the surfaces of the brain of lightly anesthetized newborn (1- to 7-day old) and adult (7- to 9-week-old) chickens (Gallus domesticus) as a measure of development of auditory processing.

A test for stimulus artifacts was conducted prior to the examination of the development of the ABR. In this test, a series of recordings were made as the body temperature of a chicken was altered by cooling. The latencies and amplitudes of peaks changed as the body temperature changed, indicating that these peaks were dependent on the physiological state of the animal and were not artifacts.

In newborn chickens, a series of early latency responses (ELRs) was observed within five milliseconds after the onset of a stimulus. Even one-day-old chicks showed ABRs. This precocity of the chicken ABR contrasts with the later development of the ABR in mammals (e.g., the onset of the ABR at four days in cats, Shipley et al., 1980, Brain Res., 182: 313-326). In adult chickens ELRs were more discrete and were compressed toward the time of the stimulus onset, a developmental modification previously reported for the ABRs of cats, rats, and humans. Detailed examinations of the changes of interwave latencies between peaks revealed that at least one of the interwave latencies (N_1 to P_{3-4}) becomes significantly shorter ($P < 0.05$) in adults (0.70 $\pm$ 0.11 msec) than it is in 1- to 7-day-old chicks (0.98 $\pm$ 0.17 msec). Among newborns, the same (N_1 to P_{3-4}) interwave latency was significantly shorter ($P < 0.05$) in 6- and 7-day-old (0.84 $\pm$ 0.10 msec) than in 1- to 3-day-old chicks (1.08 $\pm$ 0.13 msec). This suggests that in at least a part of the central auditory pathway, maturation processes, such as the formation of myelin, are occurring during the first week after hatching, and that they continue during later weeks.

This physiological evidence for postnatal maturation has recently been complemented by psychophysical studies of auditory sensitivity conducted by Gray and Rubel (1984, Assoc. Res. Otolaryngol. Abstr., 7: 4). They report improvement in high frequency hearing thresholds measured behaviorally as chickens mature during the first postnatal week. (Supported by N.I.N.C.D.S. and the Deafness Research Foundation grants to Dr. J. T. Corwin.)

158.4 CHANGES IN TONOTOPIC ORGANIZATION WITH AGE ARE DUE TO AN INTENSITY-DEPENDENT, TWO-STEP PROCESS OF HAIR CELL DAMAGE. D.A. Cotanche, L.G. Tilney* and J.C. Saunders. Depts. of Biology and Otorhinolaryngology and Human Communication, University of Pennsylvania, Philadelphia, PA 19104.

We have examined the location of pure-tone noise damage in the developing chick cochlea and have found that the position of the damage correlates with an intensity-dependent, two-step process of hair cell damage. We exposed groups of 1, 10 and 30 day old chicks to pure tones of 525, 1500 and 3000 Hz for 48 hours. For each frequency, birds were exposed to intensities of 110, 115, 120 and 125 dB/SPL. Immediately after noise exposure the cochleae were processed for scanning electron microscopy. The inner ear damage was tonotopically positioned along the cochlea in accordance with the frequency of the damage-inducing tone. However, the damage patterns for each tone occurred in two steps which were related to stimulus intensity. At low intensities, hair cells were damaged in a narrow, longitudinal strip positioned on the tall hair cell side of the cochlea. As intensity increased, a second, patch-like damage site developed in the short hair cell region. The patch of damage was located at the distal (apical) end of the longitudinal strip and, at each age, it spread distally with increasing intensity. A comparison of the damage patches across the age groups indicated that equivalent stimulus intensities produced a smaller patch in 1-day-old chicks than in 30-day-old chicks. We had to increase the intensity of the stimulus for 1-day-old chicks to acquire a damage patch similar to that found in the 30-day-old chicks. This pattern corresponds with the improvements in auditory sensitivity which occur during development in the chick cochlea (Saunders et al, Brain Res. 63: 59, 1973). The results of this study suggest that the location of hair cell damage depends on the intensity and age of the animal rather than intrinsic changes in hair cell coding within the cochlea.

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158.5 AN OPTIMAL STRATEGY FOR AUDITORY DEPRIVATION DURING THE DEVELOPMENT OF HEARING. N. K. Woolf and A. F. Ryan*. Otolaryngology Research Laboratory, University of California at San Diego Medical School, San Diego, CA 92103.

We have examined a variety of experimental procedures in order to establish a protocol which would provide the optimal degree of auditory deprivation throughout the ontogeny of hearing. The criteria to be satisfied by the chosen experimental protocol was that it must minimize the trauma to the subject, maximize the extent of isolation from environmental stimuli, maintain the auditory deficit throughout functional development and not interfere pathologically with normal morphogenesis. Based on the findings of this study an optimal strategy for providing auditory deprivation during the development of hearing has now been determined.

Subjects in these experiments were adult mongolian gerbils (Meriones unguiculatus). The effects of the various experimental procedures were reflected in changes in cochlear microphonic (CM) and N1 compound action potential (AP) thresholds. Electrophysiological responses were measured utilizing standard round window recording techniques. The pinna was not disturbed by this procedure. Auditory stimuli were provided under free field conditions.

Thresholds were established for a given subject under normal conditions and then after tying the pinna closed with a purse seine suture, or blocking the external canal with audalin impresion compound, or purse seine closure of the external canal plus various interruptions of the ossicular chain. Significantly greater threshold shifts were obtained when the external canal was tied closed and the malleus and incus ossicles were removed than under any other experimental condition. This procedure provided between 40-60 dB attenuation in auditory thresholds throughout the normal frequency range for adults.

The external canal ligation plus malleus and incus removal protocol has subsequently been applied to subjects as young as newborns. The removal of the malleus and incus ossicles required that these subjects later be stimulated by direct mechanical driving of the stapes. When examined as young adults (i.e., 45 days of age) and compared to normal subjects, the experimental animals were functionally normal and both the cochlear capsule and stapes were normally developed. Details of our experience with this procedure will be discussed.

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158.6 COMPARISON OF OLFACTORY RESPONSES IN ADULT AND LARVAL TIGER SALAMANDERS. A.H. Arzt*, W.L. Silver and J.R. Mason*. Monell Chemical Senses Center, Philadelphia, PA 19104.

The tiger salamander undergoes metamorphosis from a completely aquatic larval stage to a terrestrial adult. We have examined olfactory responses (electro-olfactograms; EOGs) in both salamander stages to determine whether olfactory function may be altered along with other changes, both to the salamander itself and to the environment inside the nasal cavity.

Both larvae and adults responded to the six amino acid solutions tested (L-arg HCl, L-cys, L-ala, L-glu, L-ile, D-ala), and for both, response magnitudes increased exponentially with logarithmic increase in stimulus concentration, even at the highest concentration (0.01M). Thresholds were lower in larvae for each of the amino acids with the exception of L-cys. Larval responses were greater than adult responses except for the highest concentrations of L-glu and L-arg.

Volatile compounds presented in air (amyl acetate, AA; limonene, Li; cyclohexanone, Cy; Butanol, Bu) elicited responses in both larvae and adults. (Only 1 of 4 larvae responded to Li). Although no differences in thresholds were seen, adult response magnitudes were higher than those of larvae at all concentrations of each odorant.

Responses were also obtained to the volatile stimuli presented in solution. Like responses to amino acid solutions, but unlike responses to volatiles in air, responses to volatiles in solution for both salamander stages increased exponentially with logarithmic increase in stimulus concentration and did not saturate. No differences in thresholds were seen. Although adult responses to AA and Li were greater than those in larvae, this difference was not as large as when AA and Li were presented in air. Response magnitudes to Cy and Bu were similar in adults and larvae.

Olfactory epithelia of aquatic larval salamanders were more responsive to amino acid solutions than terrestrial adults. Adults were more responsive than larvae to some volatiles in solution. These results suggest that alterations in olfactory function may be among the changes that occur with the metamorphosis of tiger salamanders from aquatic larvae to terrestrial adults.

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158.7 A Mechanism for Altered Chorda Tympani Taste Responses to Salts During Development: Addition of Amiloride-Sensitive Sodium Channels. D.L. Hill and T. C. Bour*. Dept. of Psychology, Univ. of Toledo, Toledo, OH 43606.

Integrated responses from the rat chorda tympani nerve change developmentally to lingually applied monochloride salts. Recordings from single fibers have revealed that response frequencies to NaCl and LiCl increase with age while frequencies to NH4Cl remain constant. These findings suggest that membrane components mediating NaCl and LiCl responses alter during development whereas components mediating NH4Cl responses do not change. Recent findings by Heck et al. (Science 223:403-405, 1984) and Teeter et al. (Neurosci. Abst. 9:1020, 1983) have indicated that a specific transduction mechanism does exist in adult mammals for NaCl and LiCl stimulation. Taste responses to NaCl and LiCl are significantly suppressed by the sodium-transport inhibitor amiloride; other salt responses are unaffected. To learn if the increasing NaCl and LiCl sensitivities during development are related to a concomitant increase in the number of amiloride-sensitive sodium channels, integrated chorda tympani responses were recorded in rats aged 12-13 days, 29-31 days and 90-110 days. Responses were recorded from 5 rats in each age group to 0.5M NaCl, LiCl, NH4Cl and KCl before and after lingual application of 500 μM amiloride hydrochloride.

Response ratios before amiloride application were similar to those reported in studies of rat developmental taste responses. However, the normal developmental changes in salt responses changed dramatically when responses were recorded immediately after amiloride application. Responses to NaCl and LiCl were unaffected by amiloride in rats aged 12-13 days; however, they were suppressed 57% and 65% in rats aged 29-31 days and 90-110 days, respectively. Responses to NH4Cl and KCl were unaffected following amiloride. Furthermore, the response ratios of NaCl and LiCl to NH4Cl in rats aged 29-31 days and 90-110 days after amiloride were similar to the ratio found without amiloride in rats aged 12-13 days. Thus, blockage of amiloride-sensitive sodium channels in postweaning and adult rats results in response ratios similar to rats less than 2 weeks old. These data suggest that NaCl and LiCl responses in early postnatal development result from non-amiloride-sensitive mechanisms and the progressive increase in NaCl and LiCl effectiveness results from a concomitant increase in amiloride-sensitive channels. (Supported by NIH #NS20538).

158.8 THE EFFECT OF ODOR DEPRIVATION ON OLFACTORY EPITHELIUM IN DEVELOPING RATS. A.I. Farbman, S.M. Ritz* and P. Brunjes. Dept. of Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60201 and Dept. of Psychology, Univ. of Virginia, Charlottesville, VA 22901.

Several groups have reported that unilateral odor deprivation of neonatal rats and mice results in a reduced volume of the ipsilateral olfactory bulb, presumably as a result of retarded or impeded development. In a recent paper, Bensen et al. (J. Neurosci., 4:638, 1984) reported that neonatal mice, cauterized in one nostril, had a 26% reduction in volume of the olfactory bulb ipsilateral to the nasal occlusion at postnatal day 30; however, they found no reduction in number of dendritic knobs at the epithelial surface and concluded that the number of mature olfactory receptors was unchanged. In our study, neonatal rats were cauterized unilaterally and allowed to survive for 30 days. Animals were anesthetized and perfused with Bouin's fixative intraventricularly. Heads were removed, decalcified and processed for paraffin embedding. Five 7 µm sections from each head were taken at approximately 600-700 µm intervals and stained routinely with hematoxylin and eosin. The total number of epithelial cell nuclei along a 200 µm length of nasal septum was counted on both the deprived and non-deprived side at the same level in the same section. In every section of each animal, the number of cells on the deprived side was significantly less than that on the control side. The average of 30 measurements on 6 animals, expressed as a ratio of cells on deprived:non-deprived, was 0.85 (S.D.= .09). In two control, unoperated littermates, the mean ratio (right side:left) of 10 measurements was 1.01 (S.D. = .08). A preliminary quantitative immunohistochemical study of olfactory marker protein (OMP) content of the deprived and non-deprived sides was made. Ten measurements of optical density were taken from different septal regions of each side at two different aperture widths on a Zeiss microspectrophotometer. The amount of OMP on the deprived side was reduced by a percentage equal to that of the cell count reduction. These results are consistent with the notion that the reduced cell number in the epithelium was due to a reduction in number of olfactory receptor cells. Further, it suggests there is a developmental interaction between pre- and post-synaptic cells in the primary olfactory pathway and both are affected by sensory deprivation during early postnatal life.

Antibody vs OMP was a gift of Dr. F. Margolis.

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158.9 GOLGI ANALYSES OF GRANULE CELL DEVELOPMENT IN NEONATAL RAT OLFACTORY BULB. Charles A. Greer. Sec. of Neurosurgery and Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

Several recent reports described subpopulations of mitral, tufted and granule cells in the adult olfactory bulb (OB) (Macrides et al., 1982; Mori et al., 1983; Orona et al., 1983). These data suggested that the external plexiform layer (EPL) of the OB might be subdivided based on the topographic distribution of the dendritic fields of these neurons (Greer et al., 1982). Neurogenesis of mitral and tufted cells is clearly dichotomized both between and within major populations of neurons. The extent to which the granule cells that form local circuits with mitral and tufted cells may vary in their timecourse of maturation is unknown. In the present report preliminary data is presented examining the hypothesis that there is a gradient in the morphological development of granule cells that corresponds to the sublaminae of the EPL and their respective dendritic constituents (mitral-deep EPL; tufted-superficial EPL).

Sprague-Dawley rats were processed for Golgi-Kopsch staining at 0, 3, 6, 9, 12 and 21 days postnatal (DPN). Serial 100um sections were cut on a vibratome and granule cells reconstructed with a camera-lucida at 100X oil immersion.

In regard to individual granule cells, a typical course of maturation was observed with dendritic extensions occurring prior to the proliferation of spines. Prior to 6 DPN no stained granule cell perikarya have been identified in the superficial portion of the granule cell layer. Rather, their average depth is 150um below the mitral cell layer. The apical dendrites extend radially through approximately 40um of the EPL, 50-70% of its width. Few dendritic spines were identified at these ages within the EPL. By 6 DPN the apical dendrites extended approximately 80um into the EPL, 80% of its width. A parallel increase in the number of spines/unit length was also observed. By 12 DPN a mixture of granule cells, with perikarya located both deep and superficial in the granule cell layer, was observed with dendritic processes extending through 50-90% of the EPL, respectively.

The data thus far suggest a dichotomous development of granule cells with those forming local circuits with mitral cells differentiating prior to those forming local circuits with tufted cells.

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158.10 EFFECTS OF DENERVATION AND TRANSECTION OF OLFACTORY PEDUNCLE ON GROWTH AND AChE and BuChE ACTIVITY IN OLFACTORY BULB OF POSTNATAL RAT. E. Meisami and M. Firoozi (SPON: W. J. Freeman). Inst. Biochem. Biophys., Univ. of Tehran and Dept. of Physiol., Univ. Calif, Berkeley, CA. 94720.

Changes in growth and activity of AChE and BuChE were investigated in olfactory bulbs (OB) of developing rats subjected to one of the following operations: Unilateral olfactory denervation (ON), unilateral transection of olfactory peduncle (OP) or combined operations (ON+OP). All operations were carried out at birth and measurements obtained at day 30. All comparisons were made between control OB and its controlateral operated counterpart. Between birth to day 30, growth (as measured by wet weight) in control OB was 9x, while in the ON and OP, growth was 8x and 5x respectively and in the ON+OP, it was 3x only. Total (per OB) activity of AChE in the control OB during the first month increased by 45x, in the ON by 38x, in the OP by 25x and in the OP+ON bulb by only 12x. During the same period,BuChE activity, increased by about 3.4x in the control bulb, 2.8x in ON, 2.1x in OP and 1.9x in OP+ON. These results suggest: 1) Postnatal OB growth is affected far more by disconnection from the brain than from the periphery. 2) Maturation and/or proliferation of cholinergic synapses in OB, as indicated by AChE activity, increases very markedly in the postnatal period (6x more than the OB growth). 3) Whereas the source of cholinergic input to OB appears to be mainly centrifugal, there must be some cholinergic activity intrinsic to OB, which continues to develop in the absence of both peripheral and central connections. 4) Source of BuChE is probably mainly intrinsic to OB (glial cells?) as there is little similarity between this enzyme and AChE during development and in response to peduncular transection.

AChE (acetylcholinesterase); BuChE (butyrylcholinesterase).

FEEDING AND DRINKING: CUES FOR NEED STATE I

159.1 PREVENTION OF FOOD-RELATED HYPERDIPSIA RETARDS BUT DOES NOT PREVENT DEVELOPMENT OF HYPERTENSION IN SHR. F.S. Kraly, L. A. Coogan*, M.S. Trattner*, J.A. Goldstein*, C. Zayfert*, S. M. Specht* and A. Cohen*. Psychology Dept., Colgate Univ., Hamilton, NY 13346.

Spontaneously hypertensive rats (SHR: n=8; Taconic Farms) and age-matched Wistar-Kyoto rats (WKY: n=8) ate similar ($p > .20$) amounts of standard pelleted chow in 24 hr when they had continuous access to food and water from 5-17 weeks of life. The SHR drank significantly ($p < .001$) more water in 24 hr throughout weeks 5-17 and as early as week 9 (SHR: 35.0 ± 1.6 ml; WKY: 28.2 ± 1.0 ml). When eating a meal of dry food after 12-hr food deprivation, SHR (n=8) typically drank earlier ($p < .01$) and drank more ($p < .001$) than did WKY (n=8) in a 1-hr test (e.g., week 5, SHR: 7.7 ± 0.5 ml/100g body weight; WKY: 4.0 ± 0.2 ml/100g) throughout development. Moreover, SHR exhibited a striking pattern of frequently ($p < .02$ vs. WKY) interrupting eating to drink during a meal.

When SHR (n=11) were prevented throughout development (weeks 4-14) from drinking more water than WKY (n=12) drank daily, the development of hypertension (measured weekly by tail-cuff to determine mean systolic pressure) was retarded ($p < .001$) and body weight gain was slowed ($p < .001$) compared to SHR (n=13) with unlimited access to water and food. For example, by week 14 SHR with unlimited access to water and food had higher ($p < .02$) blood pressure (212 ± 4 mmHg) than did SHR with restricted access to water (195 ± 6 mmHg), while WKY with unlimited access to water had even lower blood pressure (152 ± 3 mmHg). Other SHR (n=12) with unlimited access to water but restricted access to food, sufficient to enforce a slowing of body weight gain equivalent ($p > .20$) to that experienced by SHR with restricted access to water, showed retarded development of hypertension ($p < .005$; week 14: 192 ± 6 mmHg) similar to that of SHR with restricted access to water. Resumption on week 14 of unlimited access to water and food, for both previously restricted groups, produced within 1 week mean systolic blood pressures ($ps > .10$) and body weights ($ps > .05$) not different from those of SHR having unlimited access to water and food throughout development.

These findings (a) show that food-related hyperdipsia is apparent in young SHR during the development of hypertension, and (b) suggest that hyperdipsia is not a major factor contributing to the development of hypertension in SHR.

159.2 LOCAL ANESTHETIC DECREASES SATIATING POTENCY OF INTRADUODENAL INFUSION OF FATS. Danielle Greenberg*, James Gibbs, and Gerard P. Smith. (SPON: J. Sechzer). Dept. of Psychiatry, Cornell Univ. Med. Coll. and E.W. Bourne Behavioral Research Laboratory, The New York Hospital, White Plains, NY 10605.

Intraduodenal infusion of a mixture of fats inhibited sham feeding in rats (Reidelberger et al, Am. J. Physiol.244:R865, 1983). To investigate this effect further, we determined (1) whether the infusion of fat elicited the behavioral sequence of satiety and (2) the effect of mixing a local anesthetic in the fat infusion on the satiating potency of the fat infusion.

Method: Seven male Sprague Dawley rats (300-340g) were equipped with gastric cannulas for sham feeding and silastic duodenal catheters for infusion of fat. After 17h food deprivation, rats were permitted to sham feed a high carbohydrate liquid food (BioServ 50% v:v) for 90 min. Intraduodenal infusions began 12 min after sham feeding began. All infusions were 10 ml and infusion rate was 0.45 ml-min^{-1}, a rate within the normal gastric emptying rate for this mixture of fats. Infusions consisted of 3 concentrations of fat (Intralipid, Cutter Lab. 0.25, 0.5 or 1.0 kcal-ml^{-1}) or 0.15M saline. In the local anesthetic experiments, 0.45, 0.9 or 1.8 mg of tetracaine HCL (0.5%) was infused in a duodenal infusion of saline, 0.25 or 0.5 kcal-ml^{-1} of Intralipid. Sham intakes were measured every 5 min and behavior was time-sampled every minute by the method of Gibbs et al (Neuroscience Abstracts 6:530, 1980).

Result: Duodenal infusion of fat inhibited sham feeding and elicited the behavioral sequence of satiety. Tetracaine significantly reduced the satiating effect of fat ($F=16.53$ $(3,32)$ $p<.001$).

Mean Percent Inhibition of Sham Intake

Intralipid (kcal-ml^{-1})	Tetracaine (mg-10 ml^{-1})			
	0	0.45	0.9	1.8
0.25	33	8	12	18
0.50	58	40	30	38

Mean percent inhibition was calculated from 5-7 rats.

We conclude that fat not only inhibits sham feeding, but also elicits the behavioral sequence of satiety. The ability of tetracaine to reduce the satiating effect of intraduodenal infusion of fat suggests that preabsorptive mechanisms mediate part of the satiating effect of fat infused into the small intestine.

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159.3 BROWN FAT DENERVATION DOES NOT ALTER DIETARY OBESITY. J. E. Cox and J. F. Lorden. Dept. Psychology, Univ. of Alabama in Birmingham, Birmingham, AL, 35294.

Rothwell and Stock (1979) proposed that sympathetic nervous system activation of brown adipose tissue imparts metabolic resistance to obesity during overfeeding and promotes weight loss after this regimen is withdrawn. As a test of this hypothesis we compared development of and recovery from dietary obesity by intact female Sprague-Dawley rats and those sustaining denervation of interscapular brown adipose tissue (IBAT). Half of each group were subsequently maintained on high-fat mash and sweetened-condensed milk (HF-M) or Purina pellets (CHOW). Over 32 days, sham-operated H-FM rats were hyperphagic (89.8 vs 65.4 kcal/day) and gained substantially more body weight (95.8 vs 31.4 g) than did the sham-operated CHOW group. Denervates exhibited similar hyperphagia on HF-M (86.8 kcal/day) but gained significantly less weight (74.0 g, $p < .01$) than did intact rats on this diet, contrary to the direction of change predicted by the hypothesis. Switching HF-M rats to maintenance on CHOW for 8 days resulted in hypophagia and weight loss that were virtually identical in denervated and intact rats: 34.5 vs 30.0 kcal/day (NS) and 21.2 vs 22.1 g (NS), respectively. On the final day of the experiment, rats were sacrificed by decapitation and norepinephrine (NE) content of IBAT was subsequently determined by high pressure liquid chromatography with electrochemical detection. NE levels in operated rats were markedly reduced compared to those in intact animals (.032 vs .428 ug/pad, respectively; $p < .001$), indicating that the tissue had been effectively sympathectomized. Using a second set of intact rats, we also observed no indication of increased IBAT norepinephrine (NE) utilization during development of dietary obesity or recovery. Utilization was estimated by comparing IBAT NE levels after i.p. injection of either saline or alpha-methyl-p-tyrosine (250 mg/kg and 125 mg/kg, 7 and 3.5 hr prior to sacrifice, respectively). No differences in utilization were noted among CHOW-fed rats, those maintained on HF-M for 32 days, or rats switched from HF-M to CHOW on day 32 and maintained for an additional 4 days. In conclusion, we found no evidence that sympathetic activation of IBAT acts to counter weight gain in the face of excessive caloric intake or contributes to weight loss after the obesity-inducing diet is withdrawn. (Supported by NIH grants AM31805 and NS14755.)

159.4 NEONATAL GUANETHIDINE SYMPATHECTOMY AND/OR ADULT ADRENAL DEMEDULLATION DOES NOT INFLUENCE DIETARY-INDUCED OBESITY, DESPITE IMPAIRED BROWN FAT ACTIVITY. M.G. Tordoff, R.L. Oetting*, and D. Novin. Monell Chemical Senses Ctr., Philadelphia, PA 19104 & Dept. Psychol., UCLA, Ca 90024.

The effects of neonatal guanethidine (GUA) sympathectomy and adult adrenodemedullation on food intake and body weight were investigated. In Experiment 1, rats of both sexes were treated with saline (SAL) or GUA (15 injections x 50 mg/kg, SC) on Days 7-25 of life, and at weaning (Day 28) received chow or a four-component supermarket diet (SD) for 133 days (n=6/gp). During treatment, rats given GUA gained weight 33% more slowly than did controls (1.8 vs. 2.7 g/day). After treatment, chow-fed GUA-treated females regained this lost weight, and when adult, ate significantly less (56.5 + 1.9 vs 63.1±2.1 kcal/day) but weighed the same (Day 133: 297.3± 9.4 vs. 300.0±4.6 g) as SAL-treated females. Chow-fed, GUA-treated males always ate less (76.1±3.2 vs 83.2±2.5 kcal/day) weighed less (Day 133: 428.1±22.0 vs 518.0±16.4 g), and were shorter (NALs, 253±1.7 vs 267±2.5 mm) than SAL-treated males. SD-fed groups initially gained weight more slowly than did the chow-fed groups, but eventually achieved control weights (males; Day 133, SAL=520.0±10.2; GUA=441.0±20.9 g) or were significantly heavier (Day 133, SAL=390.4±4.7; GUA=385.4 ± 23.0 g) and fatter (by Lee index) than controls (females). There was no interaction of sympathectomy with diet on body weight. However, GUA-treated SD-fed rats ate fewer calories than SAL-treated, SD-fed rats during early adolescence. They also obtained a smaller proportion of their calories from chocolate-chip cookies (26 vs 38%).

In Experiments 2 and 3, female rats that were neonatally GUA-sympathectomized, adrenodemedullated, and fed chow until adult, were examined (n=4 or 5/gp). Neither form of sympathectomy nor their combination altered the development of SD-induced obesity when the diet was given between Days 93-138 of life. When the SD was replaced by chow, SD groups lost weight at the same rate.

Catecholamine histofluorescence revealed that the stomach and pancreas of GUA-treated rats in the above experiments were always devoid of noradrenergic terminals. In parallel studies, GUA-treated rats had very few or no adrenergic varicosities in brown adipose tissue (BAT), and chronic in vitro BAT oxygen consumption (but not BAT weight) was reduced by 45% compared with controls.

Taken together, these results question the importance of the sympathetic nervous system and of BAT in the development of dietary obesity.

159.5 PRENATAL EXPOSURE TO HIGH DIETARY FAT SUPPRESSES ADULT FAT INTAKE IN FEMALE BUT NOT MALE RATS. J. L. Norman and W. COLEMAN*. Department of Psychology, California State University, Chico, CA 95929.

While rats appear to regulate their body weights about a set point, the nature of the set point mechanism and the variables involved in "setting" it, including those of age and diet, are virtually unknown. This experiment was designed to investigate the effects of prenatal high dietary fat exposure on the subsequent juvenile and adult body weight, preference for dietary fat, and body fat deposits. We found that the female offspring of dams fed a high fat (HF) diet prior to and throughout gestation were influenced to gain less weight and eat less of a HF diet as adults than female offspring of dams fed a normal chow (NC) diet. This suppression of fat intake was not seen in comparable males.

The subjects were 40 offspring (20 male,20 female) of 14 Sprague Dawley dams, 8 of which had been made obese by exposure to a HF diet prior to and throughout gestation. At birth the offspring were cross-fostered to dams fed a NC diet and at weaning the offspring were placed (for 7 weeks) on one of two diets, a HF diet (5.84 kcal/g) or a NC diet (4.16 kcal/g). At the end of the 7 weeks (age 70 days) all rats were placed on NC diets for 19 weeks and then returned to the previous juvenile dietary regimen at about 7 months of age for another 15 week period.

At the end of the adult diet period, rats were killed with an overdose of anesthetic and measurements of specific gravity, perirenal fat, and epididymal fat (males only) were made. Three-factor analyses of variance (Dam Diet, Juvenile/Adult Diet, Sex) were performed on mean daily body weight changes, food intake in kcal, water intake in g, specific gravity, and fat pad assessments.

During the juvenile phase rats on the HF diet regardless of sex and Dam Diet gained significantly less and ate significantly less than their NC fed counterparts, demonstrating the well-known aversion of juvenile rats to HF diets. In the adult phase however, there was a significant Dam Diet by Sex interaction such that female offspring of HF fed dams gained significantly less weight than offspring of NC fed dams, while male offspring of HF fed dams gained significantly more than offspring of NC fed dams.

Food and water intake and specific gravity were all influenced significantly by Dam Diet, while only the Juvenile/Adult Diet influenced the measured fat deposits.

159.6 WITHDRAWN

159.7 PYLORIC BINDING SITES FOR CHOLECYSTOKININ ARE NOT NECESSARY FOR ITS SATIETY EFFECT. J.D. Falasco*, K.M.S. Joyner*, J. Gibbs and G.P. Smith. Dept. of Psychiatry, Cornell Univ. Med. Coll. and E.W. Bourne Behavioral Research Laboratory, The New York Hospital, White Plains, NY 10605.

Specific binding sites for cholecystokinin have recently been described (G.T. Smith et al, Am.J. Physiol. 246:127, 1984). To determine whether these binding sites are necessary for the satiety effect of CCK, we removed the pyloric sphincter region surgically and then investigated the satiating potency of CCK-8 on these pylorectomized rats.

Method: Four male Sprague-Dawley rats (245-460g) underwent pylorectomy. Continuity of the gut was restored by gastroduodenal anastamosis. Four rats (266-352g) served as surgical controls. When the body weights of pylorectomized rats were at least equal to their preoperative weights, all rats were adapted to the following schedule: After overnight (17h) milk diet deprivation, they were injected at 0915 with 0.15M NaCl (1 ml, ip); at 0930 they were given test diet (BioServ #078J8, 50% V/V, 0.5 Kcal/ml) and their intake was recorded over a 30 min period. At 1000 the test diet was removed and the milk diet replaced until 1630, when the milk diet was removed for deprivation purposes. Water was always available. When the intake of the test diet appeared stable for at least two consecutive days, the saline injection was substituted by an injection of CCK-8 (Squibb #SQ19844) dissolved in 1.0 ml saline in doses of 2,4, or 8 mcg-kg^{-1}. A CCK day was always preceded by a saline day. Volumes ingested following CCK injections were compared to the mean of each rat's intake following saline injection. The results were as follows:

		INTAKE (ml-30 min^{-1})			
			CCK-8 (mcg-kg^{-1})		
Group	n	Saline	2	4	8
Pylorectomized	4	19.9±2.4	16.0±2.5	7.3±2.5	5.5±2.9
Control	4	21.7±1.0	13.3±2.5	13.8±3.5	10.3±1.8

A two-way repeated measures ANOVA revealed a significant effect of dose ($F=13.0$, $p < .01$), but no group effect and no interaction. Post-hoc t tests did not reveal any significant effect of pylorectomy at any dose of CCK-8 tested compared to controls.

We conclude that the pyloric binding sites for CCK-8 are not necessary for the satiety effect of CCK-8.

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159.8 PROLONGED SATIATING EFFECTS OF CHOLECYSTOKININ AND BOMBESIN IN RATS. Stephen M. Wiener*, James Gibbs, and Gerard P. Smith Dept. of Psychiatry, Cornell Univ. Med. Coll. and E.W. Bourne Behavioral Research Laboratory, The New York Hospital, White Plains, NY 10605.

To determine whether cholecystokinin (CCK) and bombesin (BBS) exert satiety effects beyond a single meal, we measured food intakes and behaviors throughout a 140 min test period that followed systemic peptide administration.

Method: Following an overnight, 18-hour food deprivation, 14 male Sprague Dawley rats (410-560g) were allowed access to a high-carbohydrate liquid food (Bio-Serv, Inc, 40% V:V) for 140 minutes. Immediately prior to food presentation, rats were given intraperitoneal injections of the synthetic C-terminal octapeptide of CCK (CCK-8; the gift of the Squibb Institute, Princeton, NJ), tetradecapeptide BBS, or 0.15M NaCl vehicle control. Both peptides were given in doses of 2,4, and 8 ug-kg^{-1}; only one treatment was given each day. Food intake was measured before and after each meal. Each rat's behavior was recorded once a minute. Liquid food was removed 15 min after the test ended, and replaced with a milk diet (Magnolia condensed milk, 50% V:V) until food deprivation began at 1530h.

Results: (1) The satiating potency of the first meal measured as the satiety ratio (length of postprandial IMI in min/amount of food eaten at the meal in ml), was greatly increased by pretreatment with both peptides: BBS increased the satiety ratio 209% at 2 ug-kg^{-1}, 245% at 4 ug-kg^{-1}, and 248% at 8 ug-kg^{-1} ($p<0.01$ for each); CCK increased the satiety ratio by 142% at 2 ug-kg^{-1}, 151% at 4 ug-kg^{-1}, and 287% at 8 ug-kg^{-1} ($p<0.01$ for each). (2) In spite of the fact that rats took repeated meals--and satiated repeatedly--during the 140-min test period on days when they received CCK-8, BBS, or control injections, total food intake was still less than control at the end of the test period: BBS decreased total food intake by 9% at 2 ug-kg^{-1} (ns), by 21% at 4 ug-kg^{-1} ($p< 0.025$), and by 23% at 8 ug-kg^{-1} ($p<0.01$); CCK decreased total food intake 20% at 2 ug-kg^{-1} ($p<0.01$), by 22% at 4 ug-kg^{-1} (ns), and by 54% at 8 ug-kg^{-1} ($p<0.01$). Thus, rats failed to compensate for the decreased size of the first meal produced by the two peptides under these test conditions.

These results indicate that the satiety actions of systemic CCK-8 and BBS are not confined to a single meal, but extend across subsequent meals in the 140 min after systemic administration.

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159.9 ADMINISTRATION OF AN ANTI-EMETIC ATTENUATES FOOD INTAKE REDUCTION PRODUCED BY EXOGENOUS CHOLECYSTOKININ. B.O. Moore and J.A. Deutsch*. Department of Psychology, University of California, San Diego, CA 92093.

The issue of whether reduction of food intake by cholecystokinin (CCK) is due to its satiating or aversive qualities has not yet been resolved. Variables such as dose (physiological or non-physiological) and appropriateness of two-bottle aversion tests have been considered; but the relative importance or significance of these variables is still open to interpretation.

This experiment directly tested the effects of CCK by giving an anti-sickness drug to rats before administering CCK. If an anti-emetic can eliminate the effects of CCK administration and yet at the same time not affect normal food intake, this would support the hypothesis that the effects of CCK are due to its aversive rather than its satiating qualities.

Male Wistar rats were trained to drink a 50% oil-water emulsion while 17 hours hungry. After a stable baseline of consumption occurred, rats were divided into four groups: S-S (saline, saline), A-S (anti-emetic, saline), A-CCK (anti-emetic, CCK), S-CCK (saline, CCK). Rats were injected ip with the first drug 30 minutes before and with the second drug immediately before oil presentation. (The anti-emetic was 5mg/ml-kg trimethobenzamide and the CCK was at 1 of 2 doses - 10 or 20 IDU/ml-kg.)

Group S-S drank 10% more on the experimental day than its previous average baseline consumption; whereas the A-S group's intake was 7% lower. These two groups were not significantly different from each other. S-CCK (20 IDU) was 57% lower than its baseline; whereas A-CCK (20 IDU) was only 20% lower. These two groups were significantly different ($t=2.5$, $p<.05$, $df=12$). S-CCK (10 IDU) was 33% lower than its baseline; whereas A-CCK (10 IDU) was only 15% lower. Furthermore, the A-CCK groups were not significantly different from the A-S group.

This experiment demonstrates that in normal feeding (with endogenous CCK), an anti-emetic does not increase intake. And yet, administration of an anti-emetic with CCK in doses shown to reliably produce food intake reduction, does attenuate the food intake reduction. Therefore, the effects of endogenous CCK are quite different from those of exogenous CCK, thus making any previous study equating exogenous CCK effects with natural satiety problematic. Furthermore, this study corroborates other work in which CCK is shown to be aversive.

159.10 TRACING THE SENSORY PATHWAY FROM GUT TO BRAIN REGIONS MEDIATING THE ACTIONS OF CHOLECYSTOKININ ON FEEDING AND EXPLORATION. J.N. Crawley and J.Z. Kiss. (SPON: A.F. Mirsky), Clinical Neuroscience Branch and Laboratory of Cell Biology, National Institute of Mental Health, Bethesda, MD 20205.

Sulfated cholecystokinin octapeptide (CCK) inhibits food consumption and exploratory behaviors when administered intraperitoneally to rats. Lesions of the vagus nerve and nucleus tractus solitarius (NTS) abolished these behavioral effects of CCK, suggesting that CCK receptors in gut stimulate a sensory feedback pathway to the brain. Lesion studies were undertaken to determine each synapse in the pathway activated by CCK, from gut to brain regions mediating feeding and exploratory behaviors.

Bilateral midbrain knife cuts, histologically verified as destroying all rostral projections of the NTS, blocked the ability of CCK (5 ug/kg i.p.) to reduce total food consumption over a thirty minute trial. The ability of CCK to reduce exploration, as measured by approaches to a novel object, and occurrence of pauses of behavioral inactivity in a five minute test session in a novel environment, was also blocked by bilateral midbrain transection.

Discrete bilateral lesions of the paraventricular nucleus of the hypothalamus completely blocked the effects of intraperitoneal CCK on feeding, and partially blocked the effects of CCK on exploratory behavior. Control lesions 1 mm rostral to the paraventricular nucleus had no effect on the ability of CCK to reduce food consumption and exploration.

CCK appears to influence feeding and exploration through peripheral receptors, which send information through the vagus nerve to the nucleus tractus solitarius, where efferents then project rostrally. One forebrain nucleus which may receive these projections is the paraventricular nucleus of the hypothalamus, which is necessary for the manifestation of the effects of CCK on feeding behaviors and exploratory behaviors.

159.11 HEPATIC PORTAL ALLOXAN ABOLISHES THE INHIBITION OF FEEDING BY GLUCAGON, BUT NOT BY EPINEPHRINE. S. Weatherford* and S. Ritter. College of Veterinary Medicine, Washington State University, Pullman, WA 99164-6520.

Glucagon (GL) and epinephrine (EPI) are hormones which inhibit feeding. Russek and Ricotta proposed that these substances may inhibit feeding by a common effect on hepatic metabolism (Front. Horm. Res. 6: 120, 1980). We reported previously that hepatic portal injections of subdiabetogenic doses of alloxan abolish the satiety effect of GL. The goal of the present experiment was to determine whether the inhibitory effect of EPI on feeding, like that of GL, would be abolished by alloxan. Adult male rats were injected intraportally with alloxan (45, 55 or 65 mg/kg in acidified saline, pH 3.0) or with the alloxan vehicle (saline, pH 3.0). After recovery, intake of pelleted rat chow was measured in 1-hr tests after intraperitoneal injection of GL (100 ug/kg), EPI (50 ug/kg) or saline. On test days, food was removed 3 hrs prior to lights off. One hr after lights off, rats were injected with the test substance and fresh food was returned. Food intake was measured every 15 min for 1 hr. In controls, GL and EPI reduced food intake by 37% and 32% respectively, compared to intakes after saline ($p < .05$ for both). Glucagon satiety was abolished by all doses of alloxan, while inhibition of feeding by EPI was not attenuated. Alloxan treated rats ate 40% less after EPI than after saline ($p < .05$). It is noteworthy that the inhibition of feeding by EPI did not appear to result from malaise, since our dose of EPI neither caused apparent behavioral depression nor inhibited drinking induced by 24-hr water deprivation. The fact that hepatic portal alloxan has effects which are specific for GL-induced effects, suggests that EPI and GL inhibit feeding by different mechanisms. Thus, our results do not support the hypothesis of Russek and Racotta. However, our results are consistent with the report of Geary, et al. (Soc. Neurosci. Abst., 1983) that selective hepatic vagotomy abolishes inhibition of feeding by GL but not by EPI.

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159.12 **INTRAPORTAL GLUCAGON INFUSIONS: COMPARISON OF SATIETOGENIC AND GLYCEMIC EFFECTS IN THE RAT.** B.G. Welck and S. Ritter. College of Veterinary Medicine, Washington State University, Pullman, WA 99164-6520.

It has been suggested that the suppression of food intake by intraperitoneally administered glucagon is due to elevation of intrahepatic glucose concentration. As an initial examination of this idea we studied the suppression of food intake and the peripheral glycemic response to intraportally administered glucagon. Suppression of feeding by glucagon was statistically significant at 15, 30, 45 and 60 min after the start of the infusion when infusion rates of 10, 33 and 100 ug/kg/min were used. At infusion rates of 3.3 ug/kg/min, glucagon also suppressed feeding, but the suppression was significant only during the infusion period (0-30 min). Vehicle infusions did not alter food intake. Suppression of food intake varied approximately linearly with the logarithm of the rate of infusion up to a maximum of 3.0g food (45.5%) at 30 min with 100 ug/kg/min.

In a similar testing paradigm, but in the absence of food, we compared the glycemic effects of a glucagon dose which suppressed feeding (10 ug/kg/min) with a dose which had no effect on feeding (1 ug/kg/min). Glucagon or vehicle was infused for 30 min at the above rates and plasma glucose was measured from jugular blood 0, 2, 5, 10, 28 and 40 min after the start of the infusion. We found that the two glucagon doses produced hyperglycemic effects which were indistinguishable from one another. In fact, at 28 and 40 min the plasma glucose levels were at least 25 mg% higher in rats infused with the glucagon dose which was ineffective in suppressing feeding than in those infused with an effective dose. The peak glycemic response of 175 mg% occurred at 5 min after the start of the infusion with both glucagon infusion rates. Vehicle infusions had no effect. Thus, peripheral glucose levels do not appear to correlate with the suppression of food intake elicited by glucagon over an order of magnitude difference in the rate of infusion.

159.13 **PRE-MEAL DECLINE IN BLOOD GLUCOSE: A SIGNAL FOR MEAL INITIATION?** P. Brandon*, F. J. Smith*, & L. A. Campfield* (Spon: C. Enroth-Cugell). Dept. of Physiology & Engineering Sciences, Northwestern University. Chicago, Illinois 60611 and Evanston, Illinois 60201.

Louis-Sylvestre and LeMagnen have described a decline in blood glucose concentration (BG) prior to meals in free feeding rats. In order to extend their observations and test their hypothesis that the pre-meal decline in BG is or reflects a signal for meal initiation, we have continuously monitored BG and meal pattern in normal and VMH lesioned rats. 200 gm female Wistar rats were implanted with chronic cardiac cannulas. Animals were housed with free access to tap water and powdered rat chow with a 12/12 hr light/dark cycle and meal patterns were continuously recorded. Following recovery, heparin (100-200 units) was injected, the blood withdrawal cannula connected and the rat was returned to its home cage where it remained undisturbed through the experiment. 60 min later, blood withdrawal (28 ul/min) and continuous, on-line BG monitoring was begun. BG and food cup weight were displayed on the computer monitor.

In 7 experiments, no decline in BG and no meal occurred during at least 3 hours of observations; 6 of these experiments were light/dark transitions. In 10 other experiments, BG declined prior to the initiation of a meal (9 dark and 1 light). BG began to decline 9.4 ± 0.6 min before the onset of food intake. BG declined to a minimum (-10.6 ± 1.1%) at 4.8 ± 0.4 min and returned to baseline levels at 0.4 ± 0.4 min prior to the beginning of the meal. Similar declines in BG have been observed prior to 6 light phase meals in VMH lesioned rats. BG began to decline 7.3 ± 3.0 min prior to the meal. BG declined to a minimum (-8.7 ± 1.3%) at 2.2 ± 1.1 min after the beginning of the meal and returned to baseline at -0.4 ± 1.0 min prior to the end of the meal which corresponded to 17.6 ± 2.2 min after the beginning of the decline. Preliminary studies using glucose infusions suggest that partial blockade of the pre-meal BG decline alters the expected meal.

These results suggest that the pre-meal decline in BG is related to meal initiation in both intact and VMH lesioned rats. This conclusion is reinforced by the fact that, to date, we have not observed a pre-meal decline in BG without food intake nor food intake without a pre-meal decline in BG. In summary, these studies suggest that pre-meal BG decline may be among the signals for meal initiation.

159.14 INTRAVENOUS INSULIN INJECTIONS AFFECT TASTE RESPONSES IN THE RAT NUCLEUS TRACTUS SOLITARIUS. Barbara K. Giza* and Thomas R. Scott (SPON: P. Saxton). Dep't. Psychol. and Inst. for Neurosci. and Behav., U. Delaware, Newark, DE 19711.

Gustatory afferent activity is modifiable by physiological needs. Our previous work demonstrated that intravenous glucose injections decreased evoked activity in the rat nucleus tractus solitarius (NTS). Concomitant with increased blood sugar following glucose injection is an increase in endogenous insulin. We therefore studied the effect of insulin infusions on NTS responses to determine whether increasing insulin level also decreases taste sensitivity. We induced surgical levels of anesthesia in 22 undeprived female albino rats with ketaset, maintained them with chloral hydrate, respirated subjects to prevent brain movement and implanted an esophageal fistulas to prevent stimuli from entering the stomach. Heartrate served as a measure of depth of anesthesia and physiological state. We inserted etched tungsten semi-microelectrodes (500KΩ) into the NTS until we encountered robust gustatory activity evoked by chemical stimulation of the tongue. Stimuli were 1.0 M glucose, 1.0 M fructose, 0.1 M NaCl, 0.03 M HCL and 0.01 M QHCl. In each case we stimulated the whole mouth with 5.0ml of solution followed by a 50ml DH_2O rinse and a rest period of 45 seconds. We applied each stimulus four times over a 30 min. period which served to monitor the stability of our recording and to establish a pre-injection response level. At time T = 0 we injected 0.5 U/kg regular insulin or an equivalent volume of the rats' own plasma into the jugular vein and continued to monitor taste activity for the next 90 min. Integrated multiunit activity evoked by glucose declined in animals injected with insulin from a pre-injection value of 120.8, to a nadir (113.8) at 12.0-16.5 min. and recovered gradually over the next 20 min. The decrease in evoked activity over the period 12-23 min. was significant relative to pre-injection levels ($t = 3.35$; $p < .005$) and plasma control levels ($t = 2.99$; $p < .005$). Fructose responses during the same interval were significantly depressed relative to pre-injection levels ($t = 2.36$; $p < .025$). Responses to NaCl, HCl and QHCl showed only insignificant changes relative to their own pre-injection responses and to controls. These data are consistant with recent reports that insulin infusions decrease food intake. The selective depression of the more appetative tastes may provide a neural counterpart to the decreased appeal of food associated with satiety.

160.1 ACTIVATION OF ALPHA-1 ADRENERGIC AND H-1 HISTAMINERGIC RECEPTORS POTENTIATES THE STIMULATORY EFFECTS OF VASOACTIVE INTESTINAL PEPTIDE (VIP) ON CYCLIC-AMP (C-AMP) LEVELS IN MOUSE CEREBRAL CORTICAL SLICES. P.J. Magistretti, P. Hof* and M. Schorderet*. Department of Pharmacology, Centre Médical Universitaire, 1211 Geneva, Switzerland.

We have recently reported that VIP and norepinephrine (NE) acted synergistically to increase c-AMP levels in mouse cerebral cortical slices (Magistretti and Schorderet, Nature 308, 280-282, 1984). We have now examined the pharmacological characteristics of this synergism. We have observed that the rank-order of potency of several adrenergic agonists in potentiating the effects of VIP on c-AMP levels is the following : epinephrine (EC_{50} 2.2 µM) > NE (EC_{50} 5 µM) > phenylephrine (EC_{50} 10 µM) >> clonidine = isoproterenol. Furthermore the synergistic interaction between 1 µM VIP and 10 µM NE is antagonized by the specific alpha-1 adrenergic antagonist prazosin at 100 µM but not by the adenosine antagonist theophylline at 1 mM. These results support the following conclusions : (1) The synergism observed between VIP and NE in stimulating c-AMP formation is mediated by the activation by NE of adrenergic receptors of the alpha-1 type; (2) A release of adenosine induced by VIP does not account for the synergistic interaction between VIP and NE. We have also examined possible synergistic interactions between VIP and two other monoamines contained, like NE, in highly divergent neuronal systems projecting from the brain stem to the cerebral cortex, namely serotonin (5-HT) and histamine (HIS). We have observed that HIS but not 5-HT acts synergistically with VIP to increase c-AMP levels in mouse cerebral cortical slices. Furthermore, this synergistic interaction is mediated by H-1 histaminergic receptors, since it is antagonized by mepyramine but not by cimetidine. In conclusion, in view of the morphological and physiological properties of the VIP intracortical neuronal system and the noradrenergic projection to the cerebral cortex in particular, it appears that sensory stimulation may constitute a behavioral event whereby the synergism between VIP and NE may become operative and lead to a drastic increase in the levels of c-AMP within a discrete cortical volume delineated by the intersection of the tangentially organized noradrenergic fibers and a group of activated, radially oriented VIP intracortical neurons.

160.2 VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) ALTERS FIRING OF CORTICAL NEURONS AND INTERACTS WITH NOREPINEPHRINE (NE). A. Ferron, G.R. Siggins and F.E. Bloom. Div. Preclin. Neurosci. and Endocrin., Research Institute of Scripps Clinic, La Jolla, CA 92037.

Both VIP and NE are present in rat cerebral cortex and localized immunohistochemically in different fiber systems thought to project to pyramidal neurons (Morrison, et al, Brain Res. 292: 269, 1984). Previous biochemical studies (Magistretti and Schorderet, Nature 308: 280, 1984) have shown that VIP acts synergistically with norepinephrine in stimulating cyclic AMP formation in cortical slices. Therefore, we applied VIP and NE to rat sensorimotor cortical neurons by microiontophoresis to assess their responsiveness to VIP and to determine if VIP and NE might interact at the cellular level. In urethane-anesthetized, adult Sprague-Dawley rats, VIP inhibited spontaneous firing of 25% and enhanced firing in 18% of 40 fronto-parietal neurons. The remainder were not affected at iontophoretic currents up to 200 nA. These effects, obtained with ejection currents of 50-150 nA, occured in all cortical layers except layer I, but predominated in the deeper layers. NE was predominantly inhibitory in cortex, as reported previously by many researchers. Possible interactions between VIP and NE were tested by ejecting pulses of VIP before, during and after continuous administration of currents of NE small enough that they induced little or no depression of spontaneous background firing. In approximately 50% of the cells tested, ejection of VIP during NE administration (at doses subthreshold for direct effects) resulted in pronounced inhibition of firing, regardless of whether VIP alone elicited excitation, inhibition or no effect. These findings suggest that for some cortical neurons under these in vivo conditions, the direction of the direct response to VIP could depend upon the extent of ongoing NE receptor activation. The convergence of NE and VIP afferents onto common target neurons and their shared ability to activate brain cyclic AMP synthesis supports a role for cyclic AMP in this synergistic action. Supported by Fonds de la Recherche en Sante du Quebec and the USPHS (AM 26741).

160.3 EVIDENCE FOR DOPAMINE D1-RECEPTOR MEDIATED INHIBITION OF THE RELEASE OF CHOLECYSTOKININ-LIKE IMMUNOREACTIVITY (CCK-LI) BY DOPAMINE D1-RECEPTOR STIMULATION IN RAT NEOSTRIATUM D.K. Meyer* and U. Conzelmann* (SPON: W. Lichtensteiger). Dept. Pharmacol., Freiburg University, D-78 Freiburg, FRG

In the neostriatum, stimulation of dopamine D2-receptors decreases the release of several neurotransmitters (e.g. dopamine, acetylcholine or glutamate), but enhances the release of CCK-LI (Meyer and Krauss, Nature 301, 338, 1983).

Apart from the fact that stimulation of neostriatal dopamine D1-receptors activates an adenylate cyclase, nothing is known of the function of this receptor subtype.

In the present investigation, we studied whether the stimulation of these receptors affected the release of CCK-LI from slices of rat neostriatum incubated in vitro. Release of CCK-LI was induced by veratridine (3.75×10^{-6} M); CCK-LI was determined by radioimmunoassay.

Veratridine-induced release of CCK-LI from rat neostriatal slices was enhanced by dopamine (DA) (10^{-7} M), but DA (10^{-6} and 10^{-5} M) had no effect. However, in the presence of SCH 23390 (5×10^{-7} M), a selective D1-receptor antagonist, DA (10^{-7} to 10^{-5} M) only enhanced the release of CCK-LI.

In contrast, when used together with the D2-receptor antagonist domperidone (DOM; 10^{-7} M), DA (5×10^{-6} and 5×10^{-5} M) reduced the release of CCK-LI. When the D1-receptors were blocked by SCH 23390, DA plus DOM no longer had any effect on CCK-LI release. It is concluded that stimulation of dopamine D1-receptors causes a reduction of the release of CCK-LI from nerve endings in rat neostriatum.

CCK-LI is found in rat neostriatum only in afferent axons and nerve endings, but not in intrinsic neurons. In contast, dopamine D1-receptors are only situated on neurons intrinsic to the neostriatum. Therefore, the inhibitory effect of D1-receptor stimulation should be indirect, i.e. mediated by intrinsic neostriatal neurons.

We are currently investigating the mechanism of this action.

160.4 **INTERACTION BETWEEN NORADRENERGIC AND DOPAMINERGIC INNERVATION OF THE CEREBRAL CORTEX.** S.I.Harik, G.H. Doull* and R.N. Kalaria*. Dept. of Neurology, Case Western Reserve Univ. Sch. of Med., Cleveland, Ohio 44106.

Lesion of the nucleus locus ceruleus (LC), and the resultant depletion of cortical norepinephrine (NE), causes "denervation supersensitivity" of the cerebral cortex, demonstrated by increased density of β-adrenoceptors and increased isoproternol-stimulated activity of adenylate cyclase. This "denervation supersensitivity" reaches its maximum in 2 weeks, but disappears within 4 to 8 weeks after LC lesion, despite the marked persistent loss of cortical NE. We have hypothesized that such recovery may result from enhanced dopaminergic activity in the NE-depleted cortex. This hypothesis is based on the emergence of high-affinity uptake for dopamine (DA) and increased levels of cortical DA and its metabolites after LC lesion (Logan, W.J. & Harik S.I., J. Neurosci 2:394, 1982; Harik, S.I., J. Neurosci., 4:699, 1984). To test this hypothesis we assessed the development of "denervation supersensitivity" in adult male Wistar rats after either unilateral LC lesion alone, or unilateral LC lesion combined with lesion of the substantia nigra (SN) on the same side. Lesions were performed by the stereotaxic microinjection of 6-hydroxydopamine into the SN and/or LC. Rats were killed 2 or 12 weeks after lesions or sham operation and samples of the striatum and cerebral cortex were assayed for NE and DA and its metabolites to assess the efficacy of the lesions. The cerebral cortex ipsilateral to LC and SN+LC lesions was depleted of about 90% of its NE content at 2 and 12 weeks. The striatum ipsilateral to SN+LC lesions was depleted of 99% of its DA but the striatum ipsilateral to LC lesion alone was unaffected. NE and DA of contralateral structures were not different from sham-operated controls. β-adrenoceptor density was assessed by the specific binding of [^{3}H]dihydroalprenolol (DHA) to particulate fractions of the cerebral cortex. Two weeks after lesions, the cerebral cortex ipsilateral to LC lesion alone and to LC+SN lesions showed a 44% and 53% increase over the contralateral cortex. However, 12 weeks after lesions DHA Bmax in the cerebral cortex ipsilateral to LC lesion alone was only 15% higher than the contralateral side while the cerebral cortex ipsilateral to LC+SN lesions continued to show marked "denervation supersensitivity" with a DHA Bmax about 60% higher than the contralateral side. These results strongly suggest that dopaminergic innervation of the cerebral cortex plays an important role in mediating the compensatory processes that occur after chronic cerebral NE denervation and provide further evidence of the close interaction between noradrenergic and dopaminergic innervation of the cerebral cortex.

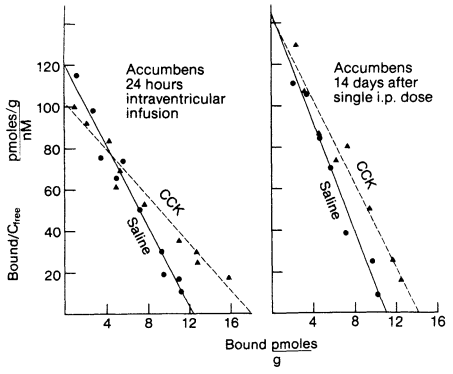
160.5 CHOLECYSTOKININ ELEVATES DOPAMINE D_2 RECEPTOR DENSITY.
A. Dumbrille-Ross and P. Seeman
Pharmacology Dept., Univ. of Toronto, Toronto, CANADA M5S 1A8

The high concentrations of cholecystokinin in the striatum and limbic regions of the brain suggest that this peptide may influence dopaminergic transmission.

The effect of cholecystokinin on dopamine D_2 receptors in the striatum and nucleus accumbens of the rat was studied using ^{3}H-spiperone.

The density (B_{max}) of D_2 receptors was elevated:

a) by 20% (accumbens) upon in vitro co-incubation with 10^{-6}M cholecystokinin. (A nonsignificant drop of 10% occurred in striatum).
b) by about 50% (accumbens) or 25% (striatum) after continuous intraventricular infusion of cholecystokinin for 24 h at 2 ng/h, and 35% (accumbens) after 14 days.
c) by 20% (accumbens) or 15% (striatum) 24 h after a single i.p. injection of 50 µg/kg cholecystokinin or caerulein, and measured 30 min to 14 days later. This prolonged action after an i.p. injection may be related to the alleviation of psychotic symptoms by cholecystokinin (Moroji et al.,Int. Pharmacopsychiat. 17: 1982; Nair et al., Progr. Neuropsychopharmacol. & Biol. Psychiat. 6: 1982). (Supported by OMHF and MRC).



160.6 SOMATOSTATIN AND CHOLECYSTOKININ OCTAPEPTIDE MODULATE THE RELEASE OF ACETYLCHOLINE AND NEUROTRANSMITTER AMINO ACIDS FROM RAT CAUDATE NUCLEUS SLICES BY DIFFERENT MECHANISMS.
S.P. Arnerić, M.P. Meeley and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

The neuropeptides somatostatin (SOM) and cholecystokinin octapeptide (CCK) increase the basal and evoked release of dopamine (DA) from rat striatum (J. Neurosci. 3:232,1983; Neurochem. Int. 4:233, 1982). DA, in turn, modulates the release of acetylcholine (ACh) and amino acid (AA) neurotransmitters within the striatum. We therefore sought to establish whether SOM and CCK modulate the release of striatal ACh and AAs. If so, are the effects mediated through the DA neuronal system?

Slices (0.3-0.5 mm) of caudate nucleus (CN) and, for comparison, cerebral cortex (CX) were prepared and incubated with Kreb's bicarbonate buffer containing bacitracin (100 uM) and physostigmine (100 uM). ACh release was measured radiochemically following preincubation with ^{3}H-choline (1 uM). Endogenous aspartate (Asp), glutamate (Glu), glycine (Gly), and gamma-amino butyric acid (GABA) were measured by high performance liquid chromatography.

Potassium (5-55 mM) produced a Ca^{2+}-dependent release of ACh, Asp, Glu, Gly and GABA from the CN and CX. The K^+-induced (35 mM) release of ACh from CN, but not CX, was reduced approximately 30% by SOM (1 uM), CCK (1 uM) and the DA agonist, apomorphine (30 uM) (n=4-9; $p < 0.05$). ACh release from CN and CX was not altered by incubation (1 uM) with m-enkephalin, vasoactive intestinal peptide, thyrotropin releasing hormone or substance P. Incubation with the DA antagonist, sulpiride (SULP), blocked the inhibition of ACh release produced by apomorphine and SOM, but not CCK. The K^+-induced release of Glu was inhibited 26% by SOM, an effect not blocked by SULP. In contrast, CCK stimulated the basal and K^+-induced release of Gly to 231% and 160% of control, respectively, an effect blocked by pretreatment with SULP.

We conclude: (1) SOM and CCK inhibit release of ACh from CN, but not CX. (2) SOM, but not CCK, modulates ACh release from CN indirectly through the DA neuronal system. (3) CCK facilitates release of Gly via DA neurons, while SOM inhibits release of Glu by a non-dopaminergic action.

160.7 NEUROTENSIN EFFECTS ON DOPAMINE METABOLISM IN MICROPUNCHED RAT BRAIN NUCLEI.
S.T. Cain, T. Ely*, C.D. Kilts*, and C.B. Nemeroff. Department of Psychiatry, Duke Univ. Me Ctr., Durham, NC 27710.

Intracisternal (IC) injections of neurotensin (NT) produce increases in the concentration of the dopamine (DA) metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the striatum, olfactory tubercles, and nucleus accumbens (J. Pharmacol. Exp. Ther. 225: 337, 1983). In the present investigation, the effects of IC NT on DOPAC, HVA, and 5-hydroxyindoleacetic acid (5-HIAA) concentrations were determined in samples of DA cell body and terminal regions obtained by a micropunch dissection technique. Sixty minutes after either 30 µg NT or saline, rats were decapitated and the brains frozen on dry ice. Brain regions examined were the substantia nigra pars reticulata (SNR), substantia nigra pars lateralis (SNL), ventral tegmental area (VTA), prefrontal cortex, cingulate cortex, lateral septum. central nucleus of the amygdala, olfactory tubercles, nucleus accumbens, and caudate nucleus. Nuclei were micropunched from 300 µm sections of the frozen brain, ultrasonically homogenized, and centrifuged. The supernatant was injected directly into an HPLC system consisting of an anion exchange column for trace enrichment and a reverse-phase column for further separation. DOPAC and HVA concentrations were approximately twice as high in the VTA as in the SNR and SNL, though this dose of NT did not alter the concentration of either DOPAC or HVA in any of the DA cell body regions. In the subortical DA terminal regions, the gradient of DOPAC concentrations was nucleus accumbens=caudate>olfactory tubercles>central amygdaloid nucleus>lateral septum. NT significantly increased ($p<.05$) DOPAC concentrations in the caudate nucleus. The concentration gradient for HVA was nucleus accumbens=caudate>central amygdaloid nucleus>olfactory tubercles>lateral septum. NT significantly increased the HVA concentration in the olfactory tubercles and nucleus accumbens ($p<.05$). The prefrontal and cingulate cortex had similar concentrations of both DOPAC and HVA. In these cortical areas, the quantities of DOPAC were half that found in the lateral septum. The HVA concentration of the cortical areas was similar to those found in the lateral septum. NT did not alter the concentration of either metabolite in the cortical areas. These experiments illustrate the importance of anatomically resolving effects when studying neurochemical mechanisms of action.
(Supported by NIMH MH-39415).

160.8 **EFFECTS OF CHRONIC NEUROLEPTIC AND ANTICHOLINERGIC THERAPY ON DOPAMINE-2 AND MUSCARINIC CHOLINERGIC RECEPTORS IN RAT STRIATUM.**
S.J. Boyson, P. McGonigle*, G.R. Luthin, B.B. Wolfe, and P.B. Molinoff. Dept. of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Chronic neuroleptic therapy in humans may be associated with the development of tardive dyskinesia, which is thought to be due to supersensitivity of dopamine-2 (D-2) receptor-mediated responses or, perhaps, subsensitivity of muscarinic (M) cholinergic receptor-related responses. Potencies of the atypical neuroleptics clozapine and thioridazine are similar at both D-2 and M receptors. It is this anticholinergic activity that has been credited for the lower rate of tardive dyskinesia associated with these atypical neuroleptics. Co-administration of anticholinergics with classical neuroleptics has been suggested as a means of preventing the development of tardive dyskinesia. Recently, Carvey, et al. (Neurology 34(Suppl. 1):104, 1984), found that, in rats, chronic administration of anticholinergic agents along with haloperidol was able to prevent the behavioral supersensitivity to the administration of apomorphine usually seen after haloperidol treatment.

We have investigated changes in D-2 and M receptor densities in rats treated for 14 days with the classical neuroleptic fluphenazine decanoate (FD; 2.5 mg/kg i.m. on day 1) with or without atropine (20 mg/kg/d), atropine alone, clozapine (20 mg/kg/d), thioridazine (20 mg/kg/d), or saline. D-2 and M receptor densities were measured in striatal membranes by Scatchard analysis of the binding of ^{3}H-spiroperidol and ^{3}H-quinuclidinylbenzilate (QNB), respectively. Clozapine, thioridazine, and atropine did not significantly alter the density of striatal D-2 receptors. The administration of FD lead to a 30% increase in the density of D-2 receptors, and this 30% increase was not prevented by the co-administration of atropine. Atropine alone increased M receptors by 20%. The co-administration of FD with atropine completely prevented this increase in M receptors. The effects of benztropine and trihexyphenidyl appear to be similar to those of atropine when co-administered with FD.

Thus, changes in the densities of D-2 and muscarinic receptors due to administration of a classical neuroleptic plus an anticholinergic are not the same as those produced by administration of atypical neuroleptics with intrinsic anticholinergic properties. Changes in individual receptor densities may not be predictable from the results of behavioral assays such as response to apomorphine.

(Supported by the Hereditary Disease Foundation, NS07272, NS18591, GM09991, GM31155, and the AHA.)

160.9 NEUROPEPTIDE Y IN THE RAT NUCLEUS ACCUMBENS: ULTRASTRUCTURAL LOCALIZATION AND SYNAPTIC INTERACTION WITH GABA-ERGIC NEURONS. V.J. Massari[1], J. Chan*[2], B. Chronwall[3], T.L. O'Donohue[3] and V.M. Pickel[2], [1]Dept. of Pharmacology, Howard Univ., Washington, DC 20059, [2]Lab. of Neurobiology, Cornell Univ. Med. Coll., , New York, NY 10021, [3]Exp. Ther. Branch, NINCDS, Bethesda, MD 20205.

We examined the electron microscopic localization of neuropeptide Y (NPY) in the adult rat nucleus accumbens to determine: (1) the ultrastructure of NPY-containing neurons and (2) the synaptic associations between NPY-labeled neurons and terminals showing immunoreactivity for the GABA-synthesizing enzyme, L-glutamate decarboxylase (GAD). The immunocytochemical markers were rabbit antisera produced against the C-terminal hexapeptide of NPY and a sheep antiserum to GAD. The antiserum to GAD was produced and generously supplied by Drs. W.H. Oertel, D.E. Schmechel, M.L. Tappaz and I.J. Kopin. The NPY and GAD antisera were independently and jointly localized in Vibratome sections from the brains of animals previously fixed by vascular perfusion with 4% acrolein and 4% paraformaldehyde in 0.1M phosphate buffer. The localization of the antisera was demonstrated either by the peroxidase-antiperoxidase (PAP) technique, or by 5-20nm colloidal gold particles attached to the secondary immunoglobulin.

The peroxidase reaction product and gold particles were localized within the cytoplasm of sparsely distributed neurons having a diameter of 10-20 um. The perikarya were further characterized by a thin rim of cytoplasm containing several dense core vesicles. The nucleus was generally round without obvious indentations and was devoid of immunoreactivity. Immunocytochemically labeled dendrites were also relatively infrequently detected. In contrast, dense labeled terminals were found in all parts of the nucleus accumbens. The labeled terminals contained numerous small clear and one or more dense core vesicles and formed primarily symmetric axodendritic synapses with proximal dendrites and occasionally with unlabeled soma. The GAD labeled terminals also formed symmetric synapses primarily with unlabeled dendrites. In studies combining peroxidase labeling for GAD with colloidal gold markers for NPY, a few (5-10%) of the dendrites postsynaptic to GAD-labeled terminals were decorated with gold particles. These results demonstrate that NPY has an ultrastructural localization comparable to that seen for other putative peptide transmitters and that the NPY-containing neurons are synaptically linked to GABA-ergic neurons in the rat nucleus accumbens. (Supported by NIH Grant HL18974, NIMH Career Award MH00078 and NSF Grant BNS7923451.)

160.10 CATECHOLAMINERGIC NEURONS IN THE MEDIAL NUCLEI OF THE SOLITARY TRACTS RECEIVE DIRECT SYNAPSES FROM GABA-ERGIC TERMINALS: COMBINED COLLOIDAL GOLD AND PEROXIDASE LABELING OF SYNTHESIZING ENZYMES. V.M. Pickel, J. Chan, T.H. Joh and V.J. Massari, Lab of Neurobio., Cornell Univ. Med. Coll., New York, NY 10021.

We sought to determine whether terminals containing γ-amino butyric acid (GABA) formed synapses with catecholaminergic neurons of the A2 group within the medial nuclei of the solitary tracts. Markers for GABA and catecholamines included antisera to their respective synthesizing enzymes, L-glutamate decarboxylase (GAD) and tyrosine hydroxylase (TH). Sheep antiserum to rat brain GAD was produced by Drs. W.H. Oertel, D.E. Schmeckel, M.L. Tappaz and I.J. Kopin. This antiserum was localized by the peroxidase-antiperoxidase (PAP) method in Vibratome sections through the medulla of adult male rats which were previously prepared by aortic arch perfusion with 100 ml of 4% acrolein plus 4% paraformaldehyde. The PAP labeled sections were subsequently incubated with rabbit antiserum to TH which was visualized by a secondary immunoglobulin bound to 5nm gold particles (Structure Probe, Inc.). Ultrastructural examination revealed good preservation of cytoplasmic membranes and retention of antigenicity for both enzymes with the combined fixatives. The peroxidase immunoreactivity for GAD was localized to unmyelinated axons and axon terminals (1-5 um in diameter). The GAD labeled terminals formed primarily symmetric axodendritic synapses with dendrites which failed to exhibit gold particles indicative of the presence of TH. However, at least 5% of the recipient dendrites were decorated with gold particles. The low percentage appeared to at least partially reflect less penetration of the gold particles as compared to PAP. Isolated terminals and perikarya also showed selective labeling for TH by the gold technique. The structure of these neurons was clearly revealed in the absence of the peroxidase product which generally obscured details of the cytoplasmic structure. The present findings demonstrate: (1) the utility of combining colloidal gold with PAP for visualization of two neurotransmitter synthesizing enzymes within the same section of tissue prepared for electron microscopy; and (2) that GABAergic neurons form direct synapses with catecholaminergic neurons of the A2 group in the caudal medulla. (Supported by NIH Grant HL18974 & NIMH Career Award MH00078). V.J.M. also received support from Dept. Pharmacology, Howard Univ. School of Med. & NSF Grant BNS7923451.

CEREBELLUM I

161.1 CONTRAST BETWEEN THE 2 MAJOR DIVISIONS AND 3 CELL TYPES OF MONKEY RED NUCLEUS. P.R. Kennedy, A.R. Gibson and J.C. Houk, Dept Physiol Northwestern Univ Med Sch, 303 E Chicago Av, Chicago, Il 60611.

Only minor differences have been found between responses in the parvocellular and magnocellular divisions of the red nucleus (Otero JB, Brain Res, 101:37-46,1976, and Larsen and Yumiya, Exp Brain Res, 40:393-404,1980). Similarily, only minor differences between the cortical projections to each division were accepted until recently (Humphrey et al, J Comp Neurol, 1984, in press).

We recorded 323 single units in 4 red nuclei in 2 monkeys, one trained to operate a finger device, and both trained to accept joint manipulation and sensory testing. At each daily recording session, a tungsten microelectrode was introduced through a chamber fixed to the head. Locations of recording sites were reconstructed histologically. Magnocellular neurons were very active during hand, foot or face movements as previously reported (Kohlerman et al, Science, 217:857, 1982, and Kennedy et al, Soc Neurosci Abstr, 9(1):224,1983). Discharge rate often exceeded 100 pps. By contrast, parvocellular neurons were weakly responsive or unresponsive during movement and in sensory testing. More than half the 55 parvocellular units were unresponsive; the others were not clearly related to any component of the movement.

In the magnocellular sample, 2 classes of responses were noted. One class had an average response rate of 116±28 pps and spontaneous rate of 36±10 pps, while the other class had an average response rate of 64±18 pps and spontaneous rate of 16±5 pps. Spike amplitudes were also different at 735±318 and 527±255 μV ($p<0.05$, $n=13$). Histological examination showed medium sized (30 to 50 μ) cells at sites where units with small spike amplitudes and low discharge rates were recorded. Large cells (50 to 90 μ) were present where units with larger spikes and higher discharge rates were recorded.

The medium sized cells may have been considered as parvocellular neurons in earlier studies. To assess this possibility, we injected WGA-HRP at various levels of the spinal cord in 2 monkeys. Dense retrograde filling of large and medium sized cells was seen. No small cells were labeled. Thus, the rubrospinal tract originates from both large and medium sized cells: On this basis, both regions can be considered part of the magnocellular division of the nucleus The parvocellular division contains only small cells which do not project to spinal levels.

To further differentiate the divisions and cell types, inputs to the nucleus were studied retrogradely and anterogradely. Injections of WGA-HRP largely confined to the magnocellular division showed very few labeled cells in the sensorimotor cortex, but many labeled cells in the interpositus nucleus of the cerebellum. In another monkey, WGA-HRP was injected into area 4, with little spread into posterior 6 or 3a. Anterograde label in the most posterior magnocellular region containing the largest cells was very light; the dorsally located medium sized cells in the mid-region were surrounded by some terminal labeling; the anterior region, containing the small cells of the parvocellular division, had extremely dense terminal labeling. Thus the parvocellular division receives heavy motor cortical input, whereas the magnocellular division receives very light cortical input, except for the dorsal medium sized cells whose cortical inputs are moderate.

In summary, our single unit and neuroanatomical data indicate that parvocellular and magnocellular divisions of the red nucleus are distinct in both properties and connections. In addition, the magnocellular division shows evidence of further specialization within the population of rubrospinal neurons.

161.2 A COMPARISON OF THE RESPONSES OF DENTATE AND INTERPOSED NEURONS DURING PERTURBED AND UNPERTURBED LOCOMOTION. A.B. Schwartz, T.J. Ebner, J.R. Bloedel, Depts. of Neurosurgery and Physiology, U. of Minn., Mpls., MN 55455

These experiments compare the responses of neurons in the dentate and interposed nuclei of precollicular, midmammillary decerebrate cats during spontaneous and perturbed treadmill walking. Integrated, rectified EMG from the biceps brachii and lateral triceps, right forelimb displacement and the discharge of antidromically identified dentate and interposed neurons were recorded simultaneously. A trigger activated at a specific phase of the step cycle was used to construct the averaged responses over 50-100 trials (1.5-3 step cycles each). Two types of perturbations timed to occur at various phases of the locomotor cycle were used, one a short braking of the treadmill belt, the other a bar placed in the path of the right forelimb during either the swing or stance phase. The responses of interposed neurons were closely related to the phase, duration and amplitude of either the extensor or flexor EMG in both the perturbed and nonperturbed trials. Modulated activity of some dentate cells during unperturbed locomotion was not well correlated to EMG activity, and perturbation caused the modulation to cease. Another group of dentate cells was relatively unmodulated during unimpeded locomotion. In many of these neurons the perturbation evoked a characteristic alteration in discharge rate independent of the step cycle phase and any evoked EMG activity. This study shows characteristic differences in the responses of interposed and dentate neurons. In general the interposed activity is correlated with the motoneuronal activity of the ipsilateral forelimb in both perturbed and unperturbed trials. In contrast dentate neuronal activity is not well related to the time course of the EMG, particularly during unperturbed locomotion. These cells are modulated principally in response to the perturbation independent of the phase of the locomotor cycle at which the perturbation is applied. These results are consistent with the hypothesis that the dentate and interposed nuclei are involved in different aspects of monitoring and correcting alterations in motor behavior. Furthermore the data indicate that the output of the dentate nucleus can be modulated by inputs from the brainstem and/or spinal cord during nonvolitional motor behavior. Supported by NIH grants NS 09447 and NS 18338.

161.3 FLOCCULAR PURKINJE CELL RESPONSE TO HIGH FREQUENCY AND SUDDEN HIGH VELOCITY OPTOKINETIC STIMULI. R.Boyle*, U.Büttner and G.Markert*. Neurology Clinic, University of Düsseldorf, D-4000 Düsseldorf, W.Germany.

Floccular Purkinje cells receive head and eye velocity inputs and their output encodes the velocity of eyes in space. We selected Purkinje cells (PCs) in the alert monkey (M.fascicularis) for their modulation to horizontal head rotation and smooth pursuit and studied their response to optokinetic stimuli (OKS), both sinusoidal and sudden exposure to a constant velocity cylinder rotation. Trained monkeys suppressed eye movements during head rotation (VOR suppression) and pursued a visual target with head-fixed. PCs were identified by their pause in simple spike discharge on occurrence of a positive-going complex spike. PC responses during VOR suppression and smooth pursuit had roughly equal sensitivities (ips per °/s) and were in phase (°) with stimulus (head/target) velocity in the same (usually ipsilateral) direction; no rate modulation was observed using head rotation in the dark (VOR dark). PC sensitivity across the frequency domain of sinusoidal OKS from 0.05-1.0 Hz (±40°/s) either remained constant or increased progressively as the frequency increased; many PCs responded vigorously up to 3.3 Hz (±20°/s) or 5.0 Hz (±15°/s). Phase of response (re cylinder velocity) at 0.1 Hz was on the average +20°, at 1.0 Hz -5°, at 2.0 Hz -50°, and at 3.3 Hz -100°. In contrast, gain of eye movements decreased from unity at about 0.5 Hz, being 0.6 at 1.0 Hz and 0.35 at 3.3 Hz; phase of eye movements (re cylinder velocity) averaged -3° at 0.1 Hz, -31° at 1.0 Hz, -62° at 2.0 Hz, and -100° at 3.3 Hz. Therefore, while the gain of eye movements declined over the higher frequencies of OKS, PC response remained high; relation between phase of response of PC and eye movements was close. Upon exposure to a 40°/s constant velocity OKS, PCs responded only with a transient rate increase during the initial rise in slow phase eye velocity; using 80 and 120°/s stimuli the transient response was followed by a maintained rate increase which persisted throughout the stimulus presentation. These results suggest that PCs are involved in the high frequency and high velocity oculomotor performances of optokinetic nystagmus, and support clinical and experimental observations following floccular damage. (Supported by DFG-SFB 200 A 2 and A.v.Humboldt-Stiftung).

161.4 PURKINJE CELL ACTIVITY IN THE FLOCCULUS OF THE ALERT RABBIT DURING NATURAL VISUAL AND VESTIBULAR STIMULATION. C.S. Leonard & J.I. Simpson, Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

The response properties of rabbit floccular Purkinje cells (P-cells) were investigated by extracellular recording while measuring eye movements during several behavioral paradigms. They were: vestibular stimulation in the dark (VD); vestibular stimulation in the light with the rabbit's visual world fixed to the earth (VL); optokinetic stimulation (OK); and vestibular stimulation in the light with the rabbit's visual world coupled to the vestibular turntable (VS). Vestibular stimulation (position triangles; 0.1 Hz ± 5°) was provided by rotating the rabbit about the earth vertical axis on a servo-controlled turntable with the animal positioned to minimize stimulation of the vertical semicircular canals. Visual stimulation (position triangles; 0.1 Hz ± 5°) was provided by rotating a patterned cylinder around the rabbit. Eye movements were measured using a magnetic search coil system. The P-cells selected for study were those having climbing fibers excited by contralaterally directed horizontal movement of the visual world. Our present results, as well as those of past investigations, showed that the large majority of such P-cells exhibit, in the VD and VL paradigms, an increase in their simple spike firing rate for contralateral turntable movement and a decrease for ipsilateral turntable movement (type II modulation). In addition, in the OK paradigm they increase their firing rate for ipsilateral movement of the visual world and decrease their firing rate for contralateral movement. Preliminary results indicate that these P-cells can be divided into two categories on the basis of their responses in the VS paradigm, during which eye movements were effectively suppressed. Under this condition, cells of one category exhibited distinct Type I modulation while the others showed virtually no modulation. For the latter category, the lack of modulation in the VS paradigm points to the absence of a head velocity signal, while the modulation patterns in the other paradigms point to an encoding of eye movement parameters. The modulation patterns of those cells that exhibited type I responses in the VS paradigm have several possible interpretations, an attractive one being that a combination of eye velocity and low speed retinal image slip signals are encoded. Supported by USPHS grant NS13742 from NINCDS.

161.5 SOMATIC SENSORY REPRESENTATION BY CLIMBING FIBER RESPONSES IN THE PARAMEDIAN LOBULE OF THE CAT CEREBELLUM. Lee T. Robertson. Neurological Sciences Institute, Good Samaritan Hosp. & Med. Ctr., Portland, OR 97209.

Earlier studies of the spatial organization of tactile information in the cerebellum have traditionally described it in terms of sensory dermatones or of parasagittal zones. However, our studies in the anterior lobe of climbing fiber responses elicited by natural stimulation reveal a more complex organization, involving a wide range of receptive fields from mainly the ipsilateral forelimb, hindlimb, and face. This study examines the climbing fiber organization in the paramedian lobule.

Data were collected from cats anesthetized with sodium pentobarbital. Microelectrodes were used to record climbing fiber response from single Purkinje cells in response to controlled mechanical stimulation of various body surfaces. On the basis of force thresholds, the units were classified as cutaneous, deep, or unresponsive. The lowest possible threshold were used to define the receptive fields.

CF responses were identified in 854 Purkinje cells, of which 54% were driven by tactile stimulation. Of the units elicited by the tactile stimulation, 50% represented areas of the ipsilateral forelimb and 41% represented fields on the hindlimb. Areas of the face were represented by only 5% of the responses and portions of the shoulder, back, or abdomen by 1.0% of the units. Split receptive fields involving both the ipsilateral forepaw and hindpaw comprised 2% and bilateral forepaw receptive fields accounted for 1%.

In general, the proportion of receptive fields of various areas of the limbs were similar between the forelimb and hindlimb, except that the wrist was represented in fields which extended more proximally, whereas the receptive fields involving the heel also included more distal parts of the paw. This distinction may correspond to a different functional role of the two limbs.

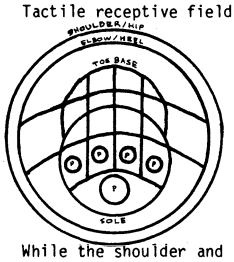
Although there was considerable interanimal variability, the forelimb representation predominated in the dorsorostral area, whereas the majority of the hindlimb representation was encountered in the ventrocaudal regions. Patches of similar representations were encountered, but no parasagittal zones were distinguished.

Only minor differences were identified between the type of tactile information conveyed to the paramedian lobule versus that received by the anterior lobe.

161.6 INFORMATION CONVEYED BY THE TACTILE RECEPTIVE FIELDS OF CLIMBING FIBERS. Gin McCollum and Lee Robertson. Neurological Sciences Institute, Portland, OR 97209.

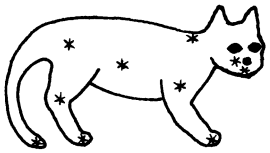
One strategy for coding skin location in a neuron array is a simple one-to-one relation between neurons and receptive fields with little overlap. Another strategy is overlapping receptive fields; then a small region is specified by the coincident firing of more than one neuron.

Tactile receptive fields of climbing fibers (Robertson, previous abstract) follow the second strategy. Overlapping receptive fields distinguish a grid of small areas on the paw as shown. The whole paw is projected flat around the paw pads (P). Most receptive fields on the paw follow the grid lines but include more than one grid area. In this way, each area is represented by a set of overlapping receptive fields.



While the shoulder and hip are analogous structures, the boundary at the elbow distinguishes foreleg receptive fields from those of the hindleg, which have a boundary at the heel. The cat heel is used for support in a way that the analogous forelimb structure, the wrist, is not.

Aside from the elbow or heel line, the boundaries are variable outside the paw area, above the toe base. Strips and rings extend various distances up the leg. The lower boundary is always at or within the wrist (fore) or the sole or toebase (hind) line. Thus these receptive fields form concentric sets centered about the paw. In general most fields not in the paw grid are arranged concentrically around certain points on the cat's body (asterisks). Split receptive fields, both fore-hind and bilateral, all involve only fields at the center of a concentric set of fields.



One would expect an organization this strict, constructed by convergence of peripheral receptive fields, to be reflected in overall climbing fiber and cerebellar function.

161.7 A GABAERGIC CEREBELLO-OLIVARY PROJECTION IN THE RAT. B. Nelson*, N.H. Barmack and E. Mugnaini (SPON: S.C. Maxson). Lab of Neuromorphol, Univ of Conn, Storrs, CT; and Neurol Sci Inst, Good Samaritan Hosp, Portland, OR.

A projection from cerebellar nuclei to inferior olive (IO) arising predominantly from small neurons in the interpositus and lateral nuclei has been described previously in several mammals (including monkeys). Small neurons showing immunoreactivity to glutamate decarboxylase (GAD) are present in these nuclei (Mugnaini & Oertel, 1981), but it is not known whether they are local circuit or projection neurons. In the present study, a ventral approach was used to inject WGA-conjugated HRP into IO of adult rats with minimal involvement of surrounding tissues. After 36-48 h, the rats were perfused with a zinc-aldehyde fixative, pH 6.5 (Mugnaini & Dahl, 1983). Coronal Vibratome sections through the extent of IO and cerebellar nuclei were processed for retrogradely transported WGA-HRP with 4-chloro-1-naphthol/H_2O_2 (blue granular reaction product). The sections were then processed with the PAP method for localizing somatal GAD immunoreactivity using the sheep antiserum of Oertel et al. (1981) with DAB as the chromogen (diffuse brown reaction product). Numerous neurons in the cerebellar nuclei demonstrated both retrogradely transported WGA-HRP and GAD immunoreactivity. The vast majority of WGA-HRP labelled cells in the cerebellar nuclei were GAD-positive small (11-12 μm) neurons usually contralateral to the injection site. Large WGA-HRP injections in IO resulted in numerous double labelled cells in the cerebellar nuclei lateralis and interpositus (posterior and anterior) and few double labelled cells in the nucleus medialis. Small injections in IO resulted in double labelled cells in restricted regions of the cerebellar nuclei, confirming that this projection is topographically organized. Studies are in progress to ascertain whether small cells that are part of the ascending (NRTP, rubral and thalamic) cerebellar nuclear projection also show GAD immunoreactivity.

This study indicates that a substantial number of projection neurons in the cerebellar nuclei may be inhibitory, in contrast to the tenet of an inhibitory outflow from the cerebellar cortex and an excitatory outflow from the deep nuclei. It is of interest that it is primarily the GAD-containing neurons of the cerebellar nuclei that project to the IO. This GABAergic projection indicates that the nuclei may participate in a negative feedback loop that regulates the activity of olivary neurons. (Supported by NIH grants NS-09904 and EY-04167.)

161.8 NEURONS IN NUCLEUS BETA OF THE CAT INFERIOR OLIVE RESPOND TO HORIZONTAL ROTATION. F.R. Robinson, M.O. Fraser*, & D.L. Tomko. Dept. of Physiol., Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

The inferior olive is necessary for behavioral adaptation to unilateral VIIIth nerve section (Llinas, et al., Science 190:1230, 1975) but little is known about the role of this nucleus in normal vestibular function. Nucleus beta (NB), a morphologically distinct subdivision of the inferior olive, receives input from the inferior and medial vestibular nuclei (e.g. Saint-Cyr and Courville, Br. Res. 165:189, 1979) and projects to a part of the vestibulocerebellum, the uvula (vermal lobule IX) (e.g. Brodal, J. Comp. Neurol. 166:417). Since climbing fiber responses to horizontal rotation have been reported in uvula Purkinje cells it seems likely that some NB neurons relay horizontal rotation information to the uvula. To test whether or not NB cells are sensitive to horizontal rotation we recorded from these neurons in barbituate anesthetized cats during rotation around the vertical (yaw) axis.

We found cells sensitive to horizontal rotation in the posteriomedial region of the inferior olive. These cells were almost always immediately ventral to cells that responded to light flashes. The position of marking lesions made among visual and vestibular olivary neurons indicates that the visual cells were in the teleceptive region of the medial accessory olive and the vestibular cells were in the underlying NB.

Neurons in NB sensitive to horizontal rotation typically fired one or two spikes at the onset of acceleration to the ipsilateral side. Above threshold there was little difference in the response of NB cells to different accelerations. In this respect they are similar to somatosensory neurons in the dorsal accessory olive that respond to the presence of a stimulus but do not seem to carry detailed information about its intensity (Gellman et al., J. Comp. Neurol. 215:228, 1983). The NB cells tested to date were not extremely sensitive to horizontal yaw rotation but may, like many cells in the vestibular nuclei, prove sensitive to other planes of rotation or to head tilt.

Supported by NASA grant NAG2-155 and NIH grant NS17585.

161.9 CHANGES IN THE RESPONSES OF ANATOMICALLY RELATED NUCLEAR NEURONS AND PURKINJE CELLS ASSOCIATED WITH THE ACTIVATION OF THE CLIMBING FIBER INPUT. C.J. McDevitt, T.J. Ebner, J.R. Bloedel, Depts. of Neurosurgery and Physiology, U. of Minn., Mpls., MN 55455

These studies examine the relationship between climbing fiber related changes in Purkinje cell responsiveness and the responses of anatomically related cerebellar interposed neurons in decerebrate, paralyzed, unanesthetized cats. Isolated nuclear neurons were antidromically identified using bipolar electrodes in the contralateral red nucleus. Anatomically related Purkinje cells were found and antidromically identified by applying 5-20 uA of cathodal current with the same microelectrode used to isolate the nuclear cell. Their relationship was further established by evoking short latency inhibitory responses in the nuclear cell following microstimuli applied with the Purkinje cell electrode. Responses in 38 of these neuronal pairs were simultaneously recorded and analyzed. Peristimulus time histograms (PSTH) showing the simple spike, complex spike and nuclear neuron responses to passive square wave displacement of the cat's ipsilateral forepaw were computed. Additional histograms were constructed by separating Purkinje and nuclear cell responses into climbing fiber and nonclimbing fiber trials based on whether a climbing fiber input to the Purkinje cell was evoked by the stimulus. Histograms from the climbing fiber trials of both cells also were aligned on the occurrence of the complex spike evoked in the Purkinje cell. The changes in the firing probability of both neurons occurring in the trials in which the climbing fiber input to the Purkinje cell was evoked were assessed by computing the gain change ratio, the quotient of the Purkinje cell or nuclear neuron response amplitude in the climbing fiber trials and the nonclimbing fiber trials. Purkinje cells in 82% (31) of pairs demonstrated changes in responsiveness when climbing fiber responses were evoked. In these pairs 77% (24) of nuclear neurons showed changes in responsiveness, 55% (17) being increased. These findings suggest that the changes in responsiveness of Purkinje cells to mossy fiber inputs produced by the action of the climbing fibers are reflected as changes in the responsiveness of cerebellar nuclear neurons. Supported by NIH grants NS 09447 and NS 18338.

161.10 CEREBELLAR REGULATION OF MULTIJOINT CAT FORELIMB TRAJECTORY. V.E. Amassian, D.E. Batson and L. Eberle. Dept. of Physiol., SUNY, Downstate Med. Ctr., Brooklyn, N.Y. 11203.

A TV-computer system was used to measure angles at shoulder (θ_{sh}), elbow (θ_{el}) and lumped digit-wrist joints (θ_{wr}) at 60 Hz during contact placing (CP). During economical CP by an intact cat, the forepaw trajectory has an initial contactual, vertical upward component followed, when the paw clears the top of the apparatus, by freepath near horizontal and near vertical-downward components. We showed that during the contactual vertical trajectory, θ_{sh} and θ_{wr} are usually linearly related to θ_{el} and inversely related to one another (J. Physiol. (1981), 310: 51-52P and (1982), 326: 53-54P). During the freepath trajectory, when the transition occurs from limb flexion to extension, $-d\theta_{sh}/dt$ and $-d\theta_{el}/dt$ reverse at about the same time; the plot of θ_{sh} versus θ_{el} shows little hysteresis, the slope, ie, the ratio of the angular velocities ($d\theta_{sh}/d\theta_{el}$) showing little change after the transition. Conservation of $d\theta_{sh}/d\theta_{el}$ despite activity predominantly in antagonist muscles and the loss of tactile drive suggests a motor 'memory' function. By contrast, $-d\theta_{wr}/dt$ reverses much earlier than $-d\theta_{el}/dt$ and then increases disproportionately, resulting in marked hysteresis in the plot of θ_{wr} versus θ_{el} and accounting for much of the horizontal component of the normal freepath movement vector.

When CP returned after massive high frequency lesions of ipsilateral N interpositus and dentatus (I-D), the freepath trajectory had increased dimensions (hypermetria) due to increased changes in θ_{sh}, θ_{el} and θ_{wr} and was altered in shape. The initial freepath trajectory was displaced upward and forward. Such distortions resulted either from $-d\theta_{el}/dt$ reversing significantly later (e.g. by 50 msec) than $-d\theta_{sh}/dt$ and, or, $d\theta_{sh}/dt$ increased disproportionately to $d\theta_{el}/dt$, resulting during extension in a marked change in $d\theta_{sh}/d\theta_{el}$ with obvious hysteresis in the plot of θ_{sh} versus θ_{el}. Locally anesthetizing joint receptors (ibid) caused similar changes. Large extirpations mainly of intermediate and lateral zone cerebellar cortex caused even greater delays in the reversal of $-d\theta_{el}/dt$; $d\theta_{el}/dt$ was reduced, the paw slowly falling onto the landing surface. Recovery occurred sooner than after I-D lesions. Thus, without I-D or joint afferent input, the motor control system cannot generate flexion-extension changes at different forelimb joints at rates appropriate for a fast, complex trajectory. Loss only of cerebellar cortex further disturbs the trajectory by releasing elbow flexion.

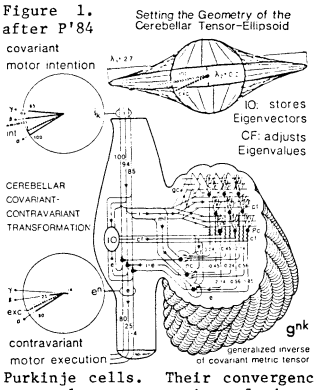
Aided by USPHS, NIH grant NS 07117.

161.11 TENSORIAL COMPUTER MOVIE OF THE GENESIS AND MODIFICATION OF CEREBELLAR NETWORKS AS DYADIC EXPANSIONS OF THE EIGENVECTORS STORED IN THE INFERIOR OLIVE. A. Pellionisz, Dept. Physiol. & Biophys., N.Y.U. Med. Ctr, 550 1st Ave, New York, NY 10016.

Cerebellar (CB) function is interpreted in tensor theory of the CNS (Pellionisz and Llinas [P&L] 1979) as a geometric transformation of covariant motor intention vectors into contravariant motor execution, that enables a coordinated action (P&L 1980). Such a metric function occurs in a curved spacetime manifold (P&L 1982), thus requiring a CF system that in effect makes the metric to be position-dependent in the state-space. Developmental changes of the structure of the organism show even more obviously that a metric-type network, whose functional geometry is to match the physical geometry of the executor system, must evolve and adapt suitably to the ever-changing status (L&P 1984).

Metaorganization, by which a geometry, eg. in the physical space, can mold another which is expressed in a different space, e.g. in a functional hyperspace (Pellionisz 1983,1984, P&L 1984), explains how such network-properties may emerge.

Figure 1. after P'84



Metaorganization is based on finding eigenvectors in one system, and composing from them a matching (duplicate or complementary) geometry. In keeping with the view on oscillatory properties (Llinas '84) it is suggested that the eigenvectors found by covariant to contravariant reentry, are implemented in the inferior olive (IO), setting the principal directions of the tensor-ellipsoid of the CB metric network. Climbing fibers (CF) then carry them to CB nuclei, both directly and via the Purkinje cells. Their convergence imprints the eigendyadic spectral representation of the required network, (generalized) inverse of the covariant metric g^{kn}, regardless of overcompleteness. Subsequent CF signals, reporting on errors of g^{kn}, adjust the eigenvalues, ie. the lengths of the ellipsoid-axes. The procedure of network-organization is demonstrated by a computer movie. -Suppt: USPHS NS13742.-

161.12 DECREASED CEREBELLAR cGMP AND FAILURE TO RESPOND TO HARMALINE IN GENETICALLY DYSTONIC (dt) RATS. J. F. Lorden, J. Lutes*, M. Beales*, and G. A. Oltmans. Dept. of Psychology, University of Alabama in Birmingham, Birmingham, AL 35294, and Dept. of Pharmacology, Chicago Medical School, North Chicago, IL 60064.

The genetically dystonic rat is an autosomal recessive mutant displaying a complex motor syndrome that includes sustained twisting movements that appear on postnatal days 9-10. The syndrome is correlated with increased glutamic acid decarboxylase activity in the deep cerebellar nuclei and increased cerebellar norepinephrine levels in comparison with unaffected littermates, but apparently normal cerebellar morphology. The tremorogenic drug harmaline was used to examine the function of the cerebellar circuitry in the dt rat. Harmaline is believed to produce a tremor of 8-12 Hz by its actions on the inferior olive and the Purkinje cells via the climbing fibers. These effects can first be detected in the rat on postnatal days 9-10 (Knowles & Phillips, 1980). Harmaline was administered (10-15 mg/kg, ip) to unrestrained dystonic rats and normal littermates at 17-19 days of age. The rats were observed for 30-60 min following injection and movements were recorded on a polygraph using a force transducer attached to a hind leg. Normal rats showed a visible tremor of the appropriate frequency within 5 min of injection. No evidence of tremor could be seen in the dt rats at either dose. Both normal and dystonic rats displayed tremors in response to oxotremorine (.5 or .75 mg/kg, ip). Thus, dt rats can display tremor but may have a defect specific to the pathway that mediates harmaline tremor.

Harmaline increases the cerebellar content of the nucleotide 3',5'-cyclic guanosine monophosphate (cGMP), a marker for Purkinje cells. Measurement of cerebellar cGMP content in 16 day old dystonic and normal littermates killed by microwave irradiation was used to assess the functional state of the Purkinje cells. There were no differences between the two groups in body weight or cerebellar weight or protein content; but cGMP levels were reduced by 60% in the dt rats in comparison with normal littermates. These findings suggest a failure in afferent input to the cerebellum or a defect in the Purkinje cells themselves. In either case neural transmission through the cerebellum may be substantially disturbed in the dystonic rat. (Supported by NS18062).

BRAIN METABOLISM I

162.1 ANALYSIS OF FUNCTIONAL SYSTEMS IN THE HUMAN BRAIN BY THE INTERCORRELATION MATRIX FOR REGIONAL RATES OF GLUCOSE METABOLISM. R. Duara, B. Horwitz, J.V. Haxby*, C.L. Grady, N. Cutler, S. Rapoport. Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health, Bethesda, MD 20205

A statistical treatment was made of regional cerebral metabolic rates for glucose ($rCMR_{glc}$), determined by positron emission tomography (PET) using [^{18}F] fluoro-deoxyglucose, in 40 healthy subjects in the resting state. Their ages ranged from 21 to 83 yr. A matrix of significant ($p<0.01$) partial correlation coefficients (r*s) was created, where whole brain glucose metabolic rate was partialled out. r*s between all pairs of regions, out of a total of 59 brain regions, were displayed (Duara et al., Soc Neurosci Abstr. 9: 1171, 1983). We proposed that these regional intercorrelations are indicative of regional interactivity.

We divided the brain into 7 bilateral systems, on a functional-anatomical basis. These systems are the ant. frontal, somato-motor-sensory, posterior parietal, visual, auditory, language and memory systems. The individual r*s from each region in a system were treated as data points and were used to obtain statistical comparisons between bilateral systems and between right and left sides within each system. A two-way analysis of variance was performed, where the factors were system and hemisphere (right or left), with repeated measures on the hemisphere factor.

The posterior parietal, ant. frontal and somato-motor-sensory systems had higher average r*s ($p<0.01$) than auditory, visual and memory systems. Significant ($p<0.01$) asymmetry in the intercorrelations of the systems was found in the posterior parietal and auditory systems, with the right being more active than the left in both cases. These results could not be demonstrated with raw $rCMR_{glc}$ values in the same subjects (Duara et al., Neurology 33 (Suppl 2): 116, 1983). We suggest that greater somato-sensory input than auditory and visual input, in the resting state, results in the greater mean r*s for the somato-motor-sensory systems, than in other systems analyzed, and that the anterior frontal and posterior parietal systems are non-specifically active association areas. Asymmetries in the posterior parietal and auditory systems were possibly related to dominant right-sided mechanisms of attention and processing of non-verbal sound, respectively, during the PET procedure.

162.2 PET STUDY OF OCCIPITAL AND SOMATOSENSORY LOBE GLUCOSE METABOLISM IN NORMAL SUBJECTS RECEIVING PHOTIC STIMULI VERSUS SOMATOSENSORY STIMULI J.C.Wu,M.S.Buchsbaum*,H.H.Holcomb ,L.E.Delisi*,E.Hazlett*
University of California, Irvine Psychiatry Department, Irvine, CA, USA 92717 & Clinical Psychobiology Branch, NIMH

The functional relationship of glucose metabolism to anatomy was examined in the response of the normal brain to photic stimuli compared to somatosensory stimuli. 34 normal subjects were studied. Each subject received 3-5 mCi of FDG immediately prior to sensory stimuli presentation. 17 normal subjects received unpleasant electrical stimulation to the right forearm. Four different intensities were given in random order 1/sec for 34 minutes. The other 17 subjects received light flashes as a stimulus. Four intensities of light flashes were presented in a randomized sequence. After being presented with the shocks or the light flashes, subjects were transferred to a ORTEC Ecat II scanner. Six to seven slices parallel to the canthomeatal line were made. Raw counts were converted to glucose (micromoles/100 gms/min) using the Sokoloff 3 constant model. An automated template program was used to analyze data. The template locations were selected using coordinates derived from the atlas of Matsui and Hirano. For the occipital lobe analysis, three slice heights were studied (48.6%, 38.9%, 29.3%) with a template that was 15 mm square (x:50%, y:85.2%). Occipital lobe metabolism for light subjects=24.9 was greater than shock subjects=19.6. Four way anova showed a significant slice x position x group effect(p less than .0015, $F=4.75$, d.f. = 3.88, 124.24). For the somatosensory lobe analysis, two slice heights were studied (78.5%, 68.2%) with a template that was 18 mm long by 9 mm wide (left,x:31%,y:26%; right,x:69%,y:26%). The left somatosensory cortex was significantly higher than for right only in shock subjects (shock:left=28.3 greater than right=25.9, light:left=25.4 vs. right=25.3). Anova showed significant hemisphere x group interaction (p less than .0071, $F=9.10$, d.f.= 1, 19). This not unexpected result by PET study confirms a strong coupling of function with the anatomical substrate in normal subjects. It is interesting to note that schizophrenics have high occipital lobe glucose metabolism without photic stimulation (unpublished data).

162.3 REGIONAL CEREBRAL GLUCOSE METABOLISM IN PATIENTS WITH ALCOHOLIC KORSAKOFF'S SYNDROME. R. M. Kessler*, E.S. Parker, C. M. Clark*, P. R. Martin*, D. T. George*, H. Weingartner*, L. Sokoloff, M. H. Ebert, and M. Mishkin. NIH, NIAAA, NIMH, Bethesda, MD 20205.

Six alcoholic male subjects diagnosed as having Korsakoff's syndrome and eight age-matched male normal volunteers were studied with ^{18}F 2-fluoro-2-deoxy-D-glucose ($2^{18}FDG$). All subjects were examined at rest with eyes covered in a quiet, darkened room. Serial plasma samples were obtained following injection of 4 to 5 mCi of $2^{18}FDG$. Consecutive tomographic slices spaced at 10mm axial increments were obtained (in-plane resolution = 1.75cm, axial resolution = 1.78cm). From these, four planes were selected from each subject, and a total of 46 regions of interest were then outlined on the four planes with rectangles of fixed size from standard templates. Glucose metabolic rates for each region were calculated with the Brooks form of the Sokoloff equation.

The mean glucose metabolic rate for the 46 regions in the Korsakoff subjects was significantly lower than that in the normal controls (5.24 $\pm$ .43 versus 6.61 $\pm$ 1.31, $p < 0.02$). A Q-component analysis, which examined each subject's regional rates relative to his mean rate, revealed two distinct patterns in the Korsakoff group. Two subjects had relatively low anterior frontal metabolism in the plane 90mm above the OM line, relatively low dorsomedial parietal metabolism, and relatively high basal ganglia and thalamic metabolism. The pattern in three other Korsakoff subjects was nearly opposite, in that the anterior frontal cortex on the 90mm plane was relatively high metabolically, whereas the basal ganglia and thalamus were relatively low. This second subgroup, however, like the first, had relatively low dorsomedial parietal metabolism. Examination of the absolute rates of regional metabolism for the Korsakoff group as a whole demonstrated significantly decreased rates in numerous cortical areas as well as in the thalamus and basal ganglia.

The finding of consistently reduced cerebral glucose metabolism in a nondemented group of subjects has not previously been reported. The identification of differing patterns of cerebral metabolism in Korsakoff's syndrome suggests the presence of subgroups with differing neuropathology. The regions with reduced metabolism for the group as a whole included, but were not restricted to, many that have been implicated in memory functions, such as medial temporal, (medial) thalamic, and medial prefrontal.

162.4 DIFFERENCES IN ASYMMETRY OF MEMORY AND BRAIN GLUCOSE METABOLISM IN SCHIZOPHRENIC, DEPRESSED, AND ALZHEIMER'S PATIENTS. W.H. Riege, E.J. Metter*, D.E. Kuhl, M.E. Phelps, and A. Kling*. V.A. Medical Center Sepulveda and UCLA School of Medicine, Los Angeles, CA.

Local cerebral metabolic glucose utilizations (LCMRGlc) were determined in moderately disabled persons with chronic schizophrenia (SCH, N=6, age 37.8$\pm$6.9 years), depression (D, N=8, 62.2$\pm$3.5 years), Alzheimer's disease (AD, N=6, 66.2$\pm$4.7 years), and in young (N=6, 30.3$\pm$4.4 years) and old (N=6, 66.6$\pm$6.9 years) controls (C), using positron emission tomography with the (18F) fluorodeoxyglucose scan method (resolution 1.2 cm). LCMRGlc were measured in 13 left and 13 right hemispheric regions and were expressed as regional l/r ratios. Persons were scanned in the resting state with eyes and ears unoccluded. Within the week of their brain scan, they were also tested on 18 tasks of verbal and modality-specific nonverbal memory.

As indices to possible left hemispheric dysfunction, the verbal memory performances of SCH were severely impaired ($F(3,7)=12.73$, $p<0.003$) but not nonverbal memory scores. Recall of story, sentences, pictures, or word lists were more difficult ($p<0.01$) for SCH than for age-matched C, and were in part correlated with left parietal LCMRGlc ($r>0.78$). However, none of the l/r metabolic ratios of SCH showed relative hemispheric asymmetry or differences against C. In D, on the other hand, the l/r ratio of posterior frontal (Broca's) regions was significantly lower than that of C ($t=2.45$, $p<0.03$). The cortical CT measure of Sylvian fissure in D was also larger on the left than on the right. Recall of story ($F=5.90$, $p<0.04$) and recognition of word lists ($F=6.26$, $p<0.02$) were impaired in D and correlated ($r>0.74$) with the posterior temporal metabolic measure.

The memory scores of AD patients were >1 SD below age-matched C in all tasks and showed relatively larger deficits in nonverbal visual, auditory, and tactual recognition. Most LCMRGlc were depressed bilaterally, although the l/r ratio of parietal cortex ($F=7.84$, $p<0.02$) was distinctly higher in AD than in C. This right parietal hypometabolism was not correlated with nonverbal memory scores. A discriminant analysis based on left and on right parietal/caudate-thalamic ratios classified 83.3% of AD, 67% of D, and 50% of SCH patients, implicating these ratios to be sensitive indices of brain metabolic differences among these small patient samples. However, the tendency for asymmetric memory dysfunctions was only tentatively reflected in regional metabolic measures.

162.5 EFFECTS OF ELECTRICAL STIMULATION OF SCIATIC NERVE ON GLUCOSE UTILIZATION IN THE SPINAL CORD AND IN THE DORSAL ROOT GANGLIA IN THE RAT. M. Kadekaro, A. Crane* and L. Sokoloff. Laboratory of Cerebral Metabolism, National Institute of Mental Health, Bethesda, MD 20205

Functional stimulation of the hypothalamo-neurohypophysial system by salt-loading of rats has been shown in qualitative (Schwartz et al. Science 205:723-725, 1979) and quantitative autoradiographic 2-deoxyglucose studies (Gross et al. Neurosci. Abstracts 8:55, 1982) to increase the rates of glucose utilization in the pituitary neural lobe but not in the paraventricular or supraoptic nuclei, where the cell bodies of that system are located. Schwartz et al. proposed that because the surface-to-volume ratios of the nerve terminals in the neural lobe are greater than those of the cell bodies in the supraoptic and paraventricular nuclei, the energy required for the same impulse activity should be greater in the nerve terminals than in the cell bodies.

In order to determine whether the cell bodies could increase their rates of glucose utilization in response to electrically-stimulated afferent input, the dorsal root ganglia of rats were chosen as a simpler model for this study. The autoradiographic 2-deoxyglucose method was used. Male Sprague-Dawley rats (300-410 g) were anesthetized with sodium pentobarbital (45 mg/kg i.p.), and the sciatic nerves on both sides were exposed, tied, and transected at the level of the gluteus muscles. The distal portion of one transected sciatic nerve was placed on bipolar platinum electrodes and stimulated via a stimulus isolation unit with pulses of 2 ms duration at a current intensity of 200-400 μA and at a frequency of 5, 10, or 15 Hz. Electrical stimulation of the sciatic nerve produced a frequency-dependent activation of glucose utilization in the dorsal horn of the spinal cord but produced no changes in glucose utilization in the dorsal root ganglion cells.

These results indicate that the cell bodies in the dorsal root ganglia do not increase their rates of glucose utilization to a sufficient degree in response to increased afferent input to be detected by the 2-deoxyglucose method.

162.6 ACUTE HYDERGINE TREATMENT SELECTIVELY INCREASES LOCAL CEREBRAL GLUCOSE UTILIZATION IN RATS. R. C. Walovitch and E. D. London, NIDA Addiction Res. Ctr., Baltimore, MD 21224.

Hydergine (H) is the trade name for co-dergocrine mesylate, which consists of four ergopeptides (dihydroergocornine, dihydroergocristine, dihydro-α-ergocriptine and dihydro-β-ergocriptine) in a ratio of 3:3:2:1. Chronic treatment of rats with H reverses the age-associated decline in forebrain hexokinase as well as the increase in lactate dehydrogenase, suggesting an enhancement of cerebral oxidative metabolism (Djuricic, B. M. and Mrsulja, B. B., Gerontology 26:99, 1980). We used the 2-deoxy-D-[1-^{14}C]-glucose (2DG) technique to determine if acute H treatment would affect local cerebral glucose utilization (LCGU), an index of oxidative metabolism and local cerebral function.

Either H (3.0 or 10.0 mg/kg, i.p.) or vehicle (propylene glycol) was injected 35 min before 2DG to conscious 12-15 mo-old male Fischer-344 rats. LCGU was measured autoradiographically in 43 brain regions, particularly those associated with motor and cognitive functions and regulation of temperature and blood pressure.

Both doses of H produced hypotension, but did not alter LCGU in regions involved with blood pressure control (raphe, paraventricular, reticular, and solitary nuclei), except the locus ceruleus. In this region, 3 and 10 mg/kg of H increased LCGU by 25% and 39%, respectively. The highest dose of H also significantly increased LCGU in areas associated with extrapyramidal function (ventrolateral thalamus, substantia nigra), tempertature control (medial preoptic and posterior hypothalamic nuclei), and motivation or cognitive function (dorsal hippocampus, subicular areas, anterior thalamus, and medial mammillary nucleus).

Some of these stimulatory effects on LCGU could reflect interactions with specific neurotransmitter systems. Effects in the locus ceruleus may reflect increases in the firing of noradrenergic neurons due to the blockade of presynaptic α_2-adrenergic receptors. In contrast, effects in the extrapyramidal motor system may be due to H's dopaminergic action. LCGU effects in the medial preoptic and posterior hypothalamic areas may relate to the hypothermic action of H. Stimulation of LCGU in the hippocampus and components of the Papez circuit are in agreement with reports that H improves performance in learning and memory testing paradigms (Loew, D. M. et al., in Aging Brain and Ergot Alkaloids, A. Agnoli et al., eds., Raven, New York, 1983). (Support in part by Sandoz Corp. fellowship to R.C.W).

162.7 DYNAMIC MEASUREMENTS OF LOCAL CORTICAL BLOOD FLOW DURING SPREADING DEPRESSION IN THE RAT. F. W. Marcoux, C. P. Taylor and J. J. Cordon*. Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

Spreading cortical depression (SD) depolarizes neocortical neurons in a propagating wave lasting 1-3 min at any particular locus in its path. During SD a transient negative shift in extracellular voltage and substantial increases in cortical glucose utilization and blood flow occur, reflecting a massive accumulation of K+ in the extracellular space and a marked increase in cerebral metabolism. However, dynamic measurements of local cortical blood flow (LCBF) related to extracellular DC potential during SD have not been reported previously. In the present study hydrogen clearance was used to monitor LCBF from a polarized Pt/Ir electrode (125 μm diameter, 1.0 mm exposed tip). A micropipette (1-10 MΩ, filled with saturated NaCl) recorded extracellular DC potential. The electrodes were placed together in the anterior neocortex of anesthetized rats. SD was initiated by brief application of a KCl crystal to the ipsilateral occipital cortex. Hydrogen clearance and DC potential before, during and after SD were stored on FM tape and later digitized by a laboratory microcomputer. LCBF was estimated sequentially from the instantaneous slope of the exponential hydrogen clearance curve. LCBF decreased dramatically, coincident with a negative shift of the DC potential which is indicative of the SD wave front. Within 30 sec, before the DC potential had returned to control, LCBF rebounded hyperemically. The increase in LCBF prior to the DC potential normalization suggests a local uncoupling of blood flow and neuronal activity. The initial decrease in LCBF may reflect neuronal inactivation. The delayed hyperemia may reflect metabolic stimulation unrelated to neuronal firing.

162.8 MODULATING CEREBRAL OXYGEN DELIVERY IN COMA FOLLOWING ACUTE DIFFUSE BRAIN INJURY. J.Cruz*, M.E.Miner, S.J.Allen*. Div.of Neurosurgery and Dept.of Anesthesiology, The University of Texas Medical School at Houston,TX 77030.

The arterio-jugular difference in oxygen contents($AVDO_2$) expresses the ratio between global cerebral O_2 metabolic rate ($CMRO_2$) and blood flow(CBF),or $AVDO_2$=$CMRO_2$/CBF(Kety S.S., J. Clin. Invest.,29:402,1950).Serial CBF studies in acute head injury have recently reinforced the concept of $AVDO_2$ as a measure of the above relationship(Obrist,W.D., J. CBF Metab., 3,Suppl.1:S67,1983).Continuous $AVDO_2$ monitoring was therefore attempted,as a means of modulating cerebral O_2 delivery.

Ten adult comatose acute head-injured patients underwent continuous monitoring of systemic arterial pressure(SAP),intracranial pressure(ICP),expired carbon dioxide($PECO_2$),arterial and jugular oxyhemoglobin saturation(SaO_2 and SjO_2).All patients had diffuse findings in the computed tomography (CT) scan of the brain.SaO_2 and SjO_2 were monitored from fiberoptic oximetry catheters placed in a femoral artery and a jugular bulb.Cerebral perfusion pressure was estimated as CPP = mean SAP - ICP,and $AVDO_2$ as $AVDSO_2$=SaO_2 - SjO_2.Modulating O_2 delivery was accomplished by:a)modifying cerebral perfusion by manipulating $PECO_2$ and/or CPP;b)modifying arterial O_2 content by manipulating the fraction of inspired O_2(FiO_2)(fig.).

Within the first 48 hours,all patients required interventions: 9 showed sudden SaO_2 drops below 90%,unexplained by cardio-pulmonary screening in 7.In the presence of normal arterial O_2 content,6 cases had SjO_2 below 48%,consistent with cerebral oligemia,and 4 had SjO_2 above 72%,consistent with cerebral hyperemia.

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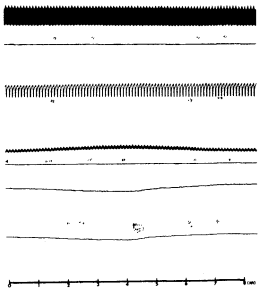
From top to bottom,SAP,$PECO_2$, ICP,SjO_2,SaO_2 recorded over 8 minutes.Scales:0-200 for SAP,and 0-100 for the others.

In this case with high SjO_2,a sudden drop in SaO_2 and simultaneous rise in $PECO_2$ associated with ICP elevation.An increase in FiO_2 from 50 to 100%,at constant $PECO_2$,was followed by ICP decline.SjO_2 changes linearly followed those of SaO_2.



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162.9 REGIONAL BLOOD-BRAIN BARRIER TRANSPORT OF GLUCOSE IN RATS WITH PORTACAVAL ANASTOMOSIS. A.M. Mans*, R.A. Hawkins and D.W. Davis*. Departments of Anesthesia and Physiology, Hershey Medical Center, The Pennsylvania State University, Hershey, PA 17033.

Rats with a portacaval anastomosis show various metabolic abnormalities, including decreased glucose utilization (by 25%) throughout the brain (1). Glucose concentrations are decreased by about 18% and 48% in the blood and brain respectively (2). Kinetically, this decrease in [brain glucose] is unexpected, and led us to wonder whether the glucose transport system might be altered in this condition. Changes have been found in other transport systems after portacaval shunting; transport by the neutral amino acid system is increased and that by the basic amino acid system is decreased (3,4). No alteration has been found in the transport of glucose using the brain uptake index technique (3). Using a more physiological approach, we measured glucose transport into brain at a regional level, in rats 5-6 weeks after establishing a portacaval anastomosis. Sham-operated rats were used as controls. [^{14}C]Glucose was infused intravenously for 15 seconds to maintain an approximate steady level in circulation; arterial blood was sampled continuously during this time to determine the integral of plasma radioactivity. The rat was decapitated and radioactivity measured in the brain by autoradiography. Plasma glucose concentrations were determined enzymatically. The clearance (PS) of glucose was calculated as: PS = (Net d.p.m. per g in brain)/(plasma integral), and the data normalized for each rat by adjusting the PS to an arbitrary plasma glucose concentration. The results showed a slight decrease of about 10% in the normalized PS in the shunted rats as compared to the control group. Overall, this difference was not statistically significant ($0.15 > p > 0.10$). Whereas glucose influx in rats with portacaval shunts was significantly reduced, this was primarily due to the low [plasma glucose]. (Supported in part by NS 16389).

1. Mans, A.M., Biebuyck, J.F., Davis, D.W., Hawkins, R.A. (1983) J. Neurochem 40, 986.
2. Mans, A.M., Biebuyck, J.F., Davis, D.W., Hawkins, R.A. (1984) J. Neurochem., in press.
3. James, J.H., Escourrou, J., Fisher, J.E. (1978) Science 200, 1395.
4. Mans, A.M., Biebuyck, J.F., Shelly, K., Hawkins, R.A. (1982) J. Neurochem. 38, 705.

162.10 MEASUREMENT OF BRAIN AND TUMOR pH IN THE RAT USING ^{14}C-DIMETHYLOXAZOLIDINEDIONE AND QUANTITATIVE AUTORADIOGRAPHY. J.B. Arnold,* L. Junck,* M.D., and D.A. Rottenberg, M.D., Department of Neurology, Memorial Sloan-Kettering Cancer Center and Cornell University Medical College, New York, NY 10021.

RG-2 glioma cells were implanted intracerebrally into young adult male CDF rats. Two weeks later, when the tumors were at least 1 mm in diameter, the animals were injected with 50 μCi ^{14}C-dimethyloxazolidinedione (^{14}C-DMO). Following a two-hour equilibration period, during which time serial arterial blood samples were analyzed for pH, pCO_2, pO_2 and ^{14}C-DMO concentration, the rats were decapitated and the brains rapidly removed and frozen. Representative 20μ cryostat sections were mounted on X-ray film and regional ^{14}C-DMO concentration measured using a computerized digitizer/image processing system. Measured arterial pH and tissue and arterial plasma ^{14}C-DMO concentrations were used to calculate an aggregate tissue pH, pH_t, for tumor tissue and normal brain regions using the Waddell-Butler equation (J. Clin. Invest. 38: 720-729, 1959). Normal brain tissue pH values were in general agreement with values in the literature obtained by other techniques, and tumor values were consistently 0.1-0.2 pH unit higher. In order to calculate tumor intracellular pH (pH_i), it is necessary to correct the tissue DMO concentration for the amount of DMO in plasma and extracellular fluid (ECF). Thus, we performed a series of quantitative autoradiographic experiments in bilaterally nephrectomized animals using ^{14}C-labeled sucrose as an extracellular space tracer. Based on values for the two-hour sucrose space, fractional tumor extracellular water volume (V_e) ranged from 0.166 to 0.265. When these values of V_e were used to calculate tumor intracellular pH, pH_i values ranged from 6.76-7.09. Our results suggest that the observed "alkalinity" of tumor tissue may reflect an increase in tumor pH_i as well as an increase in tumor extracellular space.

162.11 DIFFERENCES IN SUSCEPTIBILITY TO CEREBRAL ISCHEMIA BETWEEN THE YOUNG AND ADULT GERBILS. H. Martinez*, R. Cahn*, B.B. Mrsulja*, H. Masaoka* and I. Klatzo. National Institutes of Health, Bethesda, MD 20205.

In comparative study on effects of cerebral ischemia, the young (3 week old) and adult (12-14 week old) gerbils were subjected to 5 or 15 minute bilateral clamping of common carotid arteries. The density and distribution of ischemia was radioautographically evaluated with ^{14}C-iodoantipyrine. Morphological changes were assessed with light microscopy using cresyl violet and H & E stains. ATP, phosphocreatine, glucose, glycogen and lactid and changes were determined at the end of ischemia and during various post-ischemic periods. The ^{14}C-iodoantipyrine radioautography showed similar in density, severe ischemia in both hemispheres, with exception of central structures supplied by the vertebral system. In 5 minute ischemia, the young gerbils failed to show any morphological changes including the selective destruction of the CA1 sector of the hippocampus, which was regularly observed in the adult animals. In 15 minute ischemia, the young gerbils showed markedly lesser degree of injury in the hippocampus, cerebral cortex and striatum and they had a higher survival rate (70% v. 30%). The biochemical assays revealed a significantly lesser degree of metabolic disturbances in the young animals. Our observations indicate that different thresholds to ischemic injury between young and adult animals are based on different metabolic features in brain structures exposed to similar degree of reduction in energy supply.

162.12 DIFFERENTIAL EFFECTS OF A CNS REGULATORY CENTER ON INSULIN HYPOGLYCEMIA AND FLUOROACETATE CONVULSIONS M.A. Marrazzi, J. Wright*, J.A. Brown*, B. Frank*, T. Gluski* and P. Duquette* Dept. Pharmacol, Wayne State U. Schl. Med., Detroit, MI 48201

Our laboratory has suggested that a regulatory center in brain adjusts the convulsive response to insulin hypoglycemia (J. Pharmacol. Exper. Therap. 219:258, 1981). This hypothesis is based on the effects of gold thioglucose (GTG) on the sensitivity to insulin hypoglycemic convulsions. Following a single IP injection of GTG (0.8mg/g) into female CBA/J mice, there is a biphasic change in the sensitivity, suggesting a two component system. The percent convulsions in a population is decreased at early times (16-24 hours) and increased at later times (1-2 weeks). For both effects, the difference is in the convulsive response to equal hypoglycemic challenge, rather than in the hypoglycemic response to insulin. The sensitivity to the non-metabolic convulsions induced by Metrazol was not affected at either time, so that neither effect was a non-specific one on generalized convulsive threshold. GTG produces histological damage focused in the ventromedial hypothalamus (VMH), which was clearly evident by the time of the earliest effect on insulin hypoglycemic convulsions. Like with other VMH lesions, hyperphagia and obesity eventually result. Both effects on hypoglycemic convulsions are however prior to any significant difference in body weight. The association with the cytotoxic GTG lesion suggests that a relatively discrete brain region, a "GTG lesioned glucostat" mediates the convulsive response to insulin hypoglycemia. Such a regulatory center function is in accord with the lack of generalized power failure, which has been previously demonstrated by the lack of depletion of biochemical energy reserves (ATP and glycogen) despite convulsion and coma. Fluoroacetate induced convulsions are also metabolic convulsions. Fluoroacetate blocks the Krebs cycle at the citrate to isocitrate step,while insulin hypoglycemia inhibits all glycolysis and the Krebs cycle by decreasing glucose availability. Fluoroacetate induced convulsions are not altered by the GTG lesion at either time point. Thus, the 2 metabolic convulsions-insulin hypoglycemia and fluoroacetate-are qualitatively different. Furthermore, the "GTG lesioned glucostat" apparently does not need the Krebs cycle as blocked by fluoroacetate to operate. In addition, 5-thioglucose (5TG) simulates the early action of GTG in decreasing the sensitivity to insulin hypoglycemic convulsions but without causing the cytotoxic lesion (submitted). 5TG did not affect fluoroacetate convulsions. (NIH#RR08167)

162.13 POSITRON EMISSION TOMOGRAPHY AND COMPUTED TOMOGRAPHY IN SCHIZOPHRENICS AND CONTROLS. T.L. Jernigan, T. Sargent,III*, S.M. Stahl, N. Kusubov*, and A. Pfefferbaum*. Donner Laboratory, University of California, Berkeley, CA 94720 and Schizophrenia Biologic Research Center, Palo Alto V.A. Medical Center, Palo Alto, CA 94304.

PET and CT were obtained in 6 chronic schizophrenic patients and 6 controls. The PET facility was the Donner Laboratory 280-crystal tomograph. Both automated and manual measurements were made of local cortical ratios of 18-FDG uptake and cortical CT values. Only one of eight cortical ratios on PET was significantly different in the schizophrenics: however, interrelationships were observed between age, atrophy and regional 18-FDG uptake. The ratio of frontal to posterior 18-FDG uptake was smaller in older subjects. The presence of cerebral atrophy predicted this hypofrontal appearance in the ratios. Attempts have been made to separate the effects of diagnosis, age and atrophy on the regional pattern of 18-FDG uptake. The results of these multivariate analyses will be summarized.

This work supported by the Department of Energy and the Medical Research Service of the Palo Alto V.A. Medical Center.

Dr. Sargent and Ms. Kusubov are of the Donner Laboratory; Drs. Jernigan, Stahl and Pfefferbaum of the V.A. Drs. Stahl and Pfefferbaum are also of the Department of Psychiatry, Stanford University School of Medicine. Dr. Jernigan is now at the San Diego V.A. Medical Center and the Department of Psychiatry, University of California, San Diego, School of Medicine.

163.1 BINDING OF APAMIN TO POSTSYNAPTIC DENSITY AND SYNAPTIC MEMBRANE FRACTIONS ISOLATED FROM CANINE CEREBRAL CORTEX AND CEREBELLUM. K. Wu*, R.K. Carlin* and P. Siekevitz, Lab. Cell Biology, The Rockefeller University, New York, NY 10021.

Apamin is a 2032-dalton neurotoxin from bee venom which is a specific blocker of one class of Ca^{2+}-dependent K^+ channels (Lazdunski, Cell Calcium, 4, 421 (1983)). Apamin, kindly obtained from M. Lazdunski, was radioiodinated by the method of M. Hugues et.al., (JBC, 257, 2762 (1982)), and was used to localize the apamin receptor in subcellular fractions. Trace amounts of apamin (~0.1μg/ml) can be quantified fluorometrically using fluorescamine. The binding of [^{125}I] apamin was performed with synaptic membranes (SM) and postsynaptic density (PSD) fractions isolated from canine cerebral cortex and cerebellum. After incubation (in 20mM Tris-HCl, 5mM KCl, 0.1% BSA, pH7.5) at 0 - 4°C for 75 minutes, reaction mixtures were spun 48,000 g for 15 minutes at 4°C. The supernatants were removed, and the pellets washed with 20mM Tris-HCl buffer containing 0.1% BSA, pH7.5, and then counted in a gamma counter. Nonspecific binding was performed in the presence of large excess of unlabelled apamin (1uM). The K_D's of cerebral cortex SM and PSD fractions were 33 pM and 24 pM, respectively. The B_{max} of cerebral cortex PSD fractions (30.2 fmol/mg) were about two-fold that of cerebral cortex SM fractions (17.3 fmol/mg), indicating a concentration of receptors in the isolated cerebral cortex PSD fractions over cerebral cortex SM fractions. The value of 17.3 fmol/mg is very similar to that obtained (22 fmol/mg) for a rat SM fraction (Hugues, et. al., BBRC, 107, 1577 (1982)). Both preparations showed only single binding sites. The lattice core of the PSD, obtained by treatment with 0.5% deoxycholate or 1% N-lauroyl-Sarcosinate, still contained one half the binding sites. Covalent labelling of apamin to its receptors in SM and PSD fractions indicated that the protein has a M_r of 27K, similar to that of rat SM (Hugues, et.al., BBRC, 107, 1577 (1982)). At saturating levels of apamin (5nM), cerebellar membrane fractions had 2-2.5x the specific binding of cerebral cortex membranes, but PSD fractions isolated from cerebellar membranes showed 1/2 to 1/3 the specific binding of cerebral cortex PSDs, and also 1/2 to 1/3 the binding to cerebellar membranes, indicating a looser association of receptor to cerebellar PSD proteins. The present findings suggest that the isolated PSD contains the apamin-sensitive Ca^{2+}-dependent K^+ channel, and suggest that the PSD is an anchoring structure for some, if not all, ion channel proteins, as well as for neurotransmitter receptors, as has been shown already for the GABA and glutamate receptors. In this role, the PSD may have a modulatory influence on neurotransmission.

163.2 **PURIFICATION AND CHARACTERIZATION OF A DISTINCT CEREBELLAR FORM OF BRAIN "TYPE II" Ca^{2+}-CALMODULIN-DEPENDENT PROTEIN KINASE.** S. G. Miller* and M. B. Kennedy (SPON: D. Van Essen). Division of Biology, 216-76, Caltech, Pasadena, CA 91125.

Our laboratory has recently purified an abundant calmodulin-dependent protein kinase from rat brain (Bennett et al., 1983, J. B. C., 258, 12735). A similar brain kinase has been referred to by other groups as "Kinase II" (Yamauchi and Fujisawa, 1983, Eur. J. Biochem., 132, 15; Lai et al., 1983, Neurosci. Abs., 9, 1029). Because this kinase now appears to occur as a family of distinct but homologous forms, we will refer to the forms collectively as "Type II" Ca^{2+}-calmodulin-dependent protein kinases.

The Type II kinase originally purified from rat brain is a 650 Kdal holoenzyme composed of structurally related subunits (~9 α-subunits (50 Kdal) and ~3 β/β'-subunits (60/58 Kdal)). This form predominates in the forebrain (cortex and hippocampus) comprising approx. 75% of the total brain enzyme. We have purified a distinct form from rat cerebellum composed of similar subunits assembled in a different ratio. The MW of the cerebellar kinase is 560 Kdal, as calculated from its hydrodynamic properties. Gel electrophoresis shows that it is composed of ~8 β/β'-subunits (6, 60K; 1, 58K; 1, 56K) and ~2 α-subunits (50K). This form predominates in cerebellum and comprises approx. 10% of the total brain Type II kinase.

Monoclonal and polyclonal antibodies raised against the subunits of the forebrain kinase recognize the subunits of the cerebellar kinase on Western blots. Iodinated peptide maps of the subunits reveal a close homology between the β-subunits of the two forms, but small differences between their α-subunits.

Two differences in the properties of the forebrain and cerebellar kinases may result in functional differences between them in vivo. 85% of the cerebellar kinase is recovered in the particulate fraction of a homogenate and must be solubilized with a chaotropic salt before purification. Only ~50% of the forebrain form is particulate under the same conditions. Thus the two forms may associate differently with subcellular structures in vivo. The apparent affinity for Ca^{2+}-calmodulin differs between the forms by a factor of two. Thus the cerebellar form may be more sensitive to increases in calcium concentration than the forebrain kinase.

163.3 **SYNAPTOSOMAL POSTSYNAPTIC DENSITIES ARE LABELED BY A MONOCLONAL ANTIBODY AGAINST A SUBUNIT OF A BRAIN TYPE II Ca^{2+}-CALMODULIN-DEPENDENT PROTEIN KINASE.** M. B. Kennedy and V. L. Radice*. Division of Biology, 216-76, Caltech, Pasadena, CA 91125.

We have presented evidence that the predominant protein in postsynaptic density (PSD) fractions (Kelly and Cotman, '78, J. Cell Biol. 79, 173) is the 50K α-subunit of a brain Type II Ca^{2+}-calmodulin-dependent protein kinase (Kennedy et al., '83, P.N.A.S. 80, 7357). After centrifugation of brain homogenates, half of this kinase activity is recovered in the soluble fraction and half in the particulate fraction (Kennedy et al., '83, J. Neurosci. 3, 818). The soluble form of the kinase is a large holoenzyme composed of ~9 α-subunits (50K) and ~3 β/β'-subunits (60/58K) (Bennett et al., '83, J.B.C. 258, 12735). Both the α and β-subunits are present in isolated PSD fractions in approx. a 3 to 1 ratio. Thus, a portion of the kinase holoenzyme may be specifically immobilized in PSDs in vivo.

The relationship between the composition of the purified PSD fraction and the composition of the PSD in vivo has been a matter of dispute. The possibility of artifactual association of proteins with the PSD fraction during homogenization or treatment with detergent has not been completely ruled out. We have therefore examined the association of the kinase with PSDs by immunocytochemical techniques. We began by attempting to label PSDs in synaptosomal fractions of homogenized tissue. In this preparation, optimal labeling conditions can be worked out while avoiding the problem of inaccessibility of Abs to structures in fixed tissue. Synaptosomes were prepared by the method of Cotman et al. (J. Cell Biol. '74, 63, 441), fixed, and embedded in agarose by the method of DeCamilli et al. (J. Cell Biol. '83, 96, 1355). They were labeled by the sandwich technique with a ferritin-conjugated second Ab, after incubation with either 40 μg/ml non-specific mouse IgG (control) or 6g9 anti-α-subunit (experimental). When examined by EM, the controls showed sparse labeling of PSDs (78 ferr. grains/μ), while experimentals showed consistent heavy labeling (250 grains/μ). We are presently comparing labeling of PSDs in cortical and hippocampal synaptosomes with that in cerebellar synaptosomes which lack the 50K major PSD protein (Carlin et al., J. Cell Biol. 86, 831). We are also examining labeling of PSDs in intact fixed tissue.

163.4 SUBCELLULAR TRANSLOCATION OF Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE IN Aplysia NEURONS. Saitoh, T. and Schwartz, J.H. Howard Hughes Medical Institute and the Center for Neurobiol. & Behavior, Columbia Univ. Col. Physicians & Surgeons, New York, NY 10032.

Application of serotonin to Aplysia ganglia causes the translocation of a major 55 Kd calmodulin-binding protein from the membrane-cytoskeleton to the cytoplasm, presumably by a cAMP-dependent mechanism (Saitoh & Schwartz, PNAS 80: 6708, 1983). In vertebrate brain, the Ca^{2+}-dependent protein kinase, a prominent constituent of the postsynaptic density, has been shown to contain a major 55 Kd calmodulin-binding subunit. We first examined the identity of the 55 Kd Aplysia component and found it to be a subunit of a Ca^{2+}/calmodulin-dependent kinase.

Differential centrifugation, Sephacryl S-400 gel filtration, DEAE cellulose chromatography, or chromatography on phosphocellulose failed to separate the 55 Kd Aplysia protein from Ca^{2+}/calmodulin-dependent kinase activity, assayed with bovine Synapsin I as substrate. The kinase activity also shifts from the membrane-cytoskeleton to the cytoplasm under the same conditions as the 55 Kd protein.

We also found that the 55 Kd protein and Ca^{2+}/calmodulin-dependent kinase activity also can be released from the membrane-cytoskeleton by Ca^{2+}/calmodulin in addition to the shift caused by serotonin. Thus, the subcellular localization of this enzyme seems to be controlled by both cAMP (Saitoh & Schwartz, 1983) and by Ca^{2+}/calmodulin. The two second messengers operate through different molecular mechanisms, however. We have previously shown that cAMP does not cause the phosphorylation of the 55 Kd protein, and presumably brings about the dissociation by phosphorylating a component of the cytoskeleton, which in turn releases the 55 Kd protein; in contrast, Ca^{2+}/calmodulin causes the autophosphorylation of the 55 Kd subunit of the kinase.

At present, it is uncertain what role these translocations play within the neuron. Preliminary evidence suggests, however, that the enzyme is more active when it is released from the membrane-cytoskeleton. Thus, the subcellular movement of the kinase appears to be an early step in second messenger regulation.

163.5 ISOLATION AND CHARACTERIZATION OF A NEUROFILAMENT-ASSOCIATED CALMODULIN KINASE FROM MICROTUBULE PREPARATIONS. M.L.Vallano*, J.R. Goldenring, T.M.Buckholz*, R.J. DeLorenzo, Dept of Neurology, Yale Univ. School of Medicine, New Haven, CT 06510

The cytoskeleton plays a pivotal role in a variety of dynamic intracellular processes. Reversible phosphorylation of cytoskeletal protein may rapidly modulate interactions between cytoskeletal elements or between cytoskeletal elements and other intracellular organelles. Our laboratory has identified and characterized a calmodulin-dependent protein kinase from brain that phosphorylates microtubule-associated proteins and is present in microtubule preparations (DeLorenzo et al., 1982, Prog. Brain Res. 56, 255). Neurofilaments are also present in microtubule preparations and phosphorylation affects neurofilament/microtubule interactions. The following series of experiments was designed to determine whether calmodulin kinase is specifically associated with neurofilaments in microtubule preparations.

Neurofilaments were prepared directly from rat brain homogenates or from microtubules by differential centrifugation and gel exclusion chromatography. Negative stain electron-microscopy confirmed the presence of numerous 10 nm filaments in these preparations. Both preparations contained endogenous calmodulin kinase activity that phosphorylated microtubule and neurofilament protein in a calmodulin-dependent manner. 84% of the calmodulin kinase activity in microtubules was recovered in the filament fraction and the kinase was enriched by 26-fold. Isolation of neurofilaments directly from brain cytosol also produced a highly-enriched complex of neurofilaments and calmodulin kinase. The association between kinase and neurofilaments was stable in high-ionic strength buffer indicating that the enzyme is not adsorbed to the filaments by nonspecific electrostatic interactions. The neurofilament-associated calmodulin kinase is identical to a previously purified cytosolic enzyme (Goldenring et al., 1983, J. Biol. Chem. 258, 12632) with respect to subunit composition (52,000 and 63,000 Da), isoelectric points, calmodulin-binding subunits, autophosphorylating subunits, apparent Km's for ATP and calmodulin, substrate phosphoamino acid site pattern and phosphopeptide maps of autophosphorylating subunits.

163.6 BRAIN CALMODULIN BINDING PROTEINS: ELECTROPHORETIC AND PEPTIDE MAPPING PROPERTIES. S. D. Flanagan and B. Yost*. Division of Neurosciences, Beckman Research Institute, City of Hope, Duarte, CA 91010.

To streamline detection of brain calmodulin binding proteins, we have adapted blotting techniques for the electrophoretic transfer of proteins onto nitrocellulose filters, followed by overlay with ^{125}I-calmodulin. Autoradiography of the ^{125}I-calmodulin labeled blots allows the identification and quantitation of proteins that possess affinity for calmodulin. We investigated five protocols for suppressing nonspecific binding and for enhancing specific interactions of ^{125}I-calmodulin with electrophoretically separated proteins. Tween 20 and BSA alone, as well as combinations of BSA and poly(ethylene oxide) or hemoglobin and gelatin, were evaluated as quenching and enhancing agents. Tween 20 proved highly effective for quenching nonspecific binding and for enhancing specific ^{125}I-calmodulin binding of a 61,000 M_r rat brain protein, which was only faintly observed on blots quenched with proteins alone. An alternative, the combination of BSA followed by incubation with 15,000 to 20,000 M_r poly(ethylene oxide), proved satisfactory for the recovery of 61,000 M_r calmodulin binding activity and for the detection of calmodulin binding peptides (50,000 to 14,000 M_r) produced by CNBr cleavage or S. Aureus V-8 proteolysis of the rat brain 51,000 M_r calmodulin binding protein.

These blotting procedures for detection of calmodulin binding proteins are compatible with a variety of one-dimensional and two-dimensional electrophoresis systems, including a two-dimensional electrophoresis system utilizing urea and sodium dodecyl sulfate in the first dimension and non-urea sodium dodecyl sulfate electrophoresis in the second. The latter gel system proved useful for resolving two 61,000 M_r calmodulin binding proteins, one of which displays anomalous electrophoretic migration in the presence of urea.

Fourteen peptide fragments were detected after limited CNBr cleavage of the 51,000 M_r rat brain calmodulin binding protein, half of which retain calmodulin binding activity. A substantially larger array of calmodulin binding fragments was generated by S. Aureus V-8 proteolysis. The smallest detected calmodulin binding fragments of M_r = 19,000 and 14,000 were produced by CNBr and V-8 procedures, respectively. Supported by NS18854.

163.7 ACETYLATION-DEACETYLATION OF SYNAPTOSOMAL PEPTIDES AND Na^+ FLUX. S. Berl, A. Colon* and D.D. Clarke*. Dept. of Neurology, Mt. Sinai School of Medicine, New York, NY 10029.

Proteins were rapidly and covalently labeled with the acetyl moiety when synaptosomes (S) were incubated with(^{3}H)-acetate (HA) (Berl et al., J. Neurochem. 40:176, 1983). Labeling was decreased by veratridine/veratrine (V,100 uM), batrachotoxin (1 uM) or scorpion toxin; this effect was counteracted by tetrodotoxin (2 uM). The effect of (V) was probably due to stimulation of deacetylation of protein and perhaps by proteolytic cleavage of peptide(s) from proteins. In the absence of Na^+ the incorporation of (HA) was enhanced but the effect of (V) was abolished. The additon of NaCl (0.154 M) 5 min after incubation with (HA) resulted in deacetylation if (V) was present. Chromatography on Agarose 1.5 m of the labeled PCA precipitated protein dissolved in SDS-urea revealed at least 3 labeled peaks. (V) decreased the incorporation of (HA) into the 2nd peak. Slab gel SDS-PAGE of the column fractions revealed that the 2nd peak had many bands ranging in size from ~ 40-100 K daltons. Chromatography on Agarose 1.5 m of labeled whole (S) dissolved in SDS-urea showed at least 4 labeled peaks. Peak 4 had not been seen when PCA precipitated proteins were chromatographed. Peaks 2 and 4 showed the greatest decrease in radioactivity due to (V). Peak 4 had the highest specific activity (cpm/mg protein); the M.W.s were below 20K daltons. Labeling was markedly reduced in (S) placed in a boiling water bath for 9 min prior to the addition of the (HA). After incubation with (HA) the (S) were lysed by osmotic shock and membrane and cytosolic fractions recovered by centrifugation. The membrane fraction was dissolved in acidified chloroform: methanol = 2:1 (CM). The addition of 0.2 vol of H_2O separated a methanol-water phase containing 95% of the radioactivity. (V) decreased the incorporation of radioactivity by 60%. The cytosolic fraction was lyophilized and also extracted with (CM). Addition of 0.2 vol of H_2O yielded a methanol-water phase containing almost all the radioactivity; this was decreased by 50% by (V). The methanol-water extracts were dried in vacuo at 35°C, taken up in water and chromatographed on a Sephadex G-50 column. One major peak of radioactivity was eluted for each fraction; M.W. was in the order of 5K daltons or less. Samples were subjected to SDS-PAGE on 10-20% slab gels. Tracks were sliced, dissolved in H_2O_2 and counted. The radioactivity migrated as a single band. The Rf suggested a M.W. of 3K daltons. Peptide from membrane and cytosol migrated with a similar Rf. These studies suggest that acetylation of peptide readily occurs in synaptosomes; Na^+ flux may be involved in the deacetylation of some of these peptides. Supported by NIH grant NS 11824 and by the Clinical Center for Research in Parkinson's and Allied Disorders, grant NS 11631.

163.8 CYTOPLASMIC FILAMENTS AT THE PRESYNAPTIC ACTIVE ZONE IN RAPIDLY FROZEN CEREBELLAR CORTEX. D.M.D. Landis and T.S. Reese. Neurology Service, Massachusetts General Hospital, Boston, MA 02114; Laboratory of Neurobiology, NINCDS, NIH, at the Marine Biological Laboratory, Woods Hole, MA 02543.

Synaptic vesicles fuse with the presynaptic membrane in a restricted region of the synapse, the "active zone", during stimulation-evoked neurotransmitter release. Other vesicles presumably move through presynaptic cytoplasm to replace the synaptic vesicles lost during fusion at the active zone. It had been thought that pyramidal "dense projections", which stain heavily with ethanolic phosphotungstic acid in fixed tissue, are essential for these interactions of synaptic vesicles with the presynaptic membrane. However, when rapidly frozen adult mouse cerebellar cortex is thin sectioned after freeze-substitution fixation, a very different view is obtained of the cytoplasm in the axonal boutons of parallel fibers. The volume of axoplasm in which synaptic vesicles are distributed also has a meshwork of densely-stained fine filaments, but no dense projections. These filaments are most closely packed in the axoplasm adjacent to the widened junctional cleft, and become progressively more sparse with distance from the junction. When rapidly frozen tissue is freeze-fractured, shallow-etched, and rotary-shadowed, these filaments can be seen to abut the presynaptic plasma membrane and synaptic vesicle membrane. The filaments terminating against the presynaptic membrane at the active zone have a predominant orientation perpendicular to the membrane. The filaments tend to be straight, often intersecting, and extend from vesicle to vesicle or from vesicle to presynaptic membrane. In rotary platinum replicated preparations, the filaments appear 3-4nm in diameter, and they are often coated with irregular, globular material. Their appearance is different from that of actin, microtubules, neurofilaments, or the 5-7nm filaments in Purkinje cell dendritic spines. Thus, the pyramidal dense projections seen in aldehyde-fixed brain may represent aggregations of fine filaments. We suggest that these fine filaments in presynaptic cytoplasm may function to trap synaptic vesicles in the vicinity of synaptic junctions, and may even be necessary for their translocation to positions in the active zone where they fuse with the presynaptic membrane.

163.9 A LECTIN THAT SELECTIVELY STAINS NEUROMUSCULAR JUNCTIONS BINDS TO COLLAGEN-TAILED ACETYLCHOLINESTERASE. L.J. Scott* and J.R. Sanes (SPON: A. Chiu). Dept. of Physiol., Wash. Univ. Sch. of Med., St. Louis, MO 63110.

Fluorescein- or peroxidase-conjugated Dolichos biflorus lectin (DBA) stains mammalian neuromuscular junctions intensely without detectably staining nonsynaptic surfaces of muscle fibers or motor axons. Thus, DBA, which recognizes terminal N-acetylgalactosamine (GalNAc) residues, reveals a synapse-specific carbohydrate that contains or resembles GalNAc (Sanes and Cheney, Nature 300: 646, 1982). We are using DBA immobilized on Sepharose to isolate and characterize the molecule(s) to which DBA binds. We found that DBA-Sepharose precipitated ~15% of acetylcholinesterase (AChE) activity from a crude extract of rat skeletal muscle. GalNAc but not α-methyl-mannoside (αMM) blocked this binding. Active AChE was eluted, after binding, by GalNAc but not αMM. In control experiments, Sepharose-conjugated concanavalin A (a mannose-specific lectin that binds a wide range of glycoproteins) bound ~85% of AChE, and binding was blocked by αMM but not GalNAc; Sepharose alone bound no AChE. Thus, DBA-receptors are closely associated with a subpopulation of AChE molecules.

In rat muscle, AChE is present both in collagen-tailed, asymmetric (A) forms, and in globular (G) forms; A forms are selectively associated with the neuromuscular junction, while G forms are more widely distributed (reviewed in Massoulie and Bon, Ann. Rev. Neurosci. 5:57, 1982). Because DBA-Sepharose failed to precipitate AChE quantitatively, we asked if only some forms bind DBA. AChE eluted from DBA-Sepharose by GalNAc was entirely A form, as judged by sucrose gradient centrifugation. Asymmetric (16S) was purified >100-fold by chromatography on DBA-Sepharose. Furthermore, when AChE was separated into A and G forms by differential extraction (Younkin et al., JBC 257:13630, 1982), DBA bound no AChE in the G-rich fraction, and bound a greater proportion of AChE in the A-rich fraction than in the crude extract. (Concanavalin A bound both A and G forms.) Thus, DBA recognizes the synapse-specific (A) form of AChE. We do not yet know how the DBA-binding sugar is linked to AChE, or whether all neuromuscular DBA-receptors are associated with AChE. Interestingly, while asymmetric AChE is lost from muscle soon after denervation (Massoulie and Bon, op. cit.), fluorescein-DBA stains synaptic sites on denervated muscle fibers. (Supported by NIH and MDA.)

163.10 ABSENCE OF DIGITONIN AND SAPONIN-STEROL COMPLEXES AT ACTIVE ZONES AND ACETYLCHOLINE RECEPTOR AGGREGATES IN THE FROG NEUROMUSCULAR JUNCTION. J.W. Propst and C.P. Ko. Dept. of Biological Sciences, Univ. of Southern Calif., Los Angeles, Ca. 90089.

To increase our understanding of the distribution of cholesterol within cell membranes, the cytochemical agents Digitonin and Saponin were used as sterol probes in freeze-fracture studies of the frog neuromuscular junction. The results indicate that both agents produced sterol-specific complexes in the nerve terminal, Schwann cell and muscle membranes. However, there was a complete lack of complexes between the two double rows of particles at the presynaptic active zones (AZ's) and in the particle aggregates presumed to be acetylcholine receptors (AchR's). In this way, the results resemble those found using Filipin as a sterol probe (Nakajima, Y. and Bridgman, P.C., J. Cell Biol., 88:453-58, 1981; Ko, C.P. and Henderson, C., J. Cell Biol., 97:237a, 1983). The Digitonin-sterol complexes were scalloped in appearance and were visible on both faces of the membrane. The width of the scallops varied from 40-90 nm and the length varied from 100-500 nm. Some segments of a few terminals were seen to be lacking any complexes, while the rest of the same terminals showed the expected distribution of complexes everywhere except at the AZ's and AchR's. Saponin produced irregular bumps and pits which ranged in size from 25-60 nm in diameter. Disruption of the membrane was less evident with Saponin, due to the smaller and more diffuse complexes. These results suggest that there is a membrane lipid inhomogeneity associated with AZ's and AchR's, the functional significance of which remains to be determined.
(Supported by NIH and MDA grants).

163.11 CORRELATION BETWEEN SYNAPTIC MORPHOLOGY AND DIFFERENCES IN SENSITIVITY OF A RIBBON SYNAPSE R.D. Fields and M.H. Ellisman. Neurobiology Unit, Scripps Inst. of Oceanog., Univ. of Calif., San Diego, and the School of Med., Dept. of Neurosciences, La Jolla, CA 92093

The relationship between synaptic morphology and physiology was studied in an in vitro preparation of a sensory cell (the ampullae of Lorenzini), in which activity was monitored from the primary afferents prior to electron microscopic examination of the afferent synapses. The depth of the synaptic trough formed by evagination of the presynaptic membrane into the postsynaptic membrane was found to relate linearly to the threshold sensitivity of the sensory cell.

The rate of spontaneous activity and threshold sensitivity varied among different units, and often fluctuated in recordings from individual organs. Some units lacked spontaneous activity, others were spontaneously active but relatively insensitive, and others showed a high rate of spontaneous activity and sensitivity. Considerable variation was also found in the morphology of the 198 synapses examined from these organs. The threshold sensitivity of organs, measured immediately before fixation, was found to be a linear function of the mean depth of the synaptic troughs within the organ ($p = 0.02$), but was not related to the sensitivity of the organ measured at the start of the recording period.

These observations raise the possibility that such ribbon synapses could be structurally and functionally platic, and that these properties may be interrelated so as to modulate the efficacy of transmitter release. Other explanations could also account for these data, and this possibility needs to be tested directly.

163.12 SINGLE PYRAMIDAL NEURONS IN PIRIFORM CORTEX RECEIVE EXCITATORY INPUTS FROM FACILITATING AND NON-FACILITATING SYNAPSES ASSOCIATED WITH DIFFERENT FIBER SYSTEMS. J.M. Bower and L.B. Haberly. Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706.

We have compared the synaptic effects of two excitatory influences on piriform cortex pyramidal cells: primary olfactory tract afferents which terminate on distal segments of pyramidal cell apical dendrites, and intrinsic association fibers which terminate on proximal apical segments. Slices of rat piriform cortex (300 μm) were cut perpendicular to the laminar organization of the cortex, and maintained using standard in vitro techniques. This preparation allowed us to separately activate afferent (layer Ia) or association (layer Ib) synapses with appropriately placed tungsten microelectrodes.

The major finding of these experiments is that an EPSP evoked in distal apical dendritic segments (layer Ia) by stimulation of olfactory tract afferents produces a marked facilitation of subsequent Ia-evoked EPSPs, whereas a comparable EPSP evoked by association fibers in proximal apical segments (layer Ib) of the same pyramidal cell does not facilitate subsequent Ib-evoked EPSPs. The facilitating effect of Ia EPSPs on subsequent Ia EPSPs lasts up to 200 msec, and can increase the amplitude of the second EPSP by 50% or more. We have shown that neither the facilitation of layer Ia EPSP amplitude, nor the lack of facilitation of layer Ib EPSP amplitude, is due to a conductance change in the postsynaptic membrane. This result, combined with the finding that no facilitation of EPSPs is seen when Ia activation preceeds Ib or vice versa, suggests that the Ia facilitatory effect is presynaptic.

Ultrastructural examination of the presynaptic elements of these two fiber systems has revealed that synaptic terminals of association fibers in layer Ib contain a much higher concentration of synaptic vesicles than synaptic terminals of afferent fibers in layer Ia (Haberly & Behan, JCN 219: 448, 1983). On the basis of the residual calcium theory of facilitation (Katz & Miledi, J. Physiol. 195: 481, 1968), it can be postulated that Ca^{2+} buffering provided by the high density of vesicles in layer Ib synapses is responsible, at least in part, for the lack of facilitation.

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163.13 INTERACTIONS BETWEEN ACTIVE DENDRITIC SPINES COULD AUGMENT IMPACT OF DISTAL DENDRITIC SYNAPSES. G. M. Shepherd, R. K. Brayton*, A. Belanger*, J. P. Miller, R. Malinow, I. Segev*, J. Rinzel and W. Rall. Sect. Neuroanat., Yale U. Sch. Med., New Haven, CT 06510; IBM Watson Res. Ctr., Yorktown Heights, NY 10598; Dept. Zool., U. Calif., Berkeley, CA 94720; and Math. Res. Br., NIH, Bethesda, MD 20205.

We are interested in the large number of spines at distal dendritic locations of several neuron types, e.g. cortical pyramidal cells. Our several computations explore the possible functional implications of having excitable membrane properties at the spine head of some dendritic spines.

We find that several different kinds of functionally interesting interactions between such dendritic spines are theoretically possible, depending upon different choices of the parameters which define the morphology and the membrane properties of the dendrites and the dendritic spines. Previous computations have shown that synaptic input to a small distal dendritic branch produces a relatively large local depolarization; it was also shown that synaptic input to a dendritic spine head produces still larger depolarization of the spine head membrane (for relatively large spine stem resistance). Such depolarization of the spine head could trigger an action potential in the spine head, if that membrane has excitable properties; this could increase the charge delivered and thus provide significant synaptic amplification. Because dendritic depolarization spreads with little decrement to the spine heads of nearby spines (not already activated), it is of interest to consider how many of these neighboring spine heads may have excitable membrane and whether these will reach threshold. Using parameters such that one excited spine produces enough depolarization to trigger those neighboring spines which are excitable, there could be a chain-reaction, a saltatory propagation from one excitable spine to the next, which could spread rapidly over the dendritic surface. This would be subject to several contingencies, such as spacing of the excitable spines and timing and placement of inhibitory input. Using different parameters such that one excited spine would produce insufficient depolarization; then simultaneous activation of several excitable spines (or possibly summation from both passive and excitable spines) would be required to set-off a chain-reaction. Some implications for neural integration will be discussed.

163.14 INTERACTIONS BETWEEN ACTIVE DENDRITIC SPINES COULD GENERATE BURSTS OF SPIKES. R.Malinow & J.P.Miller, Dept. of Zoology, U.C. Berkeley, Berkeley, Ca. 94720.

We have performed computations to explore further the functional consequences of excitable membrane on the heads of dendritic spines. In addition to the propagating "chain reaction" described in the previous abstract (Shepherd, et.al.) another complex phenomenon "emerges" from the model when linear arrays of many regenerative spines are considered. Within certain parameter ranges, initiation of the first spike in the "chain reaction" is followed by subsequent re-initiation of spiking activity in the same set of spines, ie. a burst of spikes is produced. During such a burst, the peak amplitude of the spikes successively decreases, and the inter-spike polarization level increases. These bursts share many features with penicillin-induced epileptiform bursts in spinous pyramidal cells, and with complex spikes initiated in Purkinje cell dendrites by climbing fiber stimulation. A simple explanation of this phenomenon is presented, as well as a discussion of the dependence of this phenomenon upon the morphological and electrical parameters of the spines and dendrites.

Supported by NSF grant BNS-8202416 and a Sloan Foundation Fellowship to J.P.M.

EPILEPSY: FITS AND SLICES

164.1 INTERSTITIAL pH DURING PAROXYSMAL DISCHARGES AND LEAO'S DEPRESSION IN HIPPOCAMPAL FORMATION IN SITU. G.G. Somjen. Dept. of Physiology, Duke Univ., Durham, NC 27710.

It has been known for more than 40 years that interstitial fluid of the brain becomes acidic after a seizure. This study was undertaken to determine the magnitude and time course of the pH change. Double-barreled ion selective electrodes with a liquid membrane sensor were used to measure interstitial pH in fascia dentata (FD) of the hippocampal formation of rats anesthetized with urethane. $[K^+]_o$ or $[Ca^{2+}]_o$ was also measured in some experiments. The angular bundle was stimulated to provoke paroxysmal discharges (PaD) and Leao's depression (LD). Single stimuli applied to angular bundle did not detectably change tissue pH in FD. Trains of stimuli too mild to provoke either PaD or LD caused acidification not exceeding 0.04 pH units, sometimes preceded by an alkaline shift. With stronger and higher frequency stimulation PaD began during the stimulus train and continued as paroxysmal afterdischarge (PaAD). PaD was associated with acidification by 0.07 to 0.2 pH units, reaching maximum several seconds after cessation of paroxysmal firing. Acid shifts were most marked in the hilus of the FD, but they were observed in adjacent layers of the FD as well. An alkaline transient of shorter duration and smaller magnitude sometimes preceded the acidification. Episodes of LD were associated with severe interstitial acidosis, amounting to a change of 0.2 to 0.5 pH units that outlasted the negative sustained shift of potential and the changes of $[K^+]_o$ and $[Ca^{2+}]_o$. Often these acid shifts were preceded by a brief but intense wave of alkalinization. The delay of the acidification relative to the electrical and other ionic manifestations of PaD and LD support the suggestion that the accumulation of H^+ is related to the increased production of acid metabolites. Post-seizure acidification may contribute to post-ictal depression of electrical activity.

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164.2 PAROXYSMAL DISCHARGES AND SPREADING DEPRESSION IN HIPPOCAMPAL SLICES. P.G. Aitken. Dept. Physiology, Duke Univ. Med. Center, Durham, NC 27710.

Trains of stimulus pulses (1-10 sec,5-40 Hz,0.1-0.5msec, 50-150μA) were applied to afferent fibers (perforant path, mossy fibers, or Schaffer collaterals) while extracellular field potentials were recorded in the cell body layer of the fascia dentata, CA3, or CA1 zone of the hippocampal tissue slice. In CA1, paroxysmal discharges (PaD) and spreading depression (SD) could be evoked in some slices in normal (3.5mM) extracellular potassium $[K^+]_o$ and in all slices in elevated (5.5 or 7.0mM) $[K^+]_o$. In CA3, SD could be provoked but PaD were not seen. In fascia dentata, neither PaD nor SD could be provoked.

In CA1, 3 types of PaD were seen: (1) Intercurrent PaD (IPaD) consisting of compound action potentials (CAP) occurring during, but not synchronized to the pulses of, the stimulus train; (2) Tonic paroxysmal afterdischarge (PaAD) consisting of a brief (0.1-1.0 sec) burst of CAP occurring immediately upon cessation of the stimulus train, and (3) Clonic PaAD, consisting of one or more phasic bursts of CAP, riding on negative sustained potential (SP) shifts, occurring with some delay (1-5 sec) after the train. IPaD were a necessary, but not a sufficient, condition for tonic and clonic PaAD. SD, marked by a sudden, severe negative SP shift (as much as 20mv) and cessation of all neural activity, could begin during or after a stimulus train. SD was sometimes preceded by PaD.

In all 3 hippocampal subfields, $[K^+]_o$ typically rose to levels between 10 and 15mM during stimulus trains; its rise was independent of whether or not PaD were provoked. During SD in CA1 or CA3, $[K^+]_o$ would reach levels above 30mM; during recovery from SD, $[K^+]_o$ usually went below baseline by 0.8-1.6mM.

In CA1, extracellularly and intracellularly recorded action potentials occurring during IPaD and PaAD did not arise from synaptic potentials, but arose sharply from a slow, relatively long lasting (several seconds) neuronal depolarization the time course of which closely followed that of the extracellular SP shift. Intracellular records from CA1 pyramidal cells during SD reveal a severe neuronal depolarization with a time course very similar to that of the extracellular SP shift. This suggests that the neural contribution to SP shifts during SD in the hippocampus may be greater than the glial contribution. (Supported by NIH grants NS-17771 and NS-18670.)

164.3 ELECTRICAL FIELDS DIRECTLY CONTRIBUTE TO SPIKE SYNCHRONIZATION DURING CONVULSANT-INDUCED EPILEPTIFORM BURSTS.
R. W. Snow and F. E. Dudek. Dept. of Physiology, Tulane Univ. Sch. of Med., New Orleans, LA 70112.

The hypothesis that large electrical fields produced during epileptiform bursts contribute to synchronization of nearby inactive neurons has long been debated. Although recent work has suggested that recurrent excitatory chemical synapses generate epileptiform bursts in hippocampus, spike synchronization can occur when evoked neurotransmitter release is blocked with a low-Ca^{2+} solution containing Mn^{2+} or Mg^{2+}. This result suggests that electrical fields can synchronize neurons, but the importance of these interactions to synchronization of action potentials during typical convulsant-induced bursts has been questioned.

In the present study, synchronous field-potential bursts were induced in hippocampal slices with picrotoxin. Conventional intracellular recordings referenced to ground demonstrated typical paroxysmal depolarization shifts (PDS's) in all pyramidal neurons, with action potentials arising from apparent negativities superimposed on the PDS. Differential recording between intracellular and adjacent extracellular electrodes during PDS's did not show negativities before spikes, but instead revealed rapid transmembrane depolarizations (TMD's) that were due to the extracellular field potential of the population spike. These depolarizations could be more easily seen when the neuron was hyperpolarized to prevent action potentials, or when the anesthetic drug QX-314 was injected to block action potentials.

The TMD's served as spike prepotentials. Their amplitude (0.5-12 mV) was always smaller than the corresponding population spike (1-13 mV), and they were more pronounced in CA1 and CA2 than in CA3 in these slice preparations. The TMD's had a fast rate of rise (9.3±5.5 V/s, n=17), and a shorter duration at half-amplitude than the underlying PDS (1.75±0.65 msec, n=40 vs. 47.7±13.5 msec, n=22). When cells were hyperpolarized to lower their excitability, TMD's could be seen to add with the PDS and initiate spikes. Since TMD's were more obvious on the peak and falling phase of the PDS than on the rising phase, they are more likely to contribute to synchronization of the later rather than the early spikes in a burst. These experiments demonstrate that endogenous electrical fields contribute to spike synchronization in the presence of convulsant drugs even when excitatory chemical synapses are functional.

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163.4 SIMULATION OF IN VITRO SYNCHRONIZED HIPPOCAMPAL DISCHARGES OCCURRING IN THE ABSENCE OF CHEMICAL SYNAPTIC TRANSMISSION
R.D. Traub, F.E. Dudek, C.P. Taylor and W.D. Knowles. IBM Watson Research Center, Yorktown Heights, NY 10598, and Tulane University Medical Center, New Orleans, LA 70112, and Warner-Lambert Co., Ann Arbor, MI 48105.

In media with low Ca concentration that block chemical synaptic transmission, neurons in hippocampal slices can generate synchronized trains of action potentials. Each action potential occurs in phase with a large negative field potential transient representing the simultaneous discharge of a population of neurons (C.P. Taylor and F.E. Dudek, Science 218 (1982) 810-812; J.G.R. Jefferys and H.L. Haas, Nature 300 (1982) 448-450). We performed computer simulations to determine whether currents flowing in the extracellular medium might plausibly induce such synchrony. Our model consisted of a 10-by-10 array of 28-compartment neurons (R.D. Traub, Neuroscience 7 (1982) 1233-1242) embedded in a 10-by-10-by-19 array of points representing a purely resistive extracellular medium. There were no chemical synapses. Ca- and Ca-dependent currents were reduced because media used experimentally to block synaptic transmission have low Ca concentrations. If individual cells were rendered hyperexcitable (either by shifting the voltage-dependent rate functions or by injecting a small depolarizing current into each cell), then synchronization did indeed occur, together with the generation of realistic-appearing field potentials. This synchrony, caused by electrical field effects, occurred in the model with a reasonable extracellular resistivity (about 125 Ohm-cm). Our assumption of cellular hyperexcitability is reasonable, since spontaneous action potentials can occur in media that block chemical synaptic transmission. In addition, experiments demonstrate that a brief shock can evoke a prolonged afterdischarge lasting hundreds of ms in such media. Electric-field-induced synchrony was enhanced in our model by the addition of electrotonic junctions connecting small clusters of cells. Such enhancement was most striking when electrical field interactions were weakened (i.e. extracellular resistivity was decreased). Preliminary work indicates that field interactions contribute to the synchronization of individual action potentials even when chemical synapses are operative. Partially supported by N.I.H. grants NS 16683 to F.E.D. and NS 20473-01 to W.D.K.

163.5 CURRENT SOURCE DENSITY ANALYSIS OF THE CA1, CA3, AND DENTATE AREAS OF THE HIPPOCAMPUS.
B. M. Bathurst*, R. W. Snow and F. E. Dudek (SPON: J. Green). Dept. of Physiol., Tulane Univ. Sch. of Med., New Orleans, LA 70112.

Synchronous epileptiform bursts can occur in slices of rat hippocampus when chemical transmission is blocked. The large extracellular population spikes generated in the hippocampus have prompted us to consider how extracellular potential gradients may contribute to synchronization of neuronal populations. We report that the extracellular current source density generated in the CA1, CA3 and dentate regions of hippocampal slices following antidromic activation is sufficient to cause transmembrane depolarizations.

Hippocampal slices of 450 μm were prepared from 90-200 g rats. Slices were cut parallel to the alvear fibers for studies of the CA1 region and perpendicular to the long axis of the hippocampus for studies of the CA3 and dentate regions. Perfusion with saline containing 2 mM Mn^{2+}, 0.5 mM Ca^{2+} and 8 mM Mg^{2+} demonstratively blocked extracellular orthodromic responses. Antidromic stimulation of each region elicited extracellular potentials from 3-20 mV in the cell body layers. Control recordings in each region showed that the extracellular population spikes varied <10% when stimulated at 0.4 Hz. A single microelectrode was used to record extracellular potentials at 30 μm or 50 μm intervals parallel to the longitudinal axis of the dendrites, and 80 μm deep in the slice. Eight responses were averaged for a one-dimensional current source density analysis employing the methods and assumptions of Nicholson and Freeman (J. Neurophysiol. 38:356). The current source density was estimated from the second spatial derivative of the averaged potentials at each location.

Current sinks of 400 to 800 mV/mm^2 were present in the cell body layers of each region when the maximum extracellular potential at the cell body was 4 mV. The distal dendrites on both sides of the cell body layer were shown to be a current source of 300 to 700 mV/mm^2. This indicates a significant separation of extracellular charge from dendrites to somata during the peak of a population spike. Differential recording between an intracellular and an immediately extracellular electrode revealed net transmembrane depolarizations in neurons from each region during a population spike. The results provide evidence that field effect depolarizations contribute to synchronization of electrical activity in all regions of the hippocampus.

Supported by USPHS Grant NS 16683.

163.6 EFFECTS OF EXTRACELLULAR ELECTRIC FIELDS ON PENICILLIN-TREATED HIPPOCAMPAL SLICES.
M. Abu-Assal*, S.M. Bawin, M.D. Mahoney*, A.R. Sheppard and W.R. Adey. Loma Linda University and VA Medical Centers, Loma Linda, CA 92357.

Our previous experiments with hippocampal slices showed long-term changes in excitability induced by extracellular electric fields in the EEG range. This study addresses the role of these electric fields on CA1 neuronal excitability in penicillin-treated slices. Increased levels of penicillin (Pen) in the perfusing solution (0.25, 1.5 and 3 mM) resulted in dose-dependent increases in excitability as measured by the amplitude and number of peaks of the potentials evoked in the CA1 cell layer by test pulses in stratum radiatum. Short bursts (20 sec) of sinusoidal electric fields at 5 and 60 Hz with amplitude of 35-50 mV/cm were applied via AgCl electrodes to the solution surrounding the slices. These fields were similar in amplitude to the transient fields generated in brain tissue by the paroxysmal activity of epileptic neurons. In 0.25 mM Pen, the electric fields induced short-term (less than 8 min) increases in the amplitude of the evoked potential. In 1.5 mM Pen, both short-term increases and long-term decreases (longer than 8 min) were observed. In 3.0 mM Pen, only long-term depressions were seen. Thus, field effects varied with the degree of epileptiform activity in the slice. Other experiments with high levels of potassium (K+ 8.75 mM versus 6.25 mM in control solution) in the absence of Pen revealed a marked (larger than 50%) facilitation of the long-term increase in excitability following field stimulation. Since the extracellular concentration of K+ rises to similar levels during ictal episodes, field-neuron interactions may be facilitated by the ionic changes associated with epileptiform bursting. Insofar as penicillin-treated slices can be models of epileptic foci, our data suggest that extracellular fields could participate in the synchronization of neuronal discharges and/or the propagation of a seizure from an epileptic focus.

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164.7 INDUCTION OF EPILEPTIFORM ACTIVITY IN HIPPOCAMPAL SLICES BY THE PRESENTATION OF A KINDLING-LIKE STIMULUS. S.F. Stasheff*, W.A. Wilson*, and A.C. Bragdon* (SPON: B. Crain). Duke University and VA Medical Centers, Durham, NC 27705.

Kindling is an animal model which is useful for studying the mechanisms of limbic epilepsy in vivo. The hippocampus is an important focus of epileptiform activity in this and other models of limbic epilepsy. It would be useful to be able to study the development of epileptiform bursting in kindling in greater detail using an in vitro preparation. Elevated K^+, bicuculline, and penicillin have been useful for studying mechanisms of epileptiform bursting in the hippocampal slice preparation, but these models do not closely mimic the kindling process. We have found that a series of stimulus trains similar to those used in kindling induces burst firing in CA3 in hippocampal slices.

Transverse rat hippocampal slices 625 um thick were incubated in oxygenated (95% O_2 / 5% CO_2) artificial CSF containing (mM): NaCl 120, KCl 3.3, NaH_2PO_4 1.23, $NaHCO_3$ 25, $MgSO_4$ 1.2, $CaCl_2$ 1.8, dextrose 10. Pairs of slices were studied simultaneously in the same submerged chamber at 31°C. Stimuli were delivered to s. radiatum of CA3 or CA1, or s. moleculare of the dentate. Test stimuli of the intensity which evoked the maximum orthodromic response were delivered to both slices at .02 Hz. The experimental slice of each pair also received trains of 60 Hz, 2 sec duration, at twice this intensity delivered once every five minutes at least until the onset of epileptiform activity.

Hippocampal slices exposed to these kindling-like trains exhibited three types of epileptiform activity. All slices showed (1) afterdischarges following trains similar to those observed in vivo following kindling stimuli, and (2) spontaneous bursts of multiple population spikes, which may be comparable to spontaneous interictal spikes observed during kindling. The progression of these features paralleled that observed during kindling. (3) In about 2/3 of experimental slices, bursts were triggered by single stimuli. All these epileptiform activities persisted in experimental slices for up to 3 1/2 hours following the last train. None of these signs were observed in control slices. All control slices which subsequently received stimulus trains then developed epileptiform activity.

The similarities between the development of this stimulus train-induced population bursting (STIB) and the development of epileptiform activity in kindling suggest that this may be a useful in vitro model for detailed, acute studies of the kindling process.

164.8 RECURRENT INHIBITION OF DENTATE GRANULE CELLS IS SEPARABLE FROM COMMISSURAL-ASSOCIATIONAL INHIBITION IN THE HIPPOCAMPAL SLICE. A.C. Bragdon* and W.A. Wilson* (SPON: A.D. Roses). Duke University and VA Medical Centers, Durham, NC 27705.

The granule cells of the dentate gyrus are the first cells of a trisynaptic excitatory pathway through the hippocampus. Factors which control the activity of the granule cells have a major effect on the transmission of neuronal activity through the rest of the hippocampus. While synaptic inhibition of granule cells has long been thought to be recurrent, recent data suggest feed-forward inhibitory pathways may also be present. To understand the contributions of each of these, it would be advantageous to be able to study their effects separately. Using the in vitro hippocampal slice preparation we have demonstrated that we can independently activate recurrent inhibition and commissural-associational feed-forward inhibition, and we have studied the former in some detail.

500 µm, transverse hippocampal slices from 150-250 gram, male, Sprague-Dawley rats were studied in a 31°C submersion chamber perfused at 2 ml/min with artificial CSF. (NaCl 120, $NaHCO_3$ 25, NaH_2PO_4 1.23, KCl 5.0, $CaCl_2$ 2.5, $MgSO_4$ 1.5, dextrose 10; bubbled with 95% O_2, 5% CO_2. Low calcium CSF had 5.0 mM $MgCl_2$ in place of $CaCl_2$.) Orthodromic (OD) stimuli were delivered to perforant path fibers just proximal to the hippocampal fissure. Antidromic (AD) stimuli were delivered to mossy fibers in CA3 well away from area CA4. Commissural-associational (CA) stimuli were delivered to the alveus near the junction of CA3 and the dentate. Drugs were applied by bath or pressure ejection.

OD stimuli evoked EPSPs and population spikes (PSs) recorded in the granule cell layer. AD stimuli evoked PSs which persisted in low calcium CSF, but did not evoke a Ca^{++}-dependent synaptic potential in any layer of the dentate. In contrast, CA stimuli evoked a Ca^{++}-dependent, negative potential maximal in the inner molecular layer, but no PS in the granule cell layer. Pairing an OD with either an AD or a CA stimulus 5 to 20 msec earlier reduced the height of the OD-evoked PS relative to control. The AD-evoked inhibition was enhanced by chlordiazepoxide and pentobarbital, antagonized by bicuculline, and mimicked by local application of GABA at the granule cell layer.

These results show 1) mossy fibers and dentate commissural-associational fibers can be activated independently using the slice preparation, 2) both recurrent and feed-forward inhibition act on dentate granule cells, and 3) recurrent inhibition is mediated through a GABA receptor.

164.9 HIPPOCAMPAL SLICES FROM KINDLED RATS EXHIBIT ABNORMAL NEURONAL EXCITABILITY. G.L. King, R. Dingledine, J.L. Giacchino and J.O. McNamara. Dept. Pharmacology, Univ. North Carolina, Chapel Hill, NC 27514; and Depts. Medicine and Pharmacology, Duke University, Durham, NC 27710.

Although the hippocampus is thought to facilitate the development of kindling, its precise role in this model of epilepsy is unknown. The purpose of this study was to determine whether the physiology of hippocampal neurons from kindled rats is altered in a manner that might be expected to promote seizure activity in situ. Twelve rats were kindled from right lateral entorhinal cortex; 12 implanted but unstimulated rats served as controls. Within 24 hours of completion of kindling (3-12 class V seizures) slices were prepared from the right hippocampus of a control or kindled rat and incubated in medium containing either 3.5 or 7 mM K. The preparation and testing of slices, and data analyses, were done blind. There were 26-38 slices in each experimental group (control/kindled, normal/high K). All reported differences between slices from kindled and control rats were significant to at least the 0.05 level. In 7 mM K, but not 3.5 mM K, spontaneous bursts of population spikes, similar in configuration to interictal spikes, could be recorded from the CA2/3 region. A greater proportion of kindled slices developed bursts, and mean burst frequency was doubled in kindled slices. We observed no ictal-like events in any slice. Tests for the intensity of GABAergic inhibition (paired-pulse inhibition; PPI) showed a reduction of PPI in the CA1 region of kindled slices. There were three indications that, following kindling, synaptic inhibition was potentiated in the dentate. First, PPI was enhanced. Second, the orthodromic stimulus current needed to evoke a standard amplitude population spike was increased in the dentate but not in CA1. Third, the input-output (IO) curve formed by plotting population spike amplitude as a function of EPSP slope, when averaged within groups, was shifted down and to the right in the dentate but not in CA1. Several parameters were unchanged in CA1 or the dentate of kindled groups: maximum amplitudes of prevolley, population spike and field EPSP slope, and number of population spikes produced by a submaximal orthodromic stimulus. Neuronal bursting in CA2/3 together with reduced inhibition in CA1 could combine to increase the likelihood of a seizure. The enhanced synaptic inhibition in the dentate may reflect a compensatory reduction of excitatory input into CA3.

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164.10 GABAERGIC INHIBITION REGULATES SPONTANEOUS EPILEPTIFORM ACTIVITY IN HIPPOCAMPAL SLICES BATHED IN HIGH POTASSIUM. Stephen J. Korn, Nancy L. Chamberlin* and Raymond Dingledine. Dept. Pharmacology, Univ.•North Carolina, Chapel Hill, NC 27514.

Spontaneous bursts of population spikes, resembling interictal spikes in whole animal recordings, can be recorded from the CA2/CA3 region of rat hippocampal slices bathed in elevated potassium. The processes that control the intensity and duration of bursting are unknown. We tested the hypothesis that GABAergic inhibition plays a role in terminating spontaneous bursts in this in vitro model of epilepsy. Spontaneous bursts of population spikes appeared as the extracellular potassium concentration (K) was increased from 3.5 mM (normal) to 7 mM. Burst frequency and spike amplitude increased when K was raised further to 8.5 mM, but then often declined as K was raised to 10 or 11.5 mM. We measured the frequency of spontaneous bursting and the number and amplitude of population spikes within a burst in the presence of 7.0 or 8.5 mM K and drugs that influence GABAergic transmission.

Bicuculline (100 uM) invariably increased spike amplitude and the number of spikes within a burst. In contrast, several drugs that enhance GABAergic inhibition attenuated bursting, as evidenced by a reduction in the number and often the amplitude of population spikes within a burst. Pentobarbital (100 uM) nearly eliminated bursting, an effect that was blocked by bicuculline. The GABA uptake inhibitor, cis-4-OH-nipecotic acid (1 mM), substantially attenuated bursting whereas the inactive analog, 4-OH-isonipecotic acid (1 uM), was without effect. Phenobarbital (100 uM) and diazepam (10, but not 1 uM), which may also enhance GABAergic inhibition, also reduced burst intensity, but to a lesser extent than pentobarbital. All of the effects of pentobarbital, phenobarbital and cis-4-OH-nipecotic acid were reversible. These results support the hypothesis that GABAergic synaptic inhibition participates in the termination of spontaneous bursts. Furthermore, the action of bicuculline suggests that GABA-A receptors play a prominent role in this regulation. None of the above drugs consistently altered spontaneous burst frequency in 8.5 mM K, which suggests that tonic GABAergic inhibition does not suppress burst initiation under our experimental conditions.

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164.11 ROLE OF THE SODIUM/POTASSIUM PUMP AND OF EXTRACELLULAR POTASSIUM IN EXCITABILITY OF IMMATURE HIPPOCAMPAL SLICES. M.M. Haglund and P.A. Schwartzkroin. Dept. of Neurological Surgery, Univ. of Washington, Seattle, WA 98195.

Using the immature hippocampal slice preparation, we have recorded spontaneous spreading depression (SD) episodes that occur primarily in the CA1 region of 8-13 day old rabbit hippocampus. The CA1 region has a lower threshold to SDs evoked by KCl application as compared to the CA3 region. In the present study, the changes in extracellular potassium concentration $[K^+]_o$ were measured with potassium ion sensitive microelectrodes during the onset and recovery from seizure and SD episodes. The roles of the Na/K pump in "protecting" the CA3 region from pathological hyperexcitability, and in the recovery of the CA1 region from SD episodes was also investigated.

Intracellular and ion sensitive recordings were made from CA1 or CA3 stratum pyramidale. Changes in $[K^+]_o$ were complexly related to changes in membrane potential during seizure and SD episodes. Both spontaneous and evoked seizure episodes in the CA3 region were accompanied by a peak $[K^+]_o$ of 16-20 mM. The transition from normal activity to ictal activity was associated with a rise in the $[K^+]_o$, but it was unclear whether the initial increase in $[K^+]_o$ preceded membrane depolarization. During spontaneous SD episodes in the CA1 region, the $[K^+]_o$ rose to a maximum of 45-65 mM. At the onset of SD, the $[K^+]_o$ rose rapidly from resting levels before depolarization of the CA1 pyramidal cell. During recovery from SD, the membrane potential of the CA1 cell undershot its original resting membrane potential. This hyperpolarizing undershoot occurred before the $[K^+]_o$ reached its minimum level near 3 mM (baseline level of 5 mM).

We have demonstrated that pressure ejection of ouabain (10^{-5} M) onto the CA1 region of the slice blocks cell hyperpolarizing undershoots. Ouabain also increases the duration of the SD episode from 45-90 sec in normal tissue to 3-5 min in ouabain-treated tissue. Ejection of ouabain into CA3 lowers the region's threshold for seizure and SD episodes.

These results indicate that: 1) During the onset of SD, initial increases in $[K^+]_o$ occur before any apparent change in cell membrane potential; 2) Partial blockade of Na/K pump activity contributes to a decreased threshold to seizure episodes in the CA3 region; 3) The Na/K pump plays a critical role in the recovery from SD, including the hyperpolarizing undershoot that follows SD episodes.

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164.12 CURRENT SOURCE DENSITY ANALYSIS OF THE EPILEPTIFORM BURST IN CA3 HIPPOCAMPAL PYRAMIDAL CELLS. J.W. Swann, R.J. Brady, R.J. Friedman* and E.J. Smith.* Ctr. for Labs & Research, NYS Dept. of Health, Albany, NY 12201

We have previously reported the results of a one dimensional current source density analysis (CSDA) of the epileptiform burst in the hippocampal CA3 region (Swann et al., Neurosci. Abst. 9:395, 1983). In those experiments extracellular field potentials were recorded on a track perpendicular to the cell body layer across both dendritic trees. The results of those experiments suggest that there is a current sink associated with burst generation in each dendritic tree. However, it remains a possibility that extracellular current flow is not strictly perpendicular to the CA3 laminae and consequently our estimates of CSD could be inaccurate due to the presence of significant current which flows in other directions. Accordingly, we have measured field potential on the two axes orthogonal to our original recording track. In the first series of experiments reported here, recordings were made in a plane parallel to surface of the slice and roughly perpendicular to the cell body layer (depth - 50 µm). Recordings were made at 50 µm intervals on 4-6 parallel tracks (100 µm separation between tracks). In the second series of experiments variations in the burst amplitude were examined with depth. Recordings were made on a single track perpendicular to the axis of the cell body layer. At each position recordings were made at 5 regularly spaced depths (0-200 µm). In each experiment, the peak amplitude of the burst was measured and linear interpolation was used to generate isopotential contours. In all experiments the contours were largely parallel to the cell body layer. Since the direction of extracellular current flow is perpendicular to isopotential lines, our data suggest that maximum extracellular current is at right angles to the cell body layer - the direction in which we performed our original CSDA. Further support for this contention was obtained from these data by estimating CSD in tracks perpendicular to the cell body layer and comparing this to CSD which was corrected for current parallel to the cell layer. While these corrections had effects on the amplitude of CSD at some locations, the overall profile of CSD was not affected. Thus a single dimension analysis appears to provide a good estimate of CSD in hippocampal slices. Accordingly, our earlier contention that the burst is not only dendritic in origin but generated synchronously in both dendritic trees is further supported.

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164.13 INCREASES IN EXTRACELLULAR POTASSIUM AND NEGATIVE FIELD POTENTIALS PRODUCED BY GLUTAMATE IN HIPPOCAMPAL SLICES. R.J. Brady, K.L. Smith*, and J.W. Swann. Lab. of Develop. Neurophysiol., Ctr. for Labs & Research, NYS Dept. of Health Albany, NY 12201

Previous work in our laboratory has shown that the CA3 region of hippocampal slices taken from rats 9-19 days of age have an increased tendency to generate afterdischarges during epileptiform activity. Intracellular recordings show a slow depolarizing afterpotential following the downstroke of the depolarizing shift (Swann and Brady, Dev. Br. Res. 12:243-254, 1984). Correlated with this afterpotential is a prolonged negative field potential which has a current sink in the infrapyramidal portion of stratum oriens (SO), (Swann et al, Neurosci. Abst. 9(1) 395, 1983). Comparison of epileptiform activity in slices taken from mature and immature rats has shown that along with a larger extracellular slow negative field potential, the increase in $[K^+]_o$ is larger in the immature slices (Smith, K.L., et al., Neurosci. Abst. 1984). For the present investigations, a double barrelled iontophoretic electrode was positioned in a fixed array with a potassium sensitive microelectrode. The two sides of the double barrelled microelectrode were filled with 1 M glutamate and 3 M NaCl respectively, for interbarrelphoresis. Direct, post-synaptic effects of glutamate were studied in the hippocampal slice by exposure to a perfusate containing 0.2 mM Ca^{++}, 10 mM Mg^{++}, and 10^{-6} g/ml tetrodotoxin. Application of glutamate by ejection currents as low as 5 nA to the infrapyramidal portion of SO produced an extracellularly recorded negative field and a concurrent rise in $[K^+]_o$. At this position in SO, application of glutamate is able to evoke a maximal response in the immature tissue that is approximately twice as large as the maximal response of the mature tissue. The ability of glutamate to evoke an increase in $[K^+]_o$ in the low calcium, high magnesium, tetrodotoxin containing perfustate implies that the CA3 cells can respond to glutamate with a mixed ionic response. Intracellular records seem to confirm this contention. In response to glutamate the CA3 hippocampal pyramidal cells studied have primarily shown a depolarization, with an increase in membrane conductance. Occassional cells have shown a biphasic depolarizing-hyperpolarizing response with conductance increase. There have also been rare observations of completely hyperpolarizing responses with conductance increase. In this case the response was shown to have a reversal potential of approximately -80 mV. Supported by NINCDS grant #NS 18309 & NRSA fellowship #1F32 NS 07395).

164.14 EVALUATION OF CURRENT SOURCE DENSITY ANALYSIS METHODS IN HIPPOCAMPAL SLICES. E.J. Smith* and J.W. Swann (Spon: D. Poulos), Ctr. for Labs & Research, NYS Dept. of Health, Albany, NY 12201

Our laboratory is currently performing current source density analysis (CSDA) of penicillin-induced epileptiform discharges in rat hippocampal slices (Swann et al., this meeting). In experiments reported here we evaluated: 1) The use of CSDA in in-vitro slice preparations and, 2)the multielectrode electronic differentiation method of Nicholson and Llinas (Brain Res. 100:418-424, 1975). The profile of field potential and CSD about an artifical point source of current in hippocampal slices and slices composed of 4% agar were examined. Constant current square pulses (approx. 5 µA) were delivered to slices via glass micropipettes (2M NaCl). The fields consisted of circular isopotential lines whose amplitude obeyed an inverse radius relationship, $\phi = k/r$. Measurement tracks displaced 50, 100 and 200 µm from the stimulating electrode were used to record field potentials. CSD was a maximum where the measurement tract was closest to the source and experienced a reversal of sign symmetrically about this maximum value. The spatial location of these sign reversals was independent of the distance of the measuring tracks from the source but the magnitude of both the field potential and the CSD diminished with distance from the source. A model of the field potential along the measuring tracks was developed and found as $\phi = k/\sqrt{a_o^2 + x^2}$, where a_o was the measurement track offset from the source and x the distance along the track. Taking the second derivative of this equation yields an expression porportional to CSD. Excellent correlation was achieved between the experimental data and this theoretical model. The 50 µm track produced a sharp change in the sign of the CSD due to a well defined inflection point on the potential curve. This effect was blunted by moving to the 200 µm track. Indeed the profile of the field potentials measured in the 200 µm track most closely resembled those obtained from analysis of the peak amplitude of neuronally generated sources such as the epileptiform burst. That is, it best approximates the natural situation of "extended" source generators. Under these recording conditions CSDA provides an accurate and unambiguous localization of current sources.

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164.15 EXTRACELLULAR K^+ CHANGES DURING EPILEPTOGENESIS IN THE CA3 REGION OF IMMATURE RAT HIPPOCAMPAL SLICES. K.L. Smith*, R.J. Brady and J.W. Swann. (Spon: M. Pierson) Ctr. for Labs & Research, NYS Dept. of Health, Albany, NY 12201

Our laboratory is involved in an examination of penicillin's ability to produce epileptiform discharges in immature hippocampus. We have previously reported that the CA3 region of hippocampal slices taken on postnatal days 9-19 have a pronounced capacity to generate prolonged afterdischarges, which are often 20-30 sec in duration (Swann and Brady, Dev. Brain Res. 12:243-254, 1984). Associated with these afterdischarges is a slow negative field potential recorded near the cell body layer. Since in other preparations slow negative fields are thought to be the product of extracellular K^+ accumulation, we have employed K^+ sensitive microelectrode techniques to examine changes in extracellular K^+ during epileptogenesis in immature hippocampus. In immature slices the epileptiform burst is accompanied by an increase in K^+ lasting several seconds. The amplitude and rise time of this change varies with recording site - being largest and fastest just below the cell body layer (infrapyramidal) in stratum oriens. At this location the slow negative field potential is also largest. We have recorded unusually large K^+ signals from the infrapyramidal region. The epileptiform burst is followed by a 3-5 mM increase in extracellular K^+. When the burst is followed by an afterdischarge the K^+ levels increase further and these changes correlate very well with variations in the coincident negative field potentials. During prolonged afterdischarges the traditional K^+ ceiling level of 10-12 mM is exceeded. Ceiling levels in the immature CA3 region vary between 15 and 20 mM. Moreover following a prolonged afterdischarge the extracellular K^+ concentration routinely falls below baseline. These undershoots are thought to be a reflection of the presence of K^+ clearing mechanisms. Following afterdischarges the extracellular K^+ levels return slowly (1-2 min) to baseline. Epileptiform bursts which are followed by brief or prolonged afterdischarges arise from the same baseline K^+ concentrations. Thus, variations in the resting K^+ levels do not appear to play a key role in prolonged afterdischarge generation in immature hippocampus.

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164.16 FURTHER OBSERVATIONS ON NEURONAL ACTIVITY IN AREAS OF CHRONIC CORTICAL EPILEPTIFORM FOCI. J.W. Lighthall* and D.A. Prince (SPON: K.L. Chow). Dept. Neurol., Stanford Univ. Sch. of Med., Stanford, CA 94305.

Abnormal activities characteristic of epileptogenesis can be recorded in vitro in neurons of guinea pig neocortical slices obtained from areas of chronic freeze lesions (Lighthall & Prince, Neurosci. Abst. 9:907, 1983). Further experience with this model has confirmed its usefulness in studying mechanisms of chronic epileptogenesis. Chronic epileptogenic foci were produced by transdural freezing of sensorimotor cortex of guinea pigs, and neocortical slices through the lesion were subsequently (2-3 wks) cut with a vibratome and maintained in vitro using standard techniques. Stable intracellular recordings were obtained from 105 neurons in cortical lamina directly beneath the region of cortical injury and surrounding the site of a lesion. Sixty neurons generated normal orthodromic responses, consisting of short latency EPSPs < 30 msec in duration which elicited 1-2 spikes, followed in some cases by an IPSP < 250 msec in duration. One or more of three types of abnormal orthodromic responses were observed in the remaining 45 neurons. 1) Fixed latency multiple component depolarizations lasting 40-150 msec, and capable of triggering bursts of spikes were evoked in most cells. These depolarizing events responded to alterations in stimulus intensity and V_m like PSPs. 2) Some neurons exhibited an initial fixed latency EPSP followed by an all-or-none depolarizing potential. Portions of the initial EPSP were blocked in some cells during membrane hyperpolarization, as were the late depolarizing events, indicating participation of a voltage-dependent conductance in their generation. The intrinsic depolarization could generate bursts of variable latency spikes. IPSPs generally could not be demonstrated in cells which exhibited both passive and active depolarizations and superimposed spike bursts. 3) Spontaneous and orthodromic depolarization shifts (DSs) of prolonged duration (up to 2 sec) with superimposed spikes were recorded in a small population of neurons located on the periphery of the freeze lesion. Evoked DSs were always preceded by a fixed latency EPSP and could not be triggered by direct depolarization of the same neuron.

Our data indicate that both intrinsic slow membrane responses and modified synaptic input underlie epileptogenesis in the freeze lesion model of chronic cortical injury. Supported by NIH grant NS 12151 from the NINCDS.

164.17 DYE-COUPLING IS DECREASED IN AREAS OF CHRONIC CORTICAL INJURY. D.A. Prince and J.W. Lighthall*. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Previous experiments have shown that there is both dye-coupling and probable electrotonic coupling between neurons in normal guinea pig sensorimotor cortex slices maintained in vitro (Gutnick & Prince, Science 211:67, 1981; Connors et al., J. Neuroscience 3:773, 1983). Although little is known about how this form of intercellular communication is affected by cortical injury, it is possible to speculate that injury might increase electrotonic coupling among neurons and play a role in the synchronization of epileptiform burst discharge in injured neocortex (Traub & Pedley, Ann. Neurol. 10:405, 1981). To test this hypothesis we used transdural freeze lesions in the guinea pig to produce a region of injury in sensorimotor cortex and resultant chronic epileptiform foci. Abnormal neuronal activity is observed in vitro in neurons occupying the region of neocortical injury (Lighthall & Prince, Neurosci. Abst. 9:907, 1983 and this volume). We compared the incidence of dye-coupling amongst neurons filled with Lucifer Yellow (LY) in slices from normal cortex and those from chronic freeze lesions, maintained in vitro. Both lesion and control slices were placed in the same recording chamber and maintained at 37°C. Microelectrodes were filled with a mixture of 5% LY and 0.5 M $LiCl_2$ or LiAc. Penetrations were made at similar depths and in similar cortical areas in control and lesion slices. Ten of 84 intracellular injections within cortical freeze lesions yielded multiple (2-7) filled neurons. The remaining 74 injections resulted in single neuronal fills. The dye-coupling ratio (proportion of multiple/single fills) was therefore 11.9%. The dye-coupling ratio examined in normal cortex was 33.3% based on 39 intracellular injections of LY. This was similar to that found in previous experiments (Connors et al., J. Neuroscience 3:773, 1983). Testing the coupling ratios of $.0001 < P < .001$. These results show that a significant decrease in dye-coupling occurs among neurons located in a region of chronic cortical injury produced by cold, although the mechanism responsible is unclear. Our data further indicate that epileptogenic burst discharge synchronization in chronically injured neocortex is not dependent on an increase in gap junction formation as has been postulated. Supported by NIH grant NS 12151 from the NINCDS.

164.18 INTERACTION OF Na VALPROATE AND DFP ON SPONTANEOUS INTERICTAL EVENTS INDUCED BY $[K]_o$ IN RAT HIPPOCAMPAL SLICES. F.J. Lebeda, P.A. Rutecki* & D. Johnston, Dept. of Neurol., Prog. in Neurosci., Baylor Col. of Med., Houston, TX 77030.

Certain convulsants (e.g., diisopropyl phosphorofluoridate (DFP) and the putative K-channel blockers 4-aminopyridine (4AP) and tetraethylammonium (TEA)) appear to exert their effects without abolishing inhibitory synaptic activity (Lebeda et al., SFN Abstr., 1983). Elevated levels of extracellular potassium ions ($[K]_o$) also produce epileptiform activity without the loss of synaptic inhibition (Rutecki et al., SFN Abstr., 1984). To understand more about the convulsant mechanism of DFP, we compared its effects with those of 4AP and TEA at various $[K]_o$. We also studied the effects of the anticonvulsant Na valproate (VPA) on the rate of DFP- and high $[K]_o$-induced discharges.

Using continuously superfused rat hippocampal slices, conventional extracellular recording techniques monitored convulsant-induced discharges in the CA3 subfield.

Initial experiments were conducted to determine the interaction between DFP and $[K]_o$. DFP (25-35 µM) produced spontaneous discharges (ca. 0.3 Hz) at 5 mM but not at 2.5 mM $[K]_o$, in contrast to 4AP and TEA which caused discharges at both of these concentrations (Rutecki & Johnston, SFN Abstr., 1983). Moreover, unlike 4AP and TEA, which increased the discharge frequency induced by higher $[K]_o$ (7.5-9.5 mM), DFP produced either no change or a small decrease.

Another set of experiments examined the interaction of VPA and $[K]_o$. VPA (0.5-1 mM) produced a reversible decrease (≥50%) in the discharge frequency induced by 7.5 mM $[K]_o$, but was less effective in reducing the rate in 9.5 mM $[K]_o$.

Complex interactions occurred when both VPA and DFP were added to the bath. At 5 mM $[K]_o$, VPA (0.1-1 mM) reversibly decreased the apparent frequency of discharges induced by 25 µM DFP. This activity was occasionally preceded by a transient increase in discharge frequency. Furthermore, at 7.5 mM $[K]_o$, VPA produced a maintained increase in the discharge rate in the presence of DFP.

The decrease in the $[K]_o$-induced discharge frequency caused by VPA is consistent with the hypothesis that this anticonvulsant operates by altering K-mediated processes. The different results obtained at various $[K]_o$ with DFP and with 4AP or TEA indicate that DFP does not simply mimic the action of these two agents. Further study is in progress to account for the paradoxical interaction between VPA and DFP [Supported by USAMRDC DAMD17-82-C-2254, the Grass Foundation and NIH grants NS11535, NS15772 & NS18295]

164.19 LITHIUM-PILOCARPINE SEIZURES: IN VIVO STUDIES. C.F. Zorumski, R.C. Collins, J.W. Olney, D.B. Clifford. Depts. of Psychiatry and Neurology, Washington University Med. Sch., St. Louis, MO 63110.

Pilocarpine (30 mg/kg, SC) produces seizures in albino rats treated with lithium chloride (3 meq/kg, SC) (Science 220:323-5, 1983). The behavioral syndrome begins within five minutes of pilocarpine injection with signs of cholinergic stimulation (piloerection, salivation, chromodacryorrhea) and stereotyped motor movements (crouching, staring, and occasionally sniffing and chewing). Motor seizures, consisting of forelimb clonus with rearing and falling, begin by 25 minutes ($x = 24.2 \pm 2.1$ minutes) and recur every one to five minutes. After several motor seizures ($x = 5.3 \pm 0.4$) animals develop continuous head, trunk, and upper extremity jerks which persist for hours.

To study the onset and propagation of these seizures qualitative ^{14}C-2-Deoxyglucose (DG) autoradiography and multiple depth recordings were used. Compared to control animals injected with either lithium chloride (3 meq/kg, SC) or pilocarpine (30 mg/kg, SC) animals given both agents developed the typical behavioral syndrome and displayed significantly increased DG labeling in the amygdala, dentate gyrus, entorhinal cortex, septum, globus pallidus, substantia nigra, and ventrobasal thalamus. A significant decrease in DG labeling was seen in the nucleus accumbens.

Bipolar electrodes recording from the above sites as well as the caudate-putamen and neocortex revealed no epileptiform activity in control animals. However, within five minutes after pilocarpine injection in lithium treated rats baseline EEG was replaced by rhythmic low voltage activity in several sites including the hippocampus and nucleus accumbens, coincident with the behavioral state of stereotyped movements. Prior to the onset of motor seizures prominent spikes occurred in the nucleus accumbens and ventral globus pallidus. Organized ictal discharges began in ventral forebrain regions and rapidly spread to other sites. The onset of organized epileptiform discharges coincided with the onset of motor seizures.

These findings indicate that the combination of lithium and pilocarpine produces seizures which have behavioral components similar to limbic seizures produced by other toxins. However, lithium-pilocarpine seizures have a unique pattern of metabolic and electrical activation suggesting an important role for ventral forebrain sites in the propagation of limbic seizures.

164.20 LITHIUM-PILOCARPINE SEIZURES: IN VITRO HIPPOCAMPAL SLICE STUDIES. D.B. Clifford, J.W. Olney, C.F. Zorumski. Depts. of Neurology and Psychiatry, Washington University Med. Sch., St. Louis, MO 63110.

Lithium treated rats systemically injected with pilocarpine develop limbic seizures which have a unique pattern of deoxyglucose labeling and electrographic onset (see companion abstract). To investigate possible mechanisms underlying these observations, the in-vitro hippocampal slice preparation was used.

Hippocampal slices (400 µm) were prepared from male albino rats (180-250 gm) according to standard techniques. Slices were continuously perfused with oxygenated media (composition: 127 mM NaCl, 2 mM KCl, 1.5 mM $MgSO_4$, 1.5 mM $CaCl_2$, 26 mM $NaHCO_3$, 1.1 mM KH_2PO_4, 10 mM glucose, pH 7.4) at 1.5-2.0 ml/min. Extracellular field potentials were recorded from the dentate gyrus and CA_3 and CA_1 regions using 2M NaCl electrodes (1-5 MΩ). Orthodromic evoked responses were studied in CA_3 and CA_1 using stimulation of the mossy fibers and Schaffer collaterals, respectively.

Baseline activity and evoked responses were unchanged by perfusion with media containing either lithium chloride (1 mM) or pilocarpine (1.0 nM-1.0 µM). However, in combination these agents produced marked changes in the spontaneous activity of slices; at pilocarpine concentrations $\geq$ 10 nM spontaneous synchronized epileptiform bursts were noted. The bursts, which were 5-15 mV in amplitude and 50-80 msec in duration, were seen in CA_3 and CA_1 but not in the dentate gyrus. Bursts in CA_3 led those in CA_1 by several milliseconds and transection of the Schaffer collaterals eliminated bursts in CA_1 while CA_3 bursts were unaffected. At pilocarpine concentrations $\leq$1 µM the frequency of bursts was proportional to the concentration of pilocarpine. Bursts were eliminated during wash to control media but were reestablished with media containing lithium and pilocarpine.

The combination of lithium (1 mM) and pilocarpine also augmented evoked responses, as defined by the appearance of additional population spikes. CA_3 was more sensitive to this effect with augmentation at concentrations of pilocarpine as low as 1 nM. At higher concentrations of pilocarpine (> 1 µM in CA_3 and > 10 µM in CA_1) evoked potentials were diminished.

These data indicate that pilocarpine in combination with lithium produces spontaneous epileptiform discharges and augmented evoked responses in hippocampal slices. Thus the hippocampal slice is a useful preparation for studying mechanisms underlying lithium-pilocarpine epileptogenesis.

REGIONAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS

165.1 QUANTITATIVE AUTORADIOGRAPHY OF 14-C (3,3-DIMETHYL-2-BUTOXY)-METHYLPHOSPHORYLFLUORIDE (SOMAN) DISTRIBUTION IN THE RAT BRAIN. K. Traub*, K. Olson*, M. Pindzola*, and L. Spencer* (SPON: J.F. Glenn) U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010.

The importance of acetylcholinesterase (AChE) inhibition as a mechanism of toxicity of organophosphorous (OP) poisoning has been shown by many investigators. However, long lasting effects of accidental exposure to OP compounds in humans seem to be unrelated to the immediate inhibition of AChE. These effects range from sensory motor polyneuropathies to sleep and memory disturbances, acute and chronic episodes of depression and severe anxiety attacks. The probable site of action(s) and the mechanism of these disturbances remains obscure. The following study was undertaken to determine the distribution of Soman in the central nervous system of the rat using quantitative autoradiography.

Radiolabeled Soman (14-C methyl on the phosphorous), specific activity = 59 mCi/mmole, was administered to 18 male Charles River rats in a single IM dose of 0.75 LD50 (17.8 ug/Kg). The animals were sacrificed at intervals of 2 and 32 minutes and 48 hours post exposure. Diffusional artifact of the radiolabeled drug was minimized by rapid freezing of the whole animal in Freon 11 at -80oC, extraction of the brain at -20oC, cryostatic sectioning and performance of the autoradiography at -80oC. Autoradiographic exposures were made of 20 micron thick sections for periods of 50 to 300 days depending upon the signal strength in tissue areas of interest. The autoradiographs were measured using photometric methods which yielded both optical and area densities, grain counts and grain areas.

High levels of radiolabel (equivalent to 2.4x10-7 g Soman/g tissue) were found in blood and CSF after 2 and 32 min. but not 48 hrs. Preferential accumulation of radiolabel had occurred at 48 hrs in the caudate and accumbens nuclei compared to other brain areas including those rich in AChE. Both nuclei contained 4.8x10-8 equivalent g Soman/g tissue while other nuclei and cortical areas contained 1.2 to 1.8x10-8 equivalent g Soman/g tissue. Areas dominated by myelin were uniformly lower in radiolabel than areas containing neuropil or cells: myelin contained 9.6x10-9 equivalent g Soman/g tissue. It is concluded that Soman and/or its metabolites are actively extracted and retained by the caudate and accumbens nuclei and that is probably not a function of the anticholinesterase activity of Soman.

165.2 BENZODIAZEPINE AND BETA CARBOLINE BINDING SITES HAVE APPARENTLY IDENTICAL DISTRIBUTIONS IN RHESUS MONKEY BRAIN. J.B. O'Neill*+, D.P. Friedman+, and J.M. Crawley#. Laboratory of Neuropsychology+ and Clinical Neuroscience Branch#, NIMH, Bethesda, Md. 20205.

Although biochemical evidence suggests that benzodiazepine (BDZ) and beta carboline (BC) binding sites are part of the same supramolecular complex, there has not yet been an anatomical demonstration of this receptor co-localization. To gather such evidence, we performed an in vitro autoradiographic mapping study of the distribution of [^{3}H] flunitrazepam (FLN) and [^{3}H] BC binding in a rhesus monkey brain.

Slide-mounted sections of unfixed brain were pre-incubated for 30 minutes at 0°C in 50mM Tris (pH7.0), 50mM NaCl, and 1mg/ml BSA, and then incubated for 1 hr. at 0°C in 50mM Tris (pH7.0), 50mM NaCl, and either 1nM [^{3}H] FLN or 1nM [^{3}H] BC carboxylate. The sections were then quickly washed 6 times in 10mM Tris, (pH7.0), at 0°C, and dried in a stream of cool air. 1um FLN was added to the incubation media to determine nonspecific binding. Autoradiographs were produced by apposition of the sections to tritium-sensitive LKB film for 16 weeks.

The major finding is that in the monkey, specific FLN and BC binding sites have apparently identical distributions. Limbic structures such as the amygdala and the hippocampus, which have been implicated in the mediation of anxiety, were differentially labeled in identical fashion by the two ligands. The lateral, accessory, and cortical nuclei of the amygdala were heavily labeled by both. Also, there was intense labeling by both in the molecular layer of the hippocampus and in regions related to the hippocampus, such as the medial portion of the medial mamillary nucleus and the lateral dorsal and anterior nuclei of the thalamus. Futhermore, the subfields of the hippocampal formation could be distinguished on the basis of differential labeling densities of both ligands. By contrast, the locus coeruleus and the raphe nuclei, which have also been implicated in anxiety, could not be identified on the basis of labeling densities of either ligand.

The apparently identical patterns of the BDZ and BC binding suggest that these two agents are acting on the same, or closely linked, receptors. The location of especially dense labeling in certain limbic structures supplies additional evidence that these stuctures are involved in the modulation of anxiety.

165.3 IN VIVO [^{3}H]FLUNITRAZEPAM BINDING: CHARACTERIZATION AND CHANGES AFTER STRIATAL LESIONS. B.J. Ciliax, J.B. Penney, A.B. Young. Univ. of Michigan, Dept. of Neurology and Pharmacology, 1103 E. Huron, Ann Arbor, MI 48104.

In in vitro experiments, on brains from patients with Huntington's disease (HD) and in rats with striatal kainate lesions [^{3}H]flunitrazepam (FLU) binding to BDZ receptors is decreased in caudate-putamen (CP) and increased in globus pallidus (GP) and substantia nigra pars reticulata(SNr). We have investigated the feasibility of measuring these benzodiazepine (BDZ) receptor changes quantitatively in vivo.

Male Sprague-Dawley rats (200 g) were injected by IV bolus with 15 µCi [^{3}H]FLU (85 Ci/mmol) followed by constant infusion of the same substance at a rate of 15 µCi/min. Arterial blood samples were drawn at various times for measurement of plasma drug concentration. Animals were decapitated at 10, 30 or 60 min, the brains rapidly removed and portions of cortex, caudate-putamen, and cerebellum isolated by blunt dissection. Tissue radioactivity was solubilized and analyzed for tritium exhange and metabolism. Non-specific binding was determined in parallel animals pretreated with 5 mg/kg clonazepam. [^{3}H]FLU is rapidly taken up in brain and equilibrates by 30 min. Non-specific binding shows little regional variation and equilibrates almost immediately. There is a good correlation between non-specific brain binding and plasma drug concentrations.

Striatal-kainate lesioned rats were injected IV with 500 µCi [^{3}H]FLU for in vivo autoradiography. After decapitation at 10 min, brains were removed rapidly, frozen on dry ice, thin sectioned, mounted onto gelatin-coated slides, and apposed to Ultrofilm ^{3}H (LKB) for 2-10 wks. Resulting autoradiograms revealed clear regional and structural variations in binding consistent with in vitro BDZ-binding. Animals predosed with 5 mg/kg clonazepam showed virtually complete loss of structural distinction except at the lesion site where a blood brain barrier defect was seen. Quantitative densitometry showed significantly increased binding ($p<.05$, paired t-test) in GP and SNr on the lesioned side compared to the control.

This demonstrates that in vivo autoradiography of [^{3}H]-FLU binding can detect changes in receptor densities similar to those seen in in vitro studies. It should be possible to measure BDZ receptors quantitatively in human PET studies.

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165.4 LOCALIZATION OF MUSCARINIC CHOLINERGIC RECEPTOR CHANGES IN HIPPOCAMPUS AFTER SCOPOLAMINE TREATMENT OR FIMBRIA LESIONS. S.L. Carlson, M.W. Tayrien and R. Loy (SPON: D. Flood). Dept. of Anatomy, Univ. of Rochester, Rochester, NY 14642

Chronic scolpolamine (S) treatment upregulates hippocampal muscarinic receptors, but lesions of the septum or fimbria produce no consistent increase in 3H-QNB binding in rat hippocampus. This study addresses this apparent discrepancy by comparing regional binding of 3H-QNB using quantitative receptor autoradiography (AR) following fimbria lesions or S treatment. Four or 8 days following 10 mg/kg S/day, or 5 months following a unilateral knife cut of the fimbria, rats were decapitated and the brains rapidly frozen and sectioned at 6 um. Alternate coronal sections were collected for wipe scintillation spectrometric measurements and for AR analysis of total and nonspecific regional 3H-QNB binding. Slides were incubated in 2 nM 3H-QNB at 25°C for 90 min with or without 1 um atropine sulfate. Slides were exposed to LKB Ultrofilm for 2-5 weeks. Regional AR analysis was performed using a Nicon Magiscan. Receptor binding was studied in whole hippocampus as well as the following subregions: subiculum, CA1, CA3, dentate gyrus molecular layer (ML), and dentate hilus.(H). These areas were studied at 8 levels through the hippocampus.

In whole brain sections, S treatment increases specific 3H-QNB binding 15% at 4 days and 33% at 8 days relative to controls. The largest changes (21-31%) occur in the more anterior levels (dorsal hippocampus levels). Hippocampal binding increases 20% after 4 or 8 days of S treatment. In the 8 day brain, the largest increases are found in CA1, CA3 and H (23%, 28%, and 30% respectively). The fimbria lesions were complete and unilateral with the contralateral side serving as control. No change in whole hippocampal specific 3H-QNB binding is found immediately caudal to the lesion, although regionally CA3 binding is increased 30%. At a more caudal level, all subregions of the hippocampus show increased binding ipsilateral to the lesion. The largest change is in CA3 (66%), with the other subregions increasing 22-35%.

Using regional analysis, muscarinic upregulation of distinct hippocampal areas which has been obscured by analysis of whole hippocampus, may be detected. In controls, S treated and fimbria lesioned animals, the lowest specific 3H-QNB binding relative to the rest of hippocampus is found in CA3 and H at all brain levels. These regions also represent the areas of greatest upregulation in specific 3H-QNB binding compared to controls.

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165.5 AUTORADIOGRAPHY OF VIP RECEPTORS IN MAMMALIAN BRAIN M. McGrane* and T. Moody. Dept. Biochem., The George Washington University Medical School., Washington, D.C. 20037

VIP, a 28 amino acid peptide initially isolated from porcine intestine (Said, S. and Mutt, V., Science, 69: 1217, 1970), is biologically active in mammalian brain regions and excites cerebral cortex neurons. These actions may be mediated by endogenous VIP which has been detected in certain neurons in the cortex, hippocampus and hypothalamus. Upon neuronal depolarization these peptides are released where they may diffuse and activate VIP receptors. These receptors have been characterized using rat brain homogenate and ^{125}I-VIP. Here using in vitro autoradiographic techniques the discrete regional distribution of VIP receptors was investigated.

Initially, binding studies were conducted using 12 µm coronal slices of unfixed rat brain (Wolf, S. et al., Eur. J. Pharm., 87: 163, 1983). Forebrain slices bound ^{125}I-VIP with high affinity (Kd, 3nM). The ratio of specific to nonspecific binding was 3 to 1. Pharmacology studies indicated that the structurally related peptides PHI and secretin inhibited specific ^{125}I-VIP binding with IC_{50} values of 100 and 1000 nM respectively, whereas GIP had an IC_{50} of greater than 1 µM.

Autoradiographic studies were conducted using the method of Palacios et al. (Neurosci. Lett., 25: 101, 1981). Highest grain densities were present in the pineal gland, stratum griseum superficiale of the superior colliculus and the dentate gyrus. Moderate grain densities were present in the nucleus tractus solitarius, locus coeruleus, geniculate nucleus, mammilary nucleus, subiculum, medial and cortical nuclei of the amygdala, mediodorsal and anterior thalamic nuclei, cortex, striatum, nucleus accumbens, olfactory tubercle and olfactory bulb. Low grain densities were present in the cerebellum, inferior colliculus, hypothalamus, globus pallidus, and nucleus septi lateralis. Negligible grain densities were present in the corpus callosum and controls treated with 1 µM unlabeled VIP. The discrete regional distribution of ^{125}I-VIP binding sites suggest that VIP may function as an important regulatory peptide in certain brain loci.

165.6 DEMONSTRATION AND DISTRIBUTION OF KASSININ-LIKE MATERIAL (SUBSTANCE K) AND DISTRIBUTION OF IODINATED SUBSTANCE K BINDING SITES IN THE RAT CENTRAL NERVOUS SYSTEM. C.W. Shults, H. Yajima*, S. Buck, H. Gullner*, E. Burcher*, T.N.Chase and T.L. O'Donohue. Experimental Therapeutics Branch, NINCDS, Bethesda, MD 20205, Kyoto U., Kyoto, Japan, NHLBI, Bethesda, MD 20205, B.H.S. Deakin U., Australia.

To investigate whether there could be kassinin like material in the rat CNS, we generated polyclonal antisera against kassinin in rabbits. RIA with the antisera was sensitive and cross reactivity with other tachykinins, when compared to kassinin, was: substance K (also named neurokinin α, neuromedin L) 100%, eledoisin 25%, neurokinin β (also named neuromedin K) 10%, substance P 0.1%, physalaemin <0.1%. Peptides extracted from rat brain were chromatographed by reverse phase HPLC and the fractions assayed for kassinin like immunoreactivity. The major peak of kassinin like material eluted at a time different from that of synthetic kassinin, eledoisin, physalaemin, neurokinin β and substance P but coeluted with substance K (SK). Measurement of kassinin like material in brain regions indicated that kassinin like material has a distribution similar to that previously reported for substance P (Brownstein et al., Brain Res. 116:299, 1976). Measurement of both kassinin like material and SP in regions microdissected from rat brain again demonstrated similar distributions: region (SK,SP f mole/µg protein) ant. cing. cortex (0.24,0.12), n. accumbens (1.86,0.66), vent. caudate n. (1.03,0.56), dorsal caudate n. (0.54,0.39), globus pallidus (1.84,0.78), med. septal n. (0.72,0.68), lat. septal n. (1.36,0.81), bed n. of the stria terminalis (3.24,1.46), med. preoptic n. (4.68,2.67), ant. hypothalamus (2.31,1.39), paraventricular n. (2.71,1.59), habenula (2.04,1.51), med. amygdaloid n. (3.96,1.86), cent. amygdaloid n. (1.11,0.64), mamillary body (0.32,0.58), substantia nigra, reticulata (10.55,4.96), VTA (1.56,1.05), interpeduncular n. (8.07,3.79), central gray (5.18,1.68). The similar distribution for kassinin like material and SP in the rat CNS was not surprising since in the bovine striatum a gene coding for SP has also been shown to contain a sequence which codes for substance K (Nawa, H., et al., Nature 306:32, 1983).

In contrast to the similar distributions of SK and SP, the autoradiographic distributions of binding sites of SK and SP, which had been iodinated by the Bolton Hunter method, differ. Binding sites for ^{125}I-BH-SK were noted in the olfactory bulb, cortex, supra optic n., para ventricular n., certain amygdaloid n., hippocampus, medial habebula, interpeduncular n., n. of tractus solitarius, and dorsal horn of the spinal cord. The distribution of SP receptors has been reported (Shults, et al., Abstracts Soc. Neuroscience, 171, 1983; Quiron et al., Nature 303:714, 1983). The implications of distributions of peptides and binding sites will be discussed.

165.7 COMPUTER IMAGING OF DOPAMINE (D_2) AND SEROTONIN (S_2) RECEPTOR GRADIENTS IN [^{3}H]-SPIROPERIDOL AUTORADIOGRAPHS OF RAT BASAL GANGLIA OR NEOCORTEX. C. A. Altar†, H. Kim*, J. F. Marshall, Dept. Psychobiology, Univ. California, Irvine, CA 92717.

A computer-assisted image analyzer that converts autoradiographic gray values to a linear function of [^{3}H]-ligand concentration (Altar *et al, J. Neurosci. Meth.*, In Press) has enabled calculation of K_d and B_{max} values for [^{3}H]-spiroperidol binding to D_2 sites in caudate-putamen autoradiographs (Neve *et al, Brain Res.*, In Press). Measurements of [^{3}H]-spiroperidol binding to swabbed brain sections (Palacios *et al, Brain Res., 213*:277, 1981; Neve *et al., ibid*) precludes localization of gradients or quantification of D_2 or S_2 sites in discrete regions. The present study characterized the pharmacological specificity and distribution of [^{3}H]-spiroperidol binding sites in rat basal ganglia or neocortex autoradiographs.

In coronal sections (A 8.6 - 9.6), domperidone or (+)-butaclamol displaced 1.5 nM [^{3}H]-spiroperidol from caudate-putamen, n. accumbens, olfactory tubercle, claustrum, and dorsal layer 5A or medial layer 1 of cortex (IC_{50}'s = 2-80 nM). The S_2 antagonists methysergide or ketanserin displaced [^{3}H]-spiroperidol only from claustrum or cortex (IC_{50}'s = 2-14 nM). The D_2 agonist ADTN displaced the ligand from caudate-putamen (IC_{50} = 0.1 µM) but from no cortical region. The competition curve for ADTN displacement was markedly biphasic and right-shifted by 100 µM Gpp(NH)p. The D_2 antagonist (-)-sulpiride displaced [^{3}H]-spiroperidol from caudate-putamen, n. accumbens, and olfactory tubercle (IC_{50}'s = 0.14-0.7 µM) but, like ADTN, not from any cortical region. In more anterior sections (A 9.8 - 10.5), ketanserin or butaclamol, but neither ADTN nor sulpiride, displaced 25-58% of the ligand bound to medial or dorsomedial cingulate, perirhinal, or motor cortex.

In horizontal sections (D 4.5 - 5.9), a 5-fold rostral-to-caudal gradient of decreasing S_2 concentration was observed in cortical layer 1. An increasing rostral-to-caudal S_2 gradient and a quantitatively complementary 30% decreasing D_2 gradient was seen in the caudate-putamen.

We conclude that, in coronal or horizontal rat forebrain sections, [^{3}H]-spiroperidol labels D_2 sites (displaced with 10 µM (-)-sulpiride) and S_2 sites (displaced with 40 nM ketanserin) that are additive components of D_2 and S_2 sites displaced with 1 µM (+)-butaclamol. The rostral-to-caudal concentration gradients of D_2 and S_2 sites in the caudate-putamen, but not frontal cortex, correspond well with previously reported concentration gradients for dopamine and serotonin, respectively, within these forebrain regions.

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165.8 QUANTITATIVE AUTORADIOGRAPHY OF SEROTONIN-2 RECEPTORS IN RAT FOREBRAIN: COMPARISON OF SEX AND ESTROGEN EFFECTS. C. T. Fischette and B. Nock, Hoffmann-La Roche Inc., Nutley, N. J. 07110, and Rockefeller University, New York, N.Y. 10021.

The distribution and the effects of estrogen upon serotonin-2 receptors were examined in gonadectomized male and female rats using quantitative autoradiography. Rats of both sexes were gonadectomized for 1 week and injected subcutaneously with 10 µg estradiol benzoate or sesame oil at 0 and 24 hours. 48 hours after the last injection animals were decapitated, and the brains were frozen and sliced into 32µ sections. Serotonin-2 receptors were labelled with 1.0 nM 3H-Ketanserin (67Ci/mmol), while nonspecific binding was assessed by the addition of 1 µM methysergide. Specific binding accounted for 50% of total binding. Autoradiograms were generated by the apposition of tritium-sensitive ultrofilm to the labelled tissue sections, and analyzed by computer-assisted densitometry which incorporated the use of "tritiated standards" allowing the conversion of OD values to fmol/mg. All slides were stained with cresyl violet for histological verification. Areas that specifically bound 3H-Ketanserin to the greatest extent included prefrontal cortex, ventral portion of the anterior cingulate gyrus, and middle layers of cortex corresponding approximately to layers 3-4. Moderate amounts of specific binding were found in the dorsal portion of the anterior cingulate gyrus, outer and inner layers of the cortex (approximate layers 1-2,5-6), olfactory tubercle, primary olfactory cortex, ventral caudate-putamen and the dorsal raphe nucleus. Low levels of specific binding were found throughout the rest of the forebrain including the median raphe nucleus, globus pallidus, ventral pallidum/substantia innominata, and hippocampus. This pattern of distribution bears no resemblance to the distribution of serotonin-1 receptors. Binding of 3H-Ketanserin appeared to be similar in all treatment groups in the dorsal raphe nucleus. Comparison of other brain areas will be presented.

165.9 ESTROGEN INDUCTION OF PROGESTIN RECEPTORS IN MICRODISSECTED HYPOTHALAMIC AND LIMBIC NUCLEI OF THE FEMALE GUINEA PIG. J. Thornton, B. Nock*, B. McEwen, and H. Feder*. Institute of Animal Behavior, Rutgers University, Newark, N.J. 07102 and Rockefeller University, New York, N.Y. 10021.

Progesterone plays an important role in the control of sexual behavior and gonadotropin secretion in females of a number of species including the rat and guinea pig. Progesterone appears to exert much of its action through intracellular receptors located in the hypothalamus and preoptic area (HPO). The concentration of progestin receptors in these areas is regulated in part by estrogen. Treatment of ovariectomized rodents with estradiol causes an increase in progestin receptor concentration in HPO. In rats (J. Neurosci.,2:1446, 1982), the distribution of estradiol-inducible progestin receptors in HPO has been further localized using the microdissection technique of Palkovits (Brain Res.,59:499, 1973). The present study more precisely localizes where estradiol induces progestin receptors in female guinea pig HPO.

One week after ovariectomy, adult guinea pigs were given three daily injections of 20µg estradiol-17β benzoate or oil. Twenty-four hours later, females were anesthetized, perfused with 10% dimethyl sulphoxide and brains were removed and frozen. Coronal sections (300µm thick) were sliced using a cryostat. Cytosol progestin receptors were assayed in areas microdissected by the method of Palkovits. Assay procedures were similar to those previously described (J. Neurosci.,2:1446, 1982). Progestin receptors were labeled using 0.4nM of (^{3}H)R5020 (a synthetic progestin) ± 40nM unlabeled R5020.

Estradiol increased progestin receptor concentration more in some hypothalamic and limbic areas than in others. Greatest increases were in the arcuate-median eminence (by 8-9 fold) with lesser increases in the periventricular nucleus (5-6 fold), medial preoptic nucleus (5-6 fold) and ventromedial hypothalamic nucleus (3-4 fold). There was only a slight increase in the anterior hypothalamic nucleus. No induction of progestin receptors was seen in the medial amygdala. This distribution of estradiol-inducible progestin receptors is consistent with that seen in the female rat.

The more precise localization of estradiol-induced progestin receptors should facilitate future studies on the mechanism of progestin action and on the regulation of progestin receptors by neurotransmitters (Brain Res.,207: 371, 1981.

165.10 AUTORADIOGRAPHIC LOCALIZATION OF POLIOVIRUS BINDING IN HUMAN BRAIN AND SPINAL CORD USING COMPUTER-ASSISTED IMAGE ANALYSIS. R.H. Brown*, W.F. White, L. Regan*, M. Ogonowski*, D. Johnson*, H.L. Weiner*. Neurology Service, Massachusetts General Hospital, Boston, MA. 02114, and Department of Neuroscience, Children's Hospital, Boston, MA. 02115.

Autoradiographic techniques have been employed to localize receptors for a variety of ligands (eg neurotransmitters, neuropeptides, hormones); we have used autoradiography to investigate the distribution of binding sites for Type 1 (Mahoney strain) poliovirus in human neural tissue. Virus was purified to high titer, iodinated using lactoperoxidase or Bolton-Hunter techniques, and separated from free iodine and viral breakdown products by cesium chloride gradient centrifugation. Binding characteristics of the radiolabelled virus were determined using tissue preparations known to possess (HeLa cell membranes; human spinal cord homogenates; mouse-human hybrid KLEJ cells with human chromosome 19) or lack (L cell membranes, mouse spinal homogenates; KLEJ/P cells without chromosome 19) poliovirus binding activity. Autoradiographic techniques were then applied to thaw-mounted 10 micron cryostat sections of human spinal cord and cortex obtained at autopsy. The sections were incubated in phosphate buffered saline containing ^{125}-I poliovirus (10^6 infectious units per 100 ul) in the presence or absence of a 100-fold excess of unlabelled virus to determine total and nonspecific binding. After one hour (22°C) the sections were washed, rapidly dried and apposed to LKB 2208 Ultrofilm-H^3 which was developed after three weeks (Kodak D-19). The resulting autoradiograms were read in a scanning densitometer (Optronics P-1000); digitized densities were analyzed by a VAX 11/780 computer and the data was topographically displayed using a pseudo-color representation of binding density on a color raster monitor (Megatek 7255). Autoradiograms show specific binding of Type 1 poliovirus to human CNS tissue. In both spinal cord and cortex binding is most pronounced over grey matter. High levels of specific binding are seen over Rexed layers II and III of the spinal cord, regions which are rich in synaptic endings. Focal "hot spots" of binding activity were observed over the anterior horns of spinal cord.

165.11 **RECEPTOR AUTORADIOGRAPHY, GLUCOSE UTILIZATION, AND LIGHT REFRACTION IN KAINIC ACID-INDUCED NEUROTOXICITY.** L. Churchill, J.L. Jackson*, T.L. Pazdernik*, S.R. Nelson, and F.E. Samson, Dept. Anat., Pharmacol. & R.L. Smith Res. Ctr., Univ. Kansas Medical Center, Kansas City, KS 66103

Kainic acid-induced convulsions in rats produces pathological alterations in specific brain regions, mainly in the limbic system. Kainic acid-induced neurotoxicity was assessed at 72 h after injection of male Wistar rats with 12 mg/kg kainic acid, IP; convulsive activity was scored. Receptor autoradiography of [^{3}H]ligand binding to slide-mounted tissue sections revealed decrements in [^{3}H]quinuclidinyl benzilate (QNB) binding, but not [^{3}H]flunitrazepam (FLN) binding (Table 1) in rats with convulsive activity. Seventy-two hours after kainic acid-induced convulsions (Table 1), the [^{14}C]-2-deoxyglucose (2-DG) method revealed decrements in the functional activity of many brain regions. Kainic acid-induced toxicity resulted in differences in light refraction (REFR) which could be quantitated by computer-assisted densitometry utilizing a dark field source (Table 1). Hematoxylin and eosin staining verified that histological lesions occurred in frontal cortex, piriform cortex, hippocampal pyramidal cells, and lateral dorsal thalamus.

Table 1. Indicators of Kainic Acid-Induced Neurotoxicity

	% of control (mean ± S.E.M.)			
Brain Areas	QNB	FLN	2DG	REFR
Fr. Ctx.	81± 7*	97± 4	75±10*	132±19
Dors. Caud.	88± 8	97±10	81± 6*	109± 7
Pir. Ctx.	69±10*	92± 4	21± 3*	208±17*
Hip-CA1	54±17*	93± 6	54± 4*	143±17*
LD Thal.	70±9*	86±13	55± 5*	132±12*

Fr. Ctx=frontal cortex, Dors. Caud.=dorsolateral caudate, Pir. Ctx.=piriform cortex, Hip-CA1=Hippocampal body-CA1, LD Thal.=Lateral dorsal thalamus; *$P < 0.05$.

Receptor autoradiography, glucose utilization, and light refraction are useful indicators of neurotoxicity. Muscarinic receptors were reduced indicating that cholinoceptive cells are probably damaged during kainic acid-induced convulsions. This decrement of muscarinic receptors is not a general phenomena but only occurs in selective brain regions. In contrast, benzodiazepine receptors appear to be spared in kainic acid-induced brain lesions. Supported in part by U.S. Army DAMD17-83-C-3242.

165.12 TRANSMITTER CANDIDATES IN THE AREA POSTREMA: CELL CULTURE, BIOCHEMICAL AND IMMUNOCYTOCHEMICAL STUDIES. R.A. Leslie† and N.N. Osborne*. Nuffield Laboratory of Ophthalmology, Oxford University, Oxford OX2 6AW England.

High performance liquid chromatography with electrochemical detection revealed the presence of noradrenaline (NA), adrenaline (AD), serotonin (5HT) and dopamine (DA) in the bovine area postrema (AP). In addition, thin layer chromatography of dansylated derivatives of the same tissue showed GABA to be present. In order to localise some of these compounds in the bovine AP, immunocytochemical procedures were used. 5HT-like immunoreactivity was found in fibres and varicosities in basal and lateral regions of the AP, and immunoreactive varicose fibres were found on the ependymal surface. Fibres and varicosities exhibiting substance P-like immunoreactivity were localised in lateral regions of the nucleus. Cells containing dopamine-beta-hydroxylase-like immunoreactivity were found throughout the substance of the AP. The occurrence of cell profiles containing a particular substance in the AP does not necessarily mean that the substance has a functional role in the area. In order to gain some insight into this, preliminary binding studies were carried out with bovine AP membranes with the aim of establishing the presence or absence of receptors for some of these substances. Binding of the following tritiated ligands was found in membrane preparations (specific binding was determined by displacement with non-radioactive ligands indicated in parentheses): spiroperidol (haloperidol); flunitrazepam (diazepam); NA (NA, AD and phentolamine); DA (DA, NA, apomorphine and ADTN); and 5HT (5HT). Since cell culture may be a useful way to study the different properties of AP cell types and their development we have cultured cells from the dorsomedial medulla in the region of the AP of 1-5day old rabbits. Specific populations of cells were subsequently shown to take up tritiated 5HT, DA, GABA, glycine and D-aspartate. These studies are in agreement with earlier studies which indicated the presence of NA and AD in the AP, and confirm the presence of DA, 5HT and GABA in the nucleus. Similarly, the presence of binding sites for the benzodiazepines together with the occurrence of GABA and probably GABA uptake mechanisms in the AP suggest that this amino acid may be among the neurotransmitters that may mediate AP function. (†Present address: Department of Anatomy, Dalhousie University, Halifax, Canada; supported in part by the Medical Research Council of Canada).

165.13 COMPARISON OF ADENOSINE AND DOPAMINE RECEPTOR BINDING IN HUMAN CAUDATE NUCLEUS AND PUTAMEN. Charles G. New*, S. Jamal Mustafa*, Brian A. McMillen, (SPON. Wallace R. Wooles) Dept. of Pharamcology, School of Medicine, East Carolina University, Greenville, North Carolina 27834.

It is well known that dopamine neurotransmission has an important role in maintaining motor control by the extrapyramidal system. Recently, it has been reported that adenosine action in the striatum can influence extrapyramidal motor function. For example, adenosine agonists produce ataxia and sedation, which can be reversed by the xanthine adenosine antagonists. We have compared adenosine A1 receptor binding of ^{3}H-(L)-N^6-phenylisopropyladenosine (PIA) with D2 receptor binding of ^{3}H-spiperone by membranes prepared from human caudate nucleus and putamen obtained after autopsy. A large sample of globus pallidus was used in preliminary experiments to establish the characteristics of PIA binding. Scatchard analysis yielded Bmax = 1316 fmoles/mg prot and K_D = 8.7 nM. The rate constants for association (k_1) and dissociation (k_2) were 10^7 $M^{-1}min^{-1}$ and 0.03 min^{-1}, which yields k_2/k_1 = 3.0 nM. These results are in close harmony with the data reported for whole rat brain (1). The Table below shows preliminary results from right and left sides of 3 brains. The correlation between PIA and spiperone Bmax was $r = 0.497$ or $0.05 < P_{2,10} < 0.1$. These brains were from males ages 24, 36 and 37 yr who died from trauma without history of neurological or psychiatric disease. A better correlation may be obtained as a larger sample of different ages is added to the control data pool if A1 receptors decline with age as D2 receptors are claimed to do. The Table shows that the heavily dopamine innervated areas have about 4 times as many A1 adenosine receptors as D2 receptors. Whether changes in A1 receptors occurs in neurological diseases or aging remains to be demonstrated. (Supported by USPHS grant HL-27339).

	^{3}H-PIA		^{3}H-spiperone	
	Bmax	K_D	Bmax	K_D
L. Caudate	990	23.4	268	0.21
R. Caudate	712	25.4	221	0.20
L. Putamen	1046	33.7	281	0.20
R. Putamen	987	27.7	348	0.19

Bmax = fmoles/mg prot; K_D = nM; n =3.

1) Schwabe and Trost, N.-S. Arch. Pharmacol. 313,197, 1980.

165.14 IMMUNOCYTOCHEMICAL LOCALIZATION OF ADENOSINE CONTAINING NEURONS IN RAT BRAIN. K.M. Braas, A.C. Newby* and S.H. Snyder. Dept. of Neuroscience, Johns Hopkins University, School of Medicine, Baltimore, MD 21205; Dept. of Cardiology, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN, Wales.

Recent biochemical, pharmacological, electrophysiological, and behavioral studies have led to the postulation of the purine nucleoside adenosine as a neurotransmitter or neuromodulator. Regional distributions of adenosine, adenine nucleotides, adenosine receptors, and the enzymes involved in adenosine biosynthesis and degradation throughout the CNS have supported this concept. Direct measurements of adenosine levels in tissues have been limited by method sensitivity, specificity, and extensive sample preparation. While immunocytochemical techniques have been successfully employed to localize many neurotransmitters, direct anatomical localization of adenosine containing neurons required the production of specific, sensitive antisera not previously available. Rabbits were immunized with the adenosine derivative laevulinic acid ($O^{2'},3'$- adenosine-acetal), which preserved the purine and ribose rings, coupled to human serum albumin. Antibodies with high affinity and specificity for adenosine were demonstrated to be capable of detecting 1 pmol of adenosine by radioimmunoassay (Newby and Sala, Biochem. J., 208:603, 1982). More than 1000-21,000 fold concentrations of adenine nucleotides were required for displacement of adenosine binding to antisera. These antisera have now been utilized with immunocytochemical techniques to demonstrate the localizations of adenosine in rat brain sections. Rats were perfused with either 4%/% paraformaldehyde or 2.5%/% glutaraldehyde, the brains removed, immersed in the same fixative for 1 hr, and washed in buffered sucrose overnight at 4 C. Cryosections were stained with antisera using the PAP complex or avidin-biotin complex technique. Initial results indicate adenosine-like immunoreactivity in neuronal cell groups of discrete rat brain regions. Areas containing highest levels of immunoreactivity include the pyramidal cells of the hippocampus, the dentate gyrus, subnuclei of the thalamus, the lateral hypothalamus, and layers II and III of the cerebral cortex. These results correlate well with the regional distributions of adenosine previously measured by biochemical methods as well as adenosine receptor and biosynthetic enzyme distributions.

165.15 AUTORADIOGRAPHIC VISUALIZATION OF TRANSFERRIN RECEPTORS IN RAT BRAIN. M.R. Ruff*, J.M. Hill*, J.A. Danks*, and C.B. Pert (SPON: J. Trubatch). Section on Cellular Immunology, NIDR, NIH; Laboratory of Brain Evolution and Behavior, NIMH; and Section on Brain Biochemistry, NIMH, Bethesda, MD 20205.

Iron has a specific pattern of distribution in the brain (1) and functions not only in oxidative metabolism but also in the synthesis, degradation and binding of neurotransmitters. Transferrin, an iron-binding serum protein, delivers iron to cells by binding to specific receptors on cell surfaces. In addition to iron transport, transferrin may play a further role in the growth and development of the brain (2) since transferrin is known to be an essential growth factor for many tumor and other rapidly growing cell lines (3,4).

The purpose of the present study is to determine if transferrin receptors can be localized in the rat brain and to compare the pattern of distribution of transferrin receptors with that of iron.

Slide mounted sections of rat brain were preincubated 5 min in citric phosphate buffer, pH 4.8 and rinsed 15 min in 0.1 M PBS in pH 7.4 containing protease inhibitors. The sections were incubated for 3 hr at 37°C in the pH 7.4 buffer either with ^{125}I transferrin or with ^{125}I transferrin plus excess unlabelled transferrin (10^{-6} M). After incubation the sections were used to measure specifically bound transferrin by scintillation counting or to generate autoradiograms.

We have found up to 85% specific binding of labelled transferrin on brain sections and a strikingly discrete pattern of distribution of transferrin receptors is emerging. In preliminary studies the highest levels of transferrin binding are in the dentate gyrus of the hippocampal formation followed by the cerebral cortex and cerebellar cortex. Although further work is needed to determine the total pattern of distribution, the pattern thus far observed differs from that of iron. Brain iron occurs in highest concentrations in such areas as the circumventricular organs, ventral pallidum, globus pallidus, and the substantia nigra. The differences in the pattern of distribution of transferrin and iron may be a reflection of transferrin functions in the CNS beyond that of iron transport.

(1) Hill, J.M. and Switzer, R.C., Neurosci. 11:595-603 (1984); (2) Toran-Allerand, C.D., Nature 286:733-735 (1980); (3) Bishr Omary, M., Trowbridge, I.S., and Minowada, J., Nature 286:888 (1980); (4) Trowbridge, I.S. and Lopez, F., Proc. Natl. Acad. Sci, USA 79:1175 (1982).

165.16 MUSCARINIC AND NICOTINIC ACETYLCHOLINE BINDING SITES AND ACETYLCHOLINESTERASE: DISTRIBUTION IN MAMMALIAN PRIMARY VISUAL SYSTEMS AND EFFECTS OF ENUCLEATION. J.L. Fuchs, H.D. Schwark and W.T. Greenough Dept. Psychology and Neural & Behavioral Biology Program, University of Illinois, Champaign, IL 61820

The distributions of acetylcholine receptors and acetylcholinesterase (AChE) activity were investigated in carnivores (cat, dog), a lagomorph (rabbit) and rodents (squirrel, gerbil, hamster, vole, rat, mouse). Cryostat sections of unperfused tissue from the lateral geniculate nucleus (LGN), superior colliculus (SC) and visual cortex were labeled in vitro with [^{125}I]alpha-bungarotoxin (Btx) and [^{3}H]QNB. Putative nicotinic and muscarinic binding sites were identified and quantified by tritium-sensitive film autoradiography of Btx and QNB, respectively.

The distribution of QNB was quite uniform across species, whereas Btx showed considerable variation. QNB binding sites were abundant in the superficial and deep layers of primary visual cortex. In all species the pattern of Btx binding differed from that of QNB. The laminar distributions of Btx were similar in closely related species.

In the superior colliculus the highest levels of QNB and Btx binding were in the superficial gray layer, followed by the deeper gray layers. In the carnivores Btx labeling was light and lamination was indistinct in the SC and LGNd. Within species, Btx binding was denser in the LGNv than in the LGNd.

Patterns of AChE activity resembled a combination of QNB and Btx distributions in the SC and LGN. In the cortex, laminar AChE activity did not correspond well with the distribution of binding sites. In contrast to QNB and Btx binding, AChE activity was much lower in the cortex than in most subcortical regions.

Monocular and binocular enucleation effects were investigated in rats at ½, 1, 2, 4, 6, 10 and 19 days postoperative. Btx binding was decreased in the LGNd and SC one day after enucleation. Btx binding continued to decline to 2-4 days, and remained decreased 19 days after enucleation. The rapid change following enucleation indicates that Btx binding sites may be located on optic nerve terminals.

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165.17 AUTORADIOGRAPHIC LOCALIZATION OF [^{3}H]NICOTINE BINDING SITES IN THE RAT BRAIN. E. D. London[1], S. B. Waller[2] and J. K. Wamsley[3]. Neurochemistry Section, NIDA Addiction Res. Ctr.[1], and Lab. of Neurosci., NIA Gerontology Res. Ctr.[2], Baltimore MD 21224; Department of Psychiatry, Univ.of Utah Medical Ctr., Salt Lake City, UT 84132[3].

In cerebral membrane preparations, [^{3}H]nicotine ([^{3}H]N) binds specifically to two sites, of which the high affinity site has characteristics of a cholinergic receptor (Romano, C. and Goldstein, A., Science 210: 647, 1980). In this study, in vitro autoradiography was used to visualize the distribution of cerebral high affinity [^{3}H]N binding sites by light microscopy. Brains from male Sprague-Dawley rats (250-300 g) were frozen onto microtome chucks, and coronal sections were prepared for autoradiography as previously described (Wamsley, J. K. and Palacios, J. M., Handbook of Neurohemistry, Vol. II, A. Lajtha, ed. Plenum, N.Y., 1983). Slide-mounted sections (10 µm) were preincubated for 15 min in buffer (50 mM Hepes, 1.2 mM $MgSO_4$, 118 mM NaCl, 25 mM $CaCl_2$, and 4.8 mM KCl, pH 7.4) at 25° C. The sections then were labelled with 50 nM [^{3}H]N at 25° for 90 min. Nonspecific binding was determined in the presence of 1 mM unlabelled L-nicotine, 1 mM D-tubocurarine, or 1 mM hexamethonium. Incubations were stopped by rinsing the sections in buffer and then in distilled water. Sections were dried under cold dry air. Autoradiograms were prepared by exposure to LKB-Ultrofilm for 30 days at 4° C. Under the same incubation, [^{3}H]N bound to two sites in membrane preparations from rat brain sonicates, with K_D's of 50 nM and 527 nM, respectively. Therefore, specific binding in brain sections incubated with 50 nM [^{3}H]N mainly represented labelling of high affinity binding sites.

[^{3}H]N binding was heterogeneously distributed, with dense labelling in the interpeduncular nucleus, thalamic nuclei, medial habenula, ventral and dorsal nuclei of the lateral geniculate body, medial geniculate body, layer IV of the cerebral cortex, and superficial layer of the superior colliculus. Specific binding in the dentate gyrus was localized to the molecular layer. Sparse labelling and a paucity of specific binding occurred in the periaqueductal grey matter and the hypothalamus, respectively.

These findings demonstrate that [^{3}H]N can be used in light microscopic studies of cerebral high affinity [^{3}H]N binding sites, which appear to be nicotinic cholinergic receptors (Romano and Goldstein, 1980).

165.18 KINETIC ANALYSIS OF IN VIVO MUSCARINIC RECEPTOR BINDING. K.A. Frey, R.D. Hichwa*, R.L.E. Ehrenkaufer* and B.W. Agranoff. Neuroscience Laboratory and Cyclotron/PET Facility, University of Michigan, Ann Arbor, MI 48109.

Recent work in this laboratory has considered the in vivo use of radioligands for determination of regional neurotransmitter receptor densities by means of a tracer equilibrium approach. In the present study, a compartmental model describing the distribution of tracer between free, nonspecifically and specifically bound pools in rat brain, which includes exchange between tissue and intravascular pools of free tracer is presented and validated using [^{3}H]scopolamine binding to the muscarinic receptor. Rats were injected intravenously with a bolus of [^{3}H]scopolamine (500 µCi/kg, specific activity 80-90 Ci/mmol). At various intervals following injection, samples of arterial plasma and various brain regions were analyzed for scopolamine content. Composite time-activity curves for arterial plasma and brain regions were derived from a total of 18 animals studied between 1 and 180 min post-injection. The data were analyzed according to the model, using a nonlinear least-squares curve fitting procedure implemented on a PDP-11/23 computer. Parameters proportional to the rate of ligand exchange across the blood-brain barrier, nonspecific binding and the density of free receptors were estimated in cerebral cortex, striatum, hippocampus, olfactory bulb, inferior colliculus and cerebellum. The capillary permeability-surface area product, based on estimates of tracer exchange between blood and brain and literature reports of cerebral blood flow to these regions averaged 0.12 min^{-1}. The regional densities of free binding sites, estimated from the rates of ligand association, varied 5-fold between cortex and cerebellum. These results agree in general with previous in vitro and equilibrium in vivo studies at saturating ligand concentration. It is proposed that regional deviations between in vitro ligand binding capacity and the present estimates of free receptors in vivo by tracer kinetic analysis reflect receptor occupancy by endogenous neurotransmitter. It is anticipated that this in vivo experimental approach will permit quantitative imaging of regional muscarinic receptor availability in humans, using [^{11}C]scopolamine and positron emission tomography. (Supported by NIH grant NS 15655.)

165.19 INFLUENCE OF CORTICOSTERONE ON MUSCARINIC RECEPTOR SUBCLASSES IN THE CHICK EMBRYO BRAIN. F. Gremo*, A. M. Marchisio* and A. Vernadakis* (SPON: M. Reite). Institute of Human Anatomy, School of Medicine, Cagliari, Italy, and Departments of Psychiatry and Pharmacology, University of Colorado School of Medicine, Denver, Colorado 80262.

The present study was performed on retinas of chick embryos treated at day 8 of incubation with 0.02 µg of corticosterone intracerebrally. We have previously shown with the use of ^{3}H-quinuclidinylbenzilate (^{3}H-QNB) that such a treatment induced the appearance of two muscarinic binding sites in the treated retinas, whereas only one was detectable in the controls. In the present study we investigated muscarinic cholinergic receptor subclasses with agonist and antagonist binding. Agonist carbamylcholine and acetylcholine binding revealed two subpopulations of receptors, a high and a low affinity, in both treated and control retinas. However, in the hormone-treated retinas, the two subpopulations significantly differed from the controls in their affinity and in their relative percentage among the total receptor population. Moreover, using pirenzepine, an antagonist known to be able to distinguish between muscarinic cholinergic subclasses, two subpopulations were present in the hormone-treated retinas but to a single one in the controls. Pirenzepine binding in retinas from intact embryos of 7,9 and 11 days of incubation revealed one receptor subpopulation. We suggest that the hormone can either induce the appearance of a new subclass of muscarinic cholinergic receptors or favor the maturation of a population of retinal cells having these receptors.

We have advanced the hypothesis that corticosterone interferes with the maturation of a particular population of retinal neurons. However, light and electron microscopic studies did not show morphological differences between treated retinas and controls. It could be argued that the hormone unmasks a pre-existing subpopulation of muscarinic receptors, which, due to the low number of sites, is not detectable in normal conditions. However, the data on retinas of embryos at earlier and later stages of development do not support this proposal. Also the possibility that the hormone could delay the maturation of a retinal population contrasts with the results on younger embryos where the affinity for pirenzepine was similar and no evidence of two subpopulations was revealed. Thus, we speculate that the hormone affects the target cell, inducing a change in muscarinic cholinergic binding, by perhaps affecting the genome. (Partially supported by a research grant from NATO (148.80) and a Developmental Psychobiology Research Group Endowment Fund.)

165.20 INSULIN BINDING IN RAT BRAIN: QUANTITATIVE RECEPTOR AUTORADIOGRAPHY BY COMPUTER DIGITAL IMAGE ANALYSIS. E. Corp,* B. Brewitt,* D. Figlewicz,* D. Porte, Jr.,* D. Dorsa, D. Baskin, Depts. Medicine, Pharmacology, Psychology, and Biological Structure, Univ. of Washington, and VA Medical Center, Seattle, WA 98108

Recent evidence has shown that the rat brain contains insulin binding sites, but their microanatomical location is largely unknown. Therefore, we labeled frozen-dried sections of rat brain with [125]I-monoiodinated porcine insulin (0.05-0.1 nM; SA=300 µCi/µg) and determined the location of binding with LKB Ultrofilm (3-5 days' exposure). The results showed specific binding (radioactivity displaced by 1 µM unlabeled porcine insulin) in the olfactory bulbs (OB) external plexiform layer (EPL) cingulate cortex, suprachiasmatic nucleus, medial preoptic area, and choroid plexus of the lateral ventricles and fourth ventricle. Concentrations of labeled insulin bound to these regions on the slices was determined from measurements of the gray level of the corresponding autoradiographic image on LKB film with transmitted light (using a CCD camera and macro lens, A/D converter, and microcomputer), and from a standard curve of gray level/pixel vs fmols bound/mm^2, which was based on [125]I-insulin binding to rat liver slices. In the presence of 0.05 nM labeled insulin, specific binding (SB) and nonspecific binding (NSB) of the labeled insulin in fmol/mm^2 was:

Region	SB	NSB
Choroid plexus		
Fourth ventricle	2.5×10^{-2}	7.2×10^{-3}
Lateral ventricle	1.8×10^{-2}	2.8×10^{-3}
Liver	1.3×10^{-2}	7.2×10^{-3}
Medial preoptic area	8.3×10^{-3}	6.0×10^{-3}
Cingulate cortex	6.8×10^{-3}	6.0×10^{-3}
Suprachiasmatic nucleus	5.4×10^{-3}	6.0×10^{-3}
Arcuate nucleus	5.4×10^{-3}	6.0×10^{-3}

Displacement of insulin from the EPL of OB was determined by adding 0.01-1000 nM unlabeled porcine insulin to 0.1 nM labeled insulin. Specific binding to the EPL was 1.4×10^{-2} fmol/mm^2. LIGAND and EBDA computer programs showed that the high affinity binding site had a Kd = 1.8×10^{-8} M and a Bmax = 4×10^{-10} mol/mm^2. Therefore, the external plexiform layer of the rat olfactory bulb has binding sites with some pharmacological properties of insulin receptors. The high specific binding of insulin in the choroid plexus suggests that the choroid plexus may be a major site of insulin transport into the brain.

165.21 **Distribution of NMDA and quisqualate receptors in rat brain as determined by ^{3}H-D-AP5 and ^{3}H-AMPA autoradiography.** D.T. Monaghan, D. Yao*, H.J.Olverman*, J.C. Watkins* and C.W.Cotman, Dept. of Psychobiology, Univ. of Cal., Irvine CA. USA, and Dept. of Pharmacology, Univ. of Bristol, Bristol, England.

The amino acid L-glutamate is thought to be a major excitatory neurotransmitter whose action is mediated by at least 3 pharmacologically distinct receptors which are identified by their selective interaction with kainate, N-methyl-D-aspartate (NMDA), or quisqualate (QA). In order to determine the anatomical distributions of the NMDA and QA receptor classes, we have studied the binding sites for ^{3}H-D-2-amino-5-phosphonopentanoate (^{3}H-D-AP5, a selective NMDA antagonist) and ^{3}H-α-amino-3-hydroxy-5-methylisoxazole-4-propionate (^{3}H-AMPA, a QA agonist) using computer-assisted quantitative autoradiography.

Tissue sections were prepared as previously described (Monaghan et al. 1983, Nature 306: 176) and incubated with either 56nM ^{3}H-D-AP5 (custom labelled by Amersham, England, 27.3 Ci/mmole) for 20 min. at 22°C.,or 50nM ^{3}H-AMPA (27.5 Ci/mmole, New England Nuclear, Boston MA.) for 30 min. at 30°C.

^{3}H-D-AP5 and ^{3}H-AMPA each exhibit a Kd = 0.5 µM, measured in CA1 of dorsal hippocampus, but have distinct pharmacological profiles. ^{3}H-D-AP5 is potently displaced (>50% displacement) by 10 µM concentrations of NMDA, D-AP5, L-glutamate, and L-aspartate, whereas kainate, QA, AMPA, and L-2-amino-4-phosphonobutyrate (L-APB) were not potent displacers (<50%). ^{3}H-AMPA binding sites are readily displaced by QA and L-glutamate with Ki values of 0.1 and 0.7 µM, respectively. Kainate, L-serine-o-sulfate, and L-homocysteate were of intermediate potency (5 µM<IC_{50}<100 µM). D-aspartate, L-aspartate, NMDA, D-AP5, D-α-aminoadipate, L-APB displaced less than 20% of the binding at 100 µM concentrations.

Both sites exhibit their highest density in the hippocampus and their differing distributions within this structure are indistinguishable from those which we have reported for D-AP5 and AMPA- displacable ^{3}H-L-glutamate binding sites. ^{3}H-D-AP5 sites are enriched in s. radiatum, s. oriens, and inner dentate molecular layer; low levels are found in s. lucidum and s. lac.-mol. ^{3}H-AMPA sites are enriched especially in the pyramidal cell layer of hippocampus, dorsal lateral septum, and outer layers of neocortex. Low levels are found in diencephalon and brain stem. Together with our previous studies of ^{3}H-L-glutamate and ^{3}H-kainic acid binding site autoradiography, these data confirm the existence of three anatomically and pharmacologically distinct binding sites for excitatory amino acids which correspond to the three classes of excitatory amino acid receptors identified by electrophysiological/pharmacological techniques. Work supported by grant DAMD 17-83-C-3189 and by the Medical Research Council (U.K.).

165.22 RECEPTOR AUTORADIOGRAPHY: COPING WITH REGIONAL DIFFERENCES IN AUTORADIOGRAPHIC EFFICIENCY WITH TRITIUM. N.R. Taylor*, J.R. Unnerstall, R.D. Mashal*, E.B. De Souza and M.J. Kuhar (SPON: K. Dismukes). Dept. Neurosci., Johns Hopkins Univ. Sch/Med., Balto., MD 21205.

Autoradiographic studies with tritium in brain have revealed differences in efficiency between grey and white matter (Brain Res. 223: 59-67, 1981; J. Cereb. Blood Flow Metab. 3: s77-s78, 1983; Soc. Neurosci. 9: Abs#99.11, 1983). Our studies confirm these differences and suggest a practical strategy to correct for these variations.

Slide-mounted tissue sections of rat, bovine and human brain were soaked in varying concentrations of ^{3}H, ^{14}C and ^{125}I labeled amino acids. Sections were selected so that both white matter and grey matter areas were present. Usually we used bovine tissue sections containing both caudate nucleus and internal capsule as a source of grey and white matter respectively. Autoradiograms were generated with ^{3}H-sensitive film (Ultrofilm).

With ^{3}H, the optical density (OD) over the internal capsule (white matter areas) was 45% of that over caudate (grey matter areas); the radioactivity content of both regions was found to be the same after scraping tissue from the slides and measuring radioactivity. This confirms the significant difference in efficiency of tritium in producing autoradiographic grains in grey and white matter over caudate. This large difference was not found with ^{14}C or ^{125}I. The reasons for the tritium effect are 1) the proprtionately greater dry mass in white matter sections of equal thickness, and 2) the comparatively lower energy of beta-rays from tritium. Therefore, grain density or OD with ^{3}H is a function of both radioactivity concentration and tissue density.

To cope with this variation, deviations from uniform OD were measured in various brain regions and correction factors were calculated. For example, in the rat the ventral lateral thalamus, the cingulate cortex white matter and the corpus callosum were quenched 28, 40 and 45% respectively, compared to rat striatum. This procedure allows us to quantitate autoradiograms with tritium especially when comparing regional receptor densities. (Supported by USPHS MH25951, MH00053, DA00266 and a McKnight Foundation grant).

165.23 RECEPTOR AUTORADIOGRAPHY: ANALYSIS USING A PC-BASED IMAGING SYSTEM. M.J. Kuhar, P.J. Whitehouse, J.R. Unnerstall, and H. Loats*. Dept. Neuroscience, Johns Hopkins Univ. Sch/Med, Balto., MD 21205 and Loats Assoc., Inc., Westminster, MD 21157.

Receptor autoradiography is a powerful approach that combines sensitivity of measurement and a high degree of anatomical resolution in studying receptors. Several groups have successfully applied computerized image analysis systems to analyzing receptor maps. This report describes such a system. The objective was to reduce the time and effort associated with manual densitometry, and also improve discrimination by means of advanced image analysis techniques.

The system includes a DAGE 68 video camera with a NEWVICON tube, an IBM PC with 512K memory and double disk drive, an Epson FX 80 printer, 2 Sony Profeel monitors, an expansion chassis with selected boards and software, and other accessories. The system can be used to analyze positive or negative film images. Autoradiographic standards can be digitized and stored for use in quantitative analyses of autoradiographic images where optical densities need to be converted to concentrations of radioactivity or bound ligand. Editing functions are available to correct for background nonuniformities in the image lighting, thresholding ranges of optical densities, eliminating background, reducing "noise" in the digitized image and artifact removal. The video system provides accurate radiometric resolution of 256 grey levels and spatial resolution down to 20 microns using macro lenses. The user is able to select the color-coding of 15 density levels for the 256 x 256 pixel display. Statistics are provided. The format is menu driven and user friendly.

A strength of this system is that the NEWVICON tube responds linearly to light intensity. Thus, the digitized data for analysis will be a valid representation of the real data since no transformation of the data is made prior to the analysis.

The system is easy to use, allows rapid quantitation of glucose utilization and blood flow maps as well as receptor maps. Its performance compares to that of several other more expensive systems. The system significantly increases productivity and mapping resolution and discrimination. It can be used for other image analysis problems as well. The PC can also be used independently (supported by USPHS grants MH25951, DA00266, MH00053, and a McKnight Foundation grant).

165.24 AUTORADIOGRAPHICAL LOCALIZATION OF ^{3}H-DEXTROMETHORPHAN BINDING SITES IN BRAIN. D.C. Perry[1], G.L. Craviso[3], J.M. Musacchio[3] and S.H. Snyder[2]. [1]Dept. of Pharmacology, George Washington Medical Center, Washington, D.C. 20037 and [2]Dept. of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205 and [3]Dept. of Pharmacology, N.Y.U. Med. School, New York.

Dextromethorphan (DM) is a widely used non-narcotic antitussive; Craviso and Musacchio (Mol. Pharmacol., 23:619-640, 1983) recently reported a specific high-affinity binding site for ^{3}H-DM in guinea pig brain homogenates. A variety of drugs, including many different non-narcotic antitussives, exhibited high affinity to this site, but opiates such as codeine did not. We have utilized in vitro autoradiogrphic techniques to visualize ^{3}H-DM binding sites in sections of rat and guinea pig brain. Serial 10 um sections were incubated with ^{3}H-DM for 60 min at 4°C, either alone or with competing drug. After a 5 min wash, sections were dried and apposed to LKB Ultrofilm or emulsion-coated coverslips; some sections were wiped off and radioactivity counted directly. 10 nM ^{3}H-DM yelded binding of 2000 cpm per section; this was reduced to 900-110 cpm/section in the presence of 10^{-5} M fluphenazine or DM. Displacement curves with DM were shallow, with a Hill slope of 0.7 and a K_i of 15 nM. Significant displacement was seen with the non-narcotic antitussives caramiphen, dimethoxanate and carbetapentane, but not with noscapine or codeine (10^{-6} M). Binding sites were unevenly distributed, with highest labeling in areas of the hindbrain and cerebillum, moderate labeling in the stratum, hippocampus and cortex (higher in laminae III and IV), and low to moderate labeling in the thalamus, hypothalamus and midbrain. This distribution is similar to that seen in homogenates. Of particular interest are several areas of dense labeling around the floor of the fourth ventricle., including the pontine central grey region and the dorsal tegmental, hypoglossal and trigeminal nuclei. These areas may play an important role in the cough reflex, and thus the labeling by ^{3}H-DM could represent the anatomical localization of the "cough center" where DM acts to suppress coughing.

165.25 PHARMACOLOGY OF TAURINE RESPONSES OF PRIMARY AFFERENTS IN FROG SPINAL CORD. A.L. Padjen, H.M. Hassessian* and G.M. Mitsoglou*. Dept. of Pharmacology and Therapeutics, McGill University, Quebec, Canada H3G 1Y6.

Although taurine is present in the vertebrate nervous system, existence of a separate taurine receptor is less certain. We are reporting further studies on pharmacology of taurine agonists and TAG (AMBD), a compound reported to have a selective action against taurine (Yarborough et al., 1981, JPET 219, 604; Padjen et al., Neurosci. Abs. 1983).

Experiments were done on isolated hemisected frog spinal cords and dorsal root ganglia (DRG) continuously superfused with Ringer solution at 12°C. Amino acid evoked responses from terminal regions of primary afferents (DRT: indirect responses blocked by tetrodotoxin or Mn) and from DRG were recorded by means of the sucrose gap technique.

Taurine (0.1 to 2 mM) depolarized DRT but was, like glycine, essentially without effect on DRG. Distribution of responses to hypotaurine, a precursor of taurine, were just the opposite: its depolarization of DRG was five times larger than at DRT (at 1 mM). Beta-alanine was equipotent in depolarizing both sites. TAG (0.25 mM) antagonized 60-90% responses to 0.5 mM taurine (on DRT) and 40-60% responses to hypotaurine and beta-alanine without affecting responses to GABA and glycine (on DRT and DRG). Hypotaurine, as well as other responses (except those to glycine) were antagonized by bicucculine.

These results suggest that depolarizing responses to taurine and analogs on primary afferents, unlike hyperpolarizing responses on from motoneurons, are mediated by pharmacologically distinct receptor(s), differentially distributed between the terminals and somata.

Supported by MRC and ABMRF.

165.26 POSITRON EMISSION TOMOGRAPHY (PET) : IN VIVO RECEPTOR STUDIES IN BABOONS. Ph. Hantraye*, M. Maziere*, B. Maziere*, B. Guibert*, R. Guillon*, C. Loc'h*, D. Comar* and R. Naquet* (SPON : H. W. Magoun). Lab. de Physiol. nerv., CNRS, F 91190 Gif-sur-Yvette and Dept. de Biol., CEA, Service Hospitalier Frédéric-Joliot, Hôpital d'Orsay, F 91406 Orsay.

Using two specific radioligands, respectively Ro 15 1788 11 C (Ro-11C) and bromospiperone-76 Br (BSP-76 Br), benzodiazepine (BZD) and dopamine (DA) binding sites have been studied in live baboons using PET. This technique provides non invasive quantitative autoradiographic measurements. Serial images show the kinetics and the regional distribution of a previously I.V. injected radioligand. Saturability, specificity and stereospecificity of the binding are verified "in vivo" by administration of cold drugs (agonist or antagonist). After administration of 10 mCi (1nM/kg) Ro-11C (t 1/2 : 20.4 min.), the tracer is mainly localized in regions known to be rich in BZD receptors (cerebellum and neocortex). The mean maximal brain uptake in cerebellum is about 5.10^{-2} % of the injected dose/ml tissue. The localization of Ro-11C remains almost constant during the time of the experiment (80 min.). During displacement experiments (when the cold drug is injected 20 min. after Ro-11C) or in saturation experiments (coinjection of cold Ro with the labelled compound), a dose-dependent decrease of the cerebral radioactivity is observed in both cases. BSP-76 Br (3 mCi-0.2 nM/kg), a neuroleptic dopaminergic antagonist, accumulates preferentially in the striatal region where the maximal uptake is observed 1.5 hour after injection. Due to the clearance of the non-specifically bound fraction of BSP-76 Br in the cerebellum, the striatum-cerebellum ratio at 4.5 hour post injection is 2.2. The regional distribution of the cerebral radioactivity at 4.5 hour correlates very well with the anatomical localization of the DA receptors in brain. Saturation experiments with increasing concentrations of cold spiperone (0.25 and 1 mg/kg) leads to a dose-related diminution of the striatal uptake. In the same way, the binding of BSP-76Br is displaced with 1 mg/kg of cold spiperone I.V. injected.

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We thank Hoffmann-La Roche and Janssen Laboratories for kindly provinding drugs.

166.1 EFFECT OF ACTIVATED RECEPTOR DENSITY ON DESENSITIZATION. A. Rivera* and J. del Castillo* (SPON: C. Zuazaga). Lab. of Neurobiol., MSC, UPR, 201 Blvd. del Valle, Old San Juan, PR 00901

Prolonged application of an agonist to the endplate receptors results in a progressively decaying response, a process termed desensitization. Studies of this phenomena have mainly relied on measurements of the rate of decay of the induced depolarization or agonist current. The desensitization onset rates observed have varied depending on the method of agonist application: fast onsets are observed with ionophoresis and slower onsets are seen with bath application. More recently, both fast and slow components have been observed during rapid perfusion of agonists, and it has been suggested that desensitization may proceed with two different time constants. We have re-examined this problem using automatically controlled ionophoretic agonist application producing constant depolarizations of small amplitude (5-15 mV) (del Castillo, Specht & Auerbach, J. Neurosci. Res. 8: 35, 1982). Specifically, we have compared the desensitization rates obtained for equal sustained depolarizations with the ionophoretic pipette placed at different distances from the endplate region of frog sartorius muscle. At close range (risetimes of agonist test pulses 1-2 ms; sensitivities up to 3000 mV/nC) the onset rates of desensitization are up to 6X faster than those measured when the pipette is slightly withdrawn (risetimes of test pulses 5-20 ms; apparent sensitivities down to 400 mV/nC). Since a sustained response implies that the average number of active receptors also remains constant, we suggest that the different rates of desensitization observed are due to different densities of activated receptors within the total receptor population. This density would increase with close range agonist application. As higher densities would be expected to increase the frequency of reactivation of any given receptor, the faster onset rates observed at close range suggest that a receptor enters the desensitized state only after undergoing rapid reactivations. This could possibly account for the varying onset rates recorded with different methods of agonist application.

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166.2 KINETICS OF TORPEDO CALIFORNICA ACETYLCHOLINE RECEPTOR DESENSITIZATION IN RECONSTITUTED MEMBRANES. M.G. McNamee and C.A. Richardson*. (SPON: G. Hess). Dept. of Biochem. and Biophys., Univ. of Calif., Davis, CA 95616.

The rates of activation and inactivation (desensitization) of carbamylcholine-induced ion permeability have been measured in reconstituted membranes containing purified Torpedo californica acetylcholine receptor using rapid kinetics techniques with millisecond time resolution.

Acetylcholine receptor was purified by affinity chromatography and incorporated into Asolectin vesicles at a lipid:protein weight ratio of 30:1 using a cholate dialysis procedure. The agonist-stimulated influx of $^{86}Rb^+$ into the vesicles was measured using a quench-flow technique with d-tubocurarine as the quenching agent. Influx times could be varied from 15 msec to minutes. Vesicle-entrapped cations were separated from free cations by ion exchange chromatography. Inactivation was measured by first pre-incubating the vesicles with agonist in the quench flow machine and then adding $^{86}Rb^+$ and agonist and allowing influx to proceed for a fixed time before quenching.

Inactivation proceeded in two stages consisting of a fast phase ($t_{1/2}$=300 msec) and a slow phase ($t_{1/2}$=7 sec). The carbamylcholine concentration dependence of the fast phase suggested the existence of an agonist-binding site separate from the two sites that control activation. Other agonists, such as acetylcholine and suberyldicholine, and selective chemical modifications, such as disulfide reduction and alkylation, are being used to probe the relationships among the different classes of ligand binding sites. In addition, the relationships among agonist binding sites and anesthetic sites are being examined. A kinetic scheme consistent with the activation and inactivation kinetics data will be presented. (Supported by USPHS grant No. 13050).

166.3 WITHDRAWN

DRUG EFFECTS ON RECEPTORS

167.1 AMANTADINE PREVENTS DOPAMINE RECEPTOR SUPERSENSITIVITY IN RATS. J.T. Slevin, D.L. Sparks* and J. Fain* (SPON: N.H. Bass). Veterans Administration Medical Center, Department of Neurology, Lexington, KY 40536

Allen has demonstrated that amantadine HCl given to rats concurrently with haloperidol will prevent development of striatal dopamine receptor supersensitivity (Europ. J. Pharmacol. 65:313, 1980). We now report that this action of amantadine is manifest whether supersensitivity is induced by post-synaptic receptor blockade with haloperidol or by presynaptic chemical denervation with 6-OH-dopamine. One group of rats were treated concurrently with amantadine (50 mg/kg, i.p. q day) and haloperidol (2.5 mg/kg, i.p. q day) or with haloperidol alone for three weeks. Another group received a right striatal injection of 6-OH-dopamine or saline followed by a three-week course of amantadine. Receptor data was determined by Scatchard analysis of [^{3}H] spiperone binding isotherms to striatal membranes.

The amantadine + haloperidol treated group and untreated controls had similar numbers of striatal dopamine receptors (Bmax) in contrast to the 20% increase of receptor number in the haloperidol-treated rats. There was no difference in binding affinity of [^{3}H] spiperone (K_D), dopamine or DOPAC levels, or tyrosine hydroxylase activity. In the rats lesioned with 6-OH-dopamine, subsequent treatment with amantadine blocked the increase in receptor number but increased the avidity of receptor for ligand, increased dopamine and DOPAC levels, and raised the dopamine turnover ratio to normal. These data suggest that amantadine may stimulate dopamine synthesis, increase dopamine release, and have a direct agonist action on the dopamine receptor.

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167.2 ISOTOPE EXCHANGE STUDIES WITH [2-^{3}H] -HALOPERIDOL AND CATALYSIS BY A SERUM FRACTION. G. A. TRAPP. Veterans Administration Hospital and Dept. Psychiatry, La. State University Med. Cntr., Shreveport, La. 71130.

Hydrogen atoms adjacent to the carbonyl function of [2-^{3}H] -haloperidol are exchangeable with solvent water incompletely at pH 7 and more rapidly and completely at pH 8-10 in phosphate or tris buffers. Incubation of haloperidol in normal serum or plasma greatly accelerated the rate of ^{3}H loss from [2-^{3}H] -haloperiodol at pH 6-7 compared with incubation in buffer. In prolonged incubation pH 7, up to 24 hours, 22°, 95% of label was exchanged with water as shown by the decrease in radioactivity of the sample after lyophilization at various times. Ring ^{3}H-spirperidol showed no loss of isotope. The exchange proceeded to 80-95% loss of label at haloperidol concentrations 1 pg to 20 ng per ml serum. Heating serum at 70° with addition of caprylic acid to stabilize albumin decreased the reaction rate by 60%. Presence of 0.01M EDTA had no effect. The activity was present in commercial lots of Cohn fraction V from human serum but was not found in 3X crystallized serum albumin. A fraction which carries out the tritium exchange was isolated from serum by sephadex G-100 chromatography.

The chemical mechanism of exchange likely involves keto-enol transformation of haloperidol favored by the fluorobenzophenone structure and we cautiously suggest that the serum activity is an enolase.

167.3 **β-ADRENERGIC AGONIST AFFECTS ELECTRICAL ACTIVITY IN PRIMARY ANTERIOR PITUITARY CELLS: A PATCH CLAMP STUDY.**
I. Nussinovitch* and A. R. Martin. Department of Physiology, Univ. of Colorado Sch. of Med., Denver CO 80262.

Primary anterior pituitary cells dissociated from adult rat pituitary glands were cultured for short periods of time (1 - 7 days). During this period, small isolated spherical cells with a diameter of 10-20 μm were located in the culture dish and patch clamped, using the cell attached mode (Hamill et. al. 1981, Pflügers Arch. 391, 85-100). Control experiments were performed with normal saline solution in both the patch pipette and the culture dish. At a holding potential (V_H) of zero (normal resting potential), small inward and outward channel currents of less than 1 pA were usually observed. Depolarization of the membrane patch (V_H -20 to -60 mV) activated outward channels with currents of 1 - 4 pA; hyperpolarization (V_H +20 to +60 mV) revealed inward channel currents of 1 - 3 pA.

In order to study the effect on the cell membrane of a β-adrenergic agonist, isoproterenol (1 - 25 μM) was added to the solution in the patch pipette. In 13 out of 18 cells with isoproterenol in the pipette, inward channel currents were observed at the resting potential which were larger (1 - 3 pA) than in control experiments. In 8 of these cells the inward currents were accompanied by action potential currents 3 - 6 pA in amplitude. Similar action potentials were observed in only one cell in the absence of isoproterenol. In three of the cells exposed to isoproterenol, the period of inward channel activity and action potentials was followed by the appearance of outward channel currents whose activation frequency increased with time.

These results show that an adrenergic neurohormone can activate the action potential mechanism in primary anterior pituitary cells, as has been shown previously for pituitary tumor cells. The inward channel currents observed at the resting potential with isoproterenol appear to represent the initial event in such activation. The ionic nature of these inward currents is now under investigation.

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167.4 THE EFFECT OF μ, δ AND κ OPIOID RECEPTOR AGONISTS ON MONOSYNAPTIC EPSPs IN MOUSE SPINAL CORD TISSUE CULTURE.
M.Jia* and P.G.Nelson (SPON:B.Schrier). Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20205.

Previously we found that opioid peptide met-enkephalin (2-4 μM) reduced monosynaptic excitatory post-synaptic potentials (EPSPs) between spinal cord neurons. In the present experiments, we used different opioid peptides, morphiceptin, leu-enkephalin and dynorphin, which are supposed to have different affinities to μ, δ and κ receptors to study their effects on EPSPs.

The opioid peptides were applied with a mini-perfusion system from a 2-5 μM pipette situated close to the cell pair under study. The doses reported were the concentrations in the perfusing pipettes and therefore represent an upper limit of the concentration actually reaching the cells.

We found that the μ receptor agonist, morphiceptin (4 μM), reduced EPSPs in 3% of cell pairs tested (n=30), while leu-enkephalin(0.4 μM IC50, 4 μM) reduced EPSPs in 62% of cell pairs tested(n=50).The IC50 of leu-enkephalin on the EPSPs was similar to the IC50 of leu-enkephalin on the duration of DRG neuron somatic calcium-dependent action potentials(M.A.Werz and R.L.Macdonald,Brain Res. 239,315-321,1982). The κ receptor agonist, dynorphin1-9 (0.4 or 4 μM) reduced EPSPs in 37% of cell pairs tested (n=38).

Some cell pairs were tested with both dynorphin1-9 and leu-enkephalin. Although dynorphin1-9(0.4 or 4 μM) sensitivity was always accompanied by leu-enkephalin(0.4 μM) sensitivity(n=4), only 55% of leu-enkephalin (0.4 or 0.04 μM)sensitive cell pairs were dynorphin(0.4 or 4 μM)sensitive(n=11).

Statistical studies of the synaptic release process in which the coefficient of variation of the EPSP was determined showed that the mean number of transmitter quanta released per trial was reduced by leu-enkephalin, while quantal size was not affected. This suggested that the major effect of leu-enkephalin was presynaptic.

The different opioid receptors μ, δ and κ were distributed on about 3%,62% and 37% of mouse spinal cord neurons in tissue culture. Our evidence indicated that some cells only have δ receptor, but κ receptor might coexist with δ receptor.

167.5 INHIBITION OF ALPHA-ADRENERGIC RECEPTOR BINDING BY CALCIUM ANTAGONISTS.
H. Matsubayashi*, S. Kito and E. Itoga+. Third Dept. of Int. Med. Hiroshima Univ. School of Med., Hiroshima, Japan 734. +Second Dept. of Anatomy, Tohoku Univ. School of Med., Sendai, Japan 980.

The vascular smooth muscle has been so far considered to be the main site of pharmachological actions of Ca channel antagonists. It was Ehlert and Itoga that described specific binding of such drugs to neurons themselves. The authors tried to examine interactions between Ca ion channel antagonists and alpha-adrenergic receptors. In our binding experiments, ^{3}H-WB-4101 and ^{3}H-clonidine were used as radioactive ligands for $alpha_1$ and $alpha_2$ adrenergic receptor binding experiments respectively. The 10% synaptosomal fraction of the rat brain was prepared. Inhibition experiments of alpha receptor binding were done with use of various Ca ion channel antagonists as displacers. The buffer used was 50mM Tris/HCl, pH 7.7 (10mM $MgCl_2$, 0.05% ascorbic acid) and the incubation condition was 30min at 25°C. Nifedipine of 10^{-5}M had no effect on ^{3}H-clonidine specific binding. As for diltiazem, nicardipine and verapamil, concentrations of more than 10^{-6}M were needed to be effective as displacer. When 10^{-4}M Ca ion antagonists were added, diltiazem leveled down ^{3}H-clonidine binding to 51%, while nicardipine and verapamil did to 30%. The IC_{50} values obtained were 5.1×10^{-5}M for nicardipine and 2.0×10^{-5}M for verapamil. In inhibition experiments of $alpha_1$ receptor binding, nifedipine and diltiazem had no effect until the concentration was increased to 10^{-5}M. For nicardipine and verapamil, concentrations of more than 10^{-4}M were necessary to inhibit ^{3}H-WB-4101 binding. When 10^{-4}M Ca antagonists were added, verapamil and nicardipine leveled down the specific binding to 52% and 0% respectively. IC_{50} values obtained were 1.0×10^{-5}M for nicardipine and 7.0×10^{-6}M for verapamil. It was concluded that some of Ca ion channel antagonist binding sites interacted with alpha-adrenergic receptor binding mechanisms. Such interaction was definite in verapamil and nicardipine for $alpha_1$ receptor. For $alpha_2$ receptor, diltiazem, nicardipine and verapamil had inhibitory effects on specific clonidine binding. Nifedipine which has no binding affinity to calmodulin did not inhibit alpha receptor binding while verapamil and nicardipine obviously interacted with both $alpha_1$ and $alpha_2$ receptors. This was noteworthy since these two substances were known to act as calmodulin inhibitors. Attentions should be also focused on the fact that diltiazem inhibited only $alpha_2$ receptor binding.

167.6 [^{3}H]-VERAPAMIL BINDING SITES IN HEART.
M.L. Garcia,* J.P. Reuben* and G.J. Kaczorowski* (SPON: M. Cascieri). Merck Institute for Therapeutic Research, Rahway, N.J. 07065.

Specific binding sites for dihydropyridine Ca^{++} entry blockers such as [^{3}H]-nitrendipine (Nit.) have been demonstrated in cardiac, smooth and skeletal muscle as well as in neuronal tissue. Other entry blockers, such as verapamil (Ver.), cinnarizine, bepridil and diltiazem, bind to a separate site and allosterically affect dihydropyridine binding. We have demonstrated specific saturable binding of [^{3}H]-Ver. in a crude cardiac sarcolemmal membrane preparation. Membranes were incubated in 10 mM tris-HCl, pH 7.4 and [^{3}H]-Ver. (83.9 Ci/mmol) for 1 hr at 25° in the presence of 0.1% BSA and collected by filtration onto GF/C filters which had been pretreated with 0.3% polyethylenimine. Binding data was corrected for small levels of specific [^{3}H]-Ver. binding found with filters. A Scatchard analysis indicates a Kd of 50 nM and B_{max} of 1.2 pmol/mg protein for Ver. However, in the same preparation, binding of [^{3}H]-Nit. displays a Kd of 0.5 nM and B_{max} of 0.3 pmol/mg protein. Thus the ratio of Ver.:Nit. sites is 4:1. Competition studies with the stereoisomers of D-600 indicate that the (-) isomer is 1000-fold more effective than the (+) isomer in displacing [^{3}H]-Ver. (Ki = 20 nM) and complete displacement occurs. In contrast, Nit. or nisoldipine only compete 25-30% of [^{3}H]-Ver. sites (Ki = ca. 10^{-9}M). Similar results were found with diltiazem although bepridil and cinnarizine compete 100% of the binding. Because of the 4:1 ratio of sites, it is possible that only one fraction of the Ver. sites are coupled to the Nit. receptor in this membrane preparation. Purified sarcolemmal membranes highly enriched in marker enzymes (eg. Na^+,K^+-ATPase, Na^+/Ca^{++} exchange) were prepared by sucrose density gradient centrifugation and shown to be enriched in Nit. but not Ver. binding sites. However, the ratio of Ver.:Nit. sites approximates 1:1. In this preparation, Nit. and diltiazem completely release all Ver. binding. The reason for excess Ver. sites located in non-sarcolemmal membrane fractions is not clear at present, but the data would suggest that these sites are not coupled to the dihydropyridine receptor.

167.7 EFFECT OF PICROTOXININ ON BENZODIAZEPINE RECEPTOR BINDING.
R.A. Ulloque,* A.Y. Chweh, E.A. Swinyard,* and H.H. Wolf*
Department of Biochemical Pharmacology and Toxicology, College of Pharmacy, University of Utah, Salt Lake City, UT. 84112.

Picrotoxinin (Pic) has been known to exert its convulsant activity by an interaction with a specific drug receptor which is closely associated with the GABA and benzodiazepine (BDZ) receptor complex. However, the reports of the effect of Pic on BDZ receptor binding in vitro have been contradictory. Therefore, the influence of Pic on BDZ receptor binding was reinvestigated.

In mouse forebrain at 37°C in the presence of 100 mM NaCl, Pic (10 or 50 μM) significantly inhibited [^{3}H]flunitrazepam ([^{3}H]FLU) receptor binding (16.8% and 35.2% inhibition, respectively). In the absence of NaCl, the inhibition of [^{3}H]FLU binding by Pic (10 or 50 μM) was reduced (0% vs 16.8% and 19.5% vs 35.2%, respectively). At 0°C the [^{3}H]FLU binding inhibitory potency of Pic (10 or 50 μM) was also reduced as compared with that at 37°C (0% vs 16.8% inhibition and 14.5% vs 35.2% inhibition, respectively). Pic (10 or 50 μM), in the presence of NaCl, did not inhibit [^{3}H]FLU binding at either 37° or 0°C in the cerebellum. Scatchard plot analysis of [^{3}H]FLU binding to mouse forebrain membranes indicates that the inhibitory effect of Pic is most likely due to a decrease in the number of [^{3}H]FLU binding sites with no apparent change in binding affinity.

In Summary, results from the present study indicate that Pic inhibits [^{3}H]FLU binding to membranes isolated from the mouse forebrain. Pic inhibition is due to a decrease in the number of [^{3}H]FLU binding sites without altering binding affinity; it is also temperature- and anion-dependent. In contrast, Pic does not interfere with [^{3}H]FLU binding in the cerebellum. (Supported by NIH contract No. N01-NS-1-2347)

167.8 HYPEREXCITABLE CA1 CELLS IN HIPPOCAMPAL SLICES REMOVED FROM CLONAZEPAM TOLERANT RATS.
M.F. Davies and P.L. Carlen. Playfair Neuroscience Unit, Toronto Western Hospital; Addiction Research Foundation Clinical Insitute; Depts. of Medicine and Physiology, University of Toronto, Toronto, Ontario M5T 2S8.

Chronic administration of benzodiazepines to animals produces a state of tolerance to the sedative effects of the benzodiazepine. Following abrupt drug removal or administration of a benzodiazepine antagonist, animals become behaviourly hyperexcitable. We have examined the electrophysiological changes changes that occur in hippocampal slices taken from clonazepam tolerant rats.

Male Wistar rats were made tolerant to clonazepam by mixing powdered clonazepam, at increasing doses, with pulverised rat chow (10 - 50 mg/day). Tolerance was assessed by the lack of sedation and by the presence of behavioural withdrawal symptoms, including convulsions, when the benzodiazepine blocker CGS-8216 was administered (25 mg/kg i.p.).

Extracellular field potentials evoked by stimulation of Schaeffer collaterals and recorded in the the CA1 somatic layer of tolerant slices exhibited steeper I/O curves than those from control slices and, unlike control slices, a tendency for more than one population spike to be elicited by the threshold stimulation current. Recurrent inhibition elicited by alveus stimulation was impaired in tolerant slices.

No differences in the two groups were seen in the resting membrane potential (con 58.4 ± 7.69 mV (S.D.), tol 55.9 ±10.8 mV) input resistance (con 35.2 ± 11.0 MOhm, tol 34.3 ±9.4 MOhm) or spike amplitude (con 80.0 ± 10.0 mV, tol 79.4 ±mV). However, more spontaneous activity in the form of EPSPs, spikes, bursts and long-lasting but reversible depolarizing events were recorded in slices from clonazeapam-fed rats.

No tolerance was seen to clonazepam (2×10^{-9} M) in slices from tolerant animals, i.e. clonazepam reduced spontaneous activity, hyperpolarized the membrane and increased the duration and size of the post-spike afterhyperpolarizition. These effects which persisted in the state of tolerance may be a reflection of the lack of tolerance to anti-anxiety actions of benzodiazepines but not to the sedative actions. Supported by the MRC of Canada and Canadian Geriatrics Society.

167.9 ALTERATIONS IN MEMBRANE ELECTRICAL PROPERTIES OF TRIGEMINAL GANGLION NEURONS DURING GENERAL ANAESTHESIA. E. Puil and B. Gimbarzevsky*. Department of Pharmacology & Therapeutics, Faculty of Medicine, The University of British Columbia, Vancouver, B. C., V6T 1W5, Canada.

We recently have developed (Gimbarzevsky et al., Can. J. Physiol. Pharmacol. 62, 1984) fast Fourier transform methodology in order to distinguish the effects of drugs on passive membrane properties (resting input conductance, input capacitance) from their direct effect on active responses (potential- and time-dependent opening and closing of ion-selective channels). Our frequency domain analysis provided evidence that passive as well as active membrane properties of trigeminal sensory neurons of decerebrate guinea pigs may be affected during the general anaesthetic state with isoflurane. A large reduction in complex impedance was observed consistently in most of the neurons tested during administration of 1-4% isoflurane. In neurons with electrical behaviour which could be modelled by a simple, parallel RC circuit, isoflurane appeared to increase input conductance and input capacitance while the membrane time constant tended to remain unchanged. The majority of sampled neurons exhibited active properties which manifest in the frequency domain as a positive reactance. This low frequency "inductive behaviour" was modified by administration of 1-4% isoflurane which also increased resting input conductance. The above changes in membrane electrical properties presumably affect the excitability of trigeminal sensory neurons. The effects of isoflurane may be interpreted as an absorption of hydrophobic and readily polarizable molecules by the neuronal membrane.

Supported by Medical Research Council of Canada.

167.10 EFFECTS OF BW284C51 ON RESPONSES TO ACETYLCHOLINE AND CARBACHOL IN APLYSIA CALIFORNICA. Margaret G. Filbert* (SPON: Ronda Pindzola). Neurotox Br, Physio Div, US Army Med Rsch Inst of Cml Def, Aberdeen Proving Ground, MD 21010.

It is generally assumed that anti-acetylcholinesterase (anti-AChE) substances inhibit the enzyme, leading to an accumulation of acetylcholine (ACh) that is expressed as an increased amplitude and duration of the postsynaptic response. Recent evidence from our laboratory indicates that some carbamate (Filbert, et al., Soc. Neurosci. Abstr, 6: 753,1980) and organophosphate (Filbert, Soc. Neurosci. Abst. 9: 736, 1983) anti-AChE substances have direct actions on neuronal membranes. The present investigation extends the study of these agents to another class of AChE inhibitors.

1,5-bis-(4-allyldimethylammoniumphenyl)pentane-3-one, BW284C51, is a reversible, specific inhibitor of acetylcholinesterase (AChE). Using conventional intracellular recording techniques, I have examined the effects of BW284C51 on responses to ACh and carbachol in Aplysia neurons. Ach and carbachol were applied by iontophoresis from double-barreled electrodes. BW284C51 (10^{-6} - 10^{-4} M) was dissolved in artificial seawater (ASW) and applied by superfusion into a recording chamber. Membrane potential, input resistance (Ri) and agonist or passive current-voltage (I-V) relationships were monitored.

The amplitude and duration of ACh potentials increased significantly when BW284C51 was perfused into the recording chamber. After washing with drug-free ASW (up to 2 hrs), the amplitude and duration did not fully return to control levels. In contrast, carbachol potentials, elicited in the same manner, were depressed by BW284C51. Upon washout of the inhibitor with drug-free ASW, the response to carbachol was frequently larger than the control response. In the presence of BW284C51, there was an increase in the Ri that did not reverse during washout with drug-free ASW.

Therefore, BW284C51 produces several effects on cholinergic responses in the Aplysia. Responses elicited by ACh are increased in the presence of the inhibitor as would be expected from inhibition of AChE. In contrast, responses elicited by carbachol are diminished. This diminished response demonstrates a blocking action by BW284C51 on the cholinergic receptor that is independent of AChE inhibition. This blocking action is masked by the effect of AChE inhibition when ACh is used to elicit the response. After the BW284C51 is washed out, increases in Ri persist.

167.11 EFFECT OF DIISOPROPYLFLUOROPHOSPHATE ON SINGLE NICOTINIC ACETYLCHOLINE CHANNEL CURRENTS IN CLONAL G8-1 MYOTUBES. M. Adler and F.-C. T. Chang. Neurotox Br, Physiol Div, US Army Med Rsch Inst of Cml Def, APG, MD 21010.

The organophosphorous cholinesterase inhibitor, diisopropylfluorophosphate (DFP) is known to produce complex alterations of neuromuscular transmission. Low concentrations of DFP increase the amplitude and time course of endplate potentials by irreversible phosphorylation of junctional acetylcholinesterase (AChE) whereas higher DFP concentrations depress synaptic transmission by a direct and reversible action on the acetylcholine (ACh) receptor-ion channel complex. The direct actions of DFP were recently investigated on AChE-deficient G8-1 myotubes, co-cultured with ACh secreting NG108-15 neuroblastoma x glioma hybrid cells. In this system, DFP was found to produce a noncompetitive blockade of the synaptic potential amplitude and to enhance the desensitization rate of the myotube to iontophoretically applied ACh. These appeared to be independent alterations as indicated by differences in concentration-dependence and onset and washout kinetics.

To study the direct actions of DFP more precisely, we have examined its effects on single ACh channel currents by the gigaohm patch-clamp technique. G8-1 myotubes were grown in DMEM and 10% horse serum under standard cell culture conditions. Recordings were performed at 20°C on excised outside-out membrane patches. ACh (0.5 μM) and DFP (10-1000 μM) were applied by superfusion. In most patches, the ACh channel currents comprised a single amplitude distribution with a mean conductance of 41.1 ± 3.7 pS (mean ± SD, n=12). Under control conditions, the channel opening frequency was 3.1/sec at -80 mV and increased with membrane hyperpolarization, undergoing an e-fold change per 79 mV. Double exponential channel open times were observed at all membrane potentials examined: at -80 mV the two open times were 0.28 msec (fast) and 3.2 msec (slow). In the presence of 10, 100 and 1000 μM DFP, the slow component was reduced by 25, 67, and 78% respectively. The channel opening frequencies were depressed by DFP in a concentration-dependent fashion and concentrations $\geq$ 100 μM caused the channel openings to occur in short bursts separated by protracted silent periods. DFP did not alter single channel conductance or the ACh reversal potential. The DFP-induced decreases in channel open times and opening probabilities appear to account for the inhibitory actions of DFP on macroscopic channel ensembles.

167.12 EFFECT OF AN ORGANOPHOSPHATE ON EXCITABILITY IN THE RAT HIPPOCAMPUS. A.M. Williamson and J.M. Sarvey. Dept. of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Although organophosphorous (OP) agents are known to act through the blockade of acetylcholinesterase (AchE) at peripheral sites, central nervous system convulsions occur at concentrations that minimally affect peripheral transmission. While it is possible that inhibition of AchE activity and the subsequent rise in acetylcholine concentration are the primary cause of altered neuronal activity, the mechanism of centrally-mediated OP poisoning has not been clearly defined.

We have examined the effect of the OP, diisopropylfluorophosphate (DFP) and two other chemically distinct anti-AchE compounds, eserine and neostigmine, on electrical activity within the rat hippocampal slice. This preparation provides a well defined model of cortical physiology and an easily manipulated pharmacological assay system.

As measured by extracellular recording in the cell body layer of field CA1, the effect of bath application of DFP at concentrations of 0.01 to 10 μM produced no statistically significant change in the amplitude of the orthodromically evoked (stratum radiatum stimulating site) population spike. The recordings were made in a submerged slice preparation at 30°C. However, at concentrations greater than 1 μM, DFP elicited a second population spike on the late falling phase of the field potential. The second population spike elicited by 10 μM DFP appeared within 5 minutes of exposure, reached maximum amplitude within 20 minutes, was not reversible with prolonged washing (>2 hours), and was not reversed by muscarinic (atropine, 10 μM) or nicotinic (gallamine, hexamethonium, 10 μM) antagonists. DFP did not elicit spontaneous, epileptiform activity at any concentration tested. In contrast, the second population spikes caused by bath application of eserine (10 μM) or neostigmine (10 μM), were reversible in atropine (10 μM).

These data suggest that DFP has effects on excitability that are unrelated to the inhibition of acetylcholinesterase.

This work was supported by USAMRDC contract #IA02033.

167.13 SINGLE CHANNEL STUDIES OF ANTICHOLINESTERASE AGENTS IN ADULT MUSCLE FIBERS: ACTIVATION, DESENSITIZATION AND BLOCKADE OF THE ACETYLCHOLINE RECEPTOR-ION CHANNEL COMPLEX (AChR). K.-P. Shaw*, A. Akaike*, D.L. Rickett and E.X. Albuquerque. Dept. Pharmacol. & Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201.

Anticholinesterases such as pyridostigmine (PYR) (Pascuzzo et al., Mol. Pharmacol. 25:92-101, 1984) and physostigmine (PHY) (Shaw et al., Neurosci. Abs. 9:1138, 1983) have multiple direct actions on the AChR. Our present report describes a group of reversible and irreversible anticholinesterase agents which were studied to characterize their direct interactions with the AChR. Single channel currents were recorded from the perijunctional region of frog interosseal muscles at 10°C using a technique for isolation and mounting of the fibers developed in our laboratory (Allen and Albuquerque, this meeting). PHY (50-200 μM) decreased the conductance of ACh-induced channels in a dose-dependent manner. PHY (50 μM) decreased the mean channel lifetime by 42% while a larger concentration (100 μM) reduced the lifetime by 52%. Quaternary PHY (MetPHY, 200 μM) decreased the conductance of ACh-activated channels by 44% and the mean channel lifetime by 70% (at -60 mV). In addition, PHY, PYR, Neostigmine (NEO), Edrophorium (EDR) and the irreversible anticholinesterase agent, soman, had agonist effects on the ACh receptor. These weak agonists are substantially different from each other. For example, the ionic channels generated by PYR (50-100 μM) have a low conductance (< 12 pS), in contrast to PHY (0.5-5 μM) which activated channels with higher conductance. At a concentration of 5-500 μM, NEO and PYR activated channels with an increased frequency of openings associated with "flickering". All of these reversible anticholinesterase agents affected the channel via the outside surface of the membrane. In summary, various anticholinesterase agents can behave as agonists of the AChR, induce desensitization and a blockade of the ionic channel of the AChR. (Supported by USPHS Grant NS-12063 and U.S. Army Med. Res. and Develop. Command Contract DAMD-17-81-C-1279)

167.14 STEREOSELECTIVITY OF NICOTINIC RECEPTORS AND THEIR SINGLE CHANNEL PROPERTIES INDUCED BY ANATOXIN-A. C.E.Spivak[1], R. Gonzalez-Rudo*[2], H. Rapoport[3] & E.X. Albuquerque[2]. Addiction Res. Ctr.[1], Natl. Inst. Drug Abuse, Baltimore, MD 21224, Dept. Pharm. & Exp. Ther.[2], Univ. MD Sch. Med., Baltimore, MD 21201, & Dept. Chem.[3], Univ. CA, Berkeley, CA 94720.

Anatoxin-a (AnTx-a) is a semirigid, bicyclic, naturally-occurring alkaloid that is among the most potent of the known nicotinic agonists. Its semirigid structure and optical activity make it a favorable probe for elucidating the structural requirements for activating the ionic channel of the nicotinic receptor. Contracture experiments using the rectus abdominis muscle of frogs have shown that (+)-AnTx-a is >2X as potent as the racemic mixture (Mol. Pharmacol. 23:337-343, 1983). From noise analysis at endplates using frog sartorius muscles, we concluded that channel conductance (γ) induced by (+)-AnTx-a was 25% less than ACh (Mol. Pharmacol. 18:384-394,1980). With the availability of (-)-AnTx-a of >95% optical purity and single channel recording technique, we have now refined and extended these results. Comparing the two enantiomers by the contracture method we found (+)-AnTx-a to be 1000X more potent. Single channels were recorded (10°C, cell-attached patch) in dissociated interosseal muscle fibers from adult Rana pipiens and cultured rat myotubes. Slope conductances corroborated the results obtained from noise analysis. In muscle fibers, a single conductance state was observed for (+)-AnTx-a (γ=19.3±3.6 pS, mean±S.D., n=4). By contrast, γ=33.4±2.3 pS for ACh (A. Akaike & E.X. Albuquerque, results from this lab). In myoballs, (+)-AnTx-a produced γ=18.3±0.6 pS (n=3) and ACh γ=20.2±1.0 pS (n=7). The mean channel lifetime (τ) for (+)-AnTx-a was shorter than that for ACh. In the muscle, lifetimes had a single exponential distribution. The τ was 2.9±1.3 (0 to -55 mV), 3.1±1.1 (-60 to -85 mV) and 3.5±0.6 (-90 to -105 mV) msec (n=4). ACh-induced channels had τ of 9.4±1.0 (-30 to -50 mV), 15.0±1.7 (-60 to -85 mV) and 19.4±3.0 (-90 to -110 mV) msec. In myoballs, the lifetimes induced by (+)-AnTx-a had a double exponential distribution as did those induced by ACh. AnTx-a channels showed only a slight voltage-dependence and averaged 5.7±1.5 msec (n=5). By contrast, τ of ACh-induced channels was longer (10-30 msec depending on the membrane potential) (Aracava et al., Mol. Pharmacol., in press). In conclusion, although AnTx-a is at least as potent as ACh it induces channels with lower γ and shorter τ. (Support: USPHS, NS-12063 & U.S. Army Med. R. & D. Com., DAMD-17-81-C-1279)

167.15 THE INTERACTION OF PYRIDINE-2-ALDOXIME METHIODIDE (2-PAM), A REACTIVATOR OF CHOLINESTERASE, WITH THE NICOTINIC RECEPTOR OF THE FROG NEUROMUSCULAR JUNCTION. K.S. Rao* and E.X. Albuquerque. Dept. Pharmacol. & Exp. Therap., Univ. Maryland Sch. Med., Baltimore, MD 21201.

2-PAM has long been considered an effective therapy against poisoning by most organophosphate anticholinesterase (anti-ChE) agents as a reactivator of phosphorylated ChE. Early studies by Kuba et al. (J. Pharmacol. Exp. Ther. **189**:499-512, 1974) have shown that 2-PAM restored the amplitude of the endplate current (EPC) after treatment with DFP, presumably by reactivation of junctional ChE. However, there was a significant shortening of the half decay time of the EPC particularly at negative potentials suggesting that 2-PAM might have a direct effect on the AChR. The present study was thus aimed at investigating the effects of 2-PAM on EPC, MEPC and ACh-induced noise. At low concentrations, 2-PAM (10-100 μM) produced a concentration-dependent increase in the EPC and MEPC amplitude and a loss of voltage dependence of the time constant of EPC decay time constant. Although at hyperpolarized potentials (-60 to -150 mV) the decay time constant was shortened at more depolarized potentials, it became longer than control. When the concentration of 2-PAM was increased to < 1 mM, the EPC amplitude was markedly depressed in a nonlinear fashion at more negative potentials and the time constant of decay was shortened further at hyperpolarized potentials. Washing the preparation for a period of 60 min resulted in partial recovery of the peak EPC amplitude as well as the decay time constant. ACh noise analysis, at concentrations of 2-PAM ranging from 500 μM to 4 mM, showed a concentration-dependent decrease in single channel conductance (control, 19 pS; 1 μM, 10 pS; 4 μM, 7 pS) and a marked but concentration-independent decrease in channel lifetime. These effects were reversible. The slow reversibility coupled with the marked decrease in channel conductance and shortening of channel lifetime might explain at least in part some of the therapeutic value of this compound for anti-ChE poisoning. The multiple effects of 2-PAM may result from its interactions with the ionic channels associated with the nicotinic AChR. Patch clamp studies are underway to clarify these complex actions. (Supported by USPHS Grant NS-12063 and U.S. Army Med. Res. and Devel. Command Contr. DAMD-17-81-C-1279)

CHOLINERGIC RECEPTORS: MUSCARINIC RECEPTORS

168.1 MUSCARINIC MODULATION OF ACETYLCHOLINE RELEASE IS MEDIATED BY M2 BUT NOT M1 PRESYNAPTIC RECEPTORS. E.M. Meyer and D. Otero*. Department of Pharmacology, Univ. Florida, Gainesville, FL 32610.

Presynaptic muscarinic receptors appear to be important for the fine tuning of cholinergic transmission in the brain as well as periphery. We have therefore developed a synaptosomal system for measuring muscarinic release-modulation from rat cerebral cortices in order to further characterize this receptor response with respect to receptor sub-type and mechanism of action. Our results indicate that M2 but not M1 receptors are mediating this acetylcholine release-modulation, since: 1) oxotremorine, acetylcholine, and carbachol are about equally potent and efficacious inhibitors of (^{3}H)-ACh release, while pilocarpine has no effect; 2) scopolamine and atropine are similarly potent blockers of oxotremorine-induced attenuation of (^{3}H)-ACh release, while pirenzepine has no effect on these muscarinic receptors; 3) gallamine (a potential allosteric modifier of M2 but not M1 receptors) is able to block oxotremorine-induced (^{3}H)-ACh release attenuation, while dicyclomine (which acts at M1 but not M2 receptors) has no effect; and 4) liposomal delivery of GppNHp, the non-hydrolyzable GTP analog, into synaptosomes appears to reduce the potency of oxotremorine with respect to release-inhibition.

Several properties of this M2-mediated ACh release-inhibition from synaptosomes might not be predicted from simple binding studies: 1) K^+-induced depolarization significantly reduces the potency of all of the M2 agonists, while equal concentrations of Na^+ extracellularly increase the efficacy of the release-modulation induced by oxotremorine; and 2) gallamine appears to act as a competitive blocker at presynaptic receptor sites.

With respect to mechanism of action of M2 receptors on ACh-release, we find: 1) voltage-dependent calcium channels appear be involved (release triggered by calcium ions entering through other channels is not modulated); 2) phosphatidylinositol turnover as well as levels of cyclic AMP and cyclic GMP do not appear to be involved; 3) modulation is prevented by combined treatment with tetrodotoxin and high extracellular Na^+ levels (reducing spontaneous depolarization of nerve terminals; and 4) potassium channels may be involved (4-aminopyridine but not TEA can block modulation). Our working hypothesis is that receptor activation hyperpolarizes nerve terminals through K^+ channel activation, followed by reduced voltage-dependent calcium ion entry.

168.2 ARE MUSCARINIC CHOLINERGIC RECEPTORS COUPLED TO THE PHOSPHOINOSITIDE-CA^{++} SYSTEM BY AN UNKNOWN GUANINE NUCLEOTIDE REGULATORY PROTEIN? J.R. Hepler*, T. Evans*, S.L. Brown*, J.H. Brown, and T.K. Harden. Univ. North Carolina Sch. Med., Chapel Hill, NC 27514 and U. Cal. San Diego, La Jolla, CA 92093.

Stimulation of muscarinic cholinergic receptors (MR) on 1321N1 astrocytoma cells results in marked increases in the breakdown of phosphoinositides (PI) and the unidirectional efflux of $^{45}Ca^{++}$ from $^{45}Ca^{++}$-prelabelled cells. Cholinergic agonists also attenuate cAMP accumulation through a Ca^{++}-dependent activation of phosphodiesterase (PDE). No MR-mediated inhibition of adenylate cyclase occurs. However, 1321N1 cells express the inhibitory guanine nucleotide regulatory protein (N_i) of adenylate cyclase since a 41,000 Mr protein is ^{32}P-labelled in the presence of ^{32}P-NAD and pertussis toxin (PT). N_i also is apparently functional since guanine nucleotide-mediated inhibition of forskolin-stimulated adenylate cyclase activity is readily observed. PT blocks guanine nucleotide-mediated inhibition of forskolin-stimulated adenylate cyclase activity, but has no effect on MR-mediated activation of PDE. Thus, N_i is apparently not involved in MR coupling to PDE. Furthermore, PT does not block MR-stimulated PI turnover or Ca^{++} efflux. However, further experiments suggest that MR in these cells do couple to a guanine nucleotide regulatory protein. That is, GTP-sensitive high affinity binding of MR agonists has been detected in washed 1321N1 membranes in competition binding experiments with ^{3}H-QNB. The relative capacity of a series of agonists for inducing the GTP-sensitive high affinity binding state correlated strongly with their relative efficacy for the stimulation of PI turnover and Ca^{++} efflux. PT pretreatment had no effect on this GTP-sensitive agonist binding suggesting that N_i is not involved. These data thus suggest that an unknown guanine nucleotide binding protein, presumably different from N_i, may be involved in coupling MR to the PI-Ca^{++} system. Supported by GM 29536 and HL 28143.

168.3 THE MUSCARINIC RECEPTOR COUPLED TO PHOSPHOINOSITIDE HYDROLYSIS IS NOT THE PUTATIVE M_1 RECEPTOR. J. Heller Brown and S. Brown Masters*. University of California, San Diego, Division of Pharmacology. La Jolla, CA 92093.

The muscarinic antagonist pirenzepine does not bind with equal affinity to all muscarinic receptors (MR). MR have been classified as M_1 and M_2 receptor subtypes, having high and low affinity for pirenzepine. It is postulated that the M_1 receptor is coupled to phosphoinositide (PhI) metabolism and the M_2 receptor to adenylate cyclase inhibition but evidence for such a functional difference between M_1 and M_2 receptor has not been presented. We used dissociated embryonic chick heart cells to compare the MR mediating inhibition of adenylate cyclase to that through which PhI hydrolysis is stimulated (JBC 259:3777, 1984). The cAMP response was assessed by measuring muscarinic inhibition of isoproterenol-stimulated cyclic AMP formation and the PhI response by measuring the accumulation of [^{3}H]inositol 1-phosphate in the presence of lithium. Atropine antagonizes both the cyclic AMP and PhI responses with a K_I of ~1nM. These K_I values are nearly identical to the K_D (1.8 nM) determined by atropine competition for [^{3}H]QNB or [^{3}H]NMS binding sites on intact chick heart cells. Pirenzepine, like atropine, competes for all radioligand binding sites on those cells with a single apparent affinity (K_D=22 nM). Pirenzepine antagonizes the effect of carbachol on cyclic AMP formation with a K_I (42 nM) close to the K_D determined by radioligand binding. However the K_I for pirenzepine antagonism of carbachol-stimulated PhI hydrolysis is much higher (250 nM). Thus, the MR coupled to PhI hydrolysis has relatively low affinity for pirenzepine rather than the high affinity predicted for an M_1 receptor. The muscarinic agonists, McN-A-343 and AHR-602 are thought to have greater activity at the M_1 receptor. McN-A-343 and AHR-602 cause less than 10% of the maximal PhI response elicited by carbachol or oxotremorine-M (OXO-M). McN-A-343 and AHR-602 are more efficacious at inhibiting cyclic AMP formation since they produce 40-50% of the maximal response seen with carbachol or OXO-M. Thus, in chick heart cells, where MR binding and muscarinic effects on cyclic AMP and PhI metabolism can be measured under nearly identical conditions the predominant receptor state has relatively high affinity for pirenzepine and appears to mediate adenylate cyclase inhibition while the muscarinic receptor regulating PhI metabolism is relatively insensitive to putatively selective M_1 receptor agonists and antagonists.

Supported by HL 28143.

168.4 **(3H)PIRENZEPINE BINDING TO MUSCARINIC RECEPTORS SOLUBILIZED FROM RAT CEREBRAL CORTEX.** **G.R. Luthin and B.B. Wolfe, University of Pennsylvania, Dept. of Pharmacology, Phila., PA 19104.** **Supported by GM 31155, GM-09991 and AHA.**

Pirenzepine (PZ), an antagonist at muscarinic cholinergic receptors, potently inhibits the ability of muscarinic agonists to increase phosphoinositide breakdown, but less potently inhibits the effects of muscarinic agonists on adenylate cyclase activity (Gil and Wolfe, Soc. Neurosci. Abstract, 1984). It was shown that (^{3}H)PZ labelled a lower density of muscarinic receptor sites than (^{3}H)quinuclidinylbenzilate (QNB) did in rat brain membranes. The latter difference between (^{3}H)PZ and (^{3}H)QNB binding in brain is thought to reflect the existence of M_1 and M_2 subtypes of the muscarinic receptor, or alternatively, differential coupling of muscarinic receptors to cellular effector mechanisms. In this study, we compared the binding of (^{3}H)PZ and (^{3}H)QNB to muscarinic receptors solubilized from rat cerebral cortical membranes using 1% digitonin followed by centrifugation. Approximately 25 to 30% of the total (^{3}H)QNB binding sites were solubilized from the membranes. The material present in the 105,000 x g supernatant bound (^{3}H)QNB in a saturable fashion. PZ inhibited binding of (^{3}H)QNB by the solubilized receptors, with a K_i of 270 nM and a Hill slope of 1. (^{3}H)PZ labelled solubilized receptors, with a K_d of 130 nM, but (^{3}H)PZ labelled only 30% of the number of (^{3}H)QNB binding sites. The association and dissociation constants for (^{3}H)PZ binding to solubilized receptors were 3.2 x $10^6 M^{-1} min^{-1}$ and 0.13 min^{-1}, respectively, at 32°C. Muscarinic antagonists inhibited (^{3}H)PZ and (^{3}H)QNB binding to solubilized receptors with Hill slopes of 1. The muscarinic agonist carbachol inhibited (^{3}H)PZ and (^{3}H)QNB binding to solubilized receptors with a Hill slope of 0.7. The binding data for carbachol was computer-modelled, and was best described using a 2-binding site model. GTP did not alter the relative density of high- and low-affinity carbachol binding sites. Pre-incubation of solubilized receptors at 32°C for 1 hour did not alter the relative density of (^{3}H)PZ or (^{3}H)QNB binding sites. Together, these data demonstrate that (^{3}H)PZ binding sites can be solubilized from rat brain. Solubilization appeared to disrupt coupling mechanisms which can modulate agonist interactions with membrane-bound cortical receptors. Carbachol appeared to discriminate two forms of solubilized receptor labelled by (^{3}H)PZ and (^{3}H)QNB. Therefore, binding site heterogeneity discriminated both by agonists and antagonists was present in the solubilized preparation. The study of solubilized muscarinic receptors should contribute to our understanding of the mechanisms responsible for muscarinic receptor binding heterogeneity.

168.5 **SELECTIVITY OF PIRENZEPINE FOR TWO MUSCARINIC RECEPTOR-MEDIATED RESPONSES.** **Daniel W. Gil and Barry B. Wolfe. Dept. of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.**

On the basis of radioligand binding studies, the existence of subtypes of the muscarinic cholinergic receptor has been proposed, but their function is unknown. As other investigators have reported, we find that pirenzepine, a muscarinic receptor antagonist, distinguishes high (15 nM) and low (260 nM) affinity binding sites in rat brain while atropine is nonselective (0.4 nM). In other receptor systems (e.g. α-adrenergic), separate receptor subtypes modulate separate biochemical responses such as phosphoinositide (PI) breakdown and adenylate cyclase activation or inhibition. Muscarinic receptors also modulate these responses and the possibility that these are associated with distinct muscarinic receptor subtypes was investigated. If this is the case, pirenzepine should exhibit different potencies in assays of the two responses.

Assays of muscarinic receptor-mediated calcium-independent PI breakdown and inhibition of cAMP production were carried out with rat brain, where both responses can be measured and where the high affinity pirenzepine binding has been observed. The accumulation of (^{3}H)-inositol 1-phosphate in slices preincubated with (^{3}H)-inositol and LiCl was measured as an assay of PI breakdown and was linear for 30 min. Adenylate cyclase activity was measured for 500 sec in striatal homogenates and maximal inhibition was 30%.

The K_i value for pirenzepine inhibition of muscarinic receptor-mediated PI breakdown, determined by Schild analysis, is 21 nM. In assays of muscarinic receptor-mediated inhibition of cAMP production, pirenzepine is 15-fold less potent (K_i = 310 nM). Atropine is equipotent in the two assays (K_i = 0.8-1.9 nM). The muscarinic receptor agonists acetylcholine and carbachol are also nonselective and oxotremorine and McN A343 are partial agonists in these studies. In earlier studies using the rat parotid and ventricle a 19-fold selectivity of pirenzepine for the inhibition of PI breakdown was observed. However, the potency of pirenzepine at these peripheral muscarinic receptors was 6-7 fold lower than that observed in the CNS.

These studies suggest that the site in the CNS that has high affinity (15 nM) for pirenzepine in radioligand binding studies mediates PI breakdown while a low affinity site (260 nM) mediates inhibition of cAMP production. It also appears from these data that the properties of muscarinic receptors may not be identical in the CNS and peripheral tissues. (Supported by GM 31155 and the American Heart Association.)

168.6 CHARACTERIZATION OF PUTATIVE M_1 AND M_2 MUSCARINIC CHOLINERGIC RECEPTOR BINDING SITES IN RAT CEREBRAL CORTICAL AND MYOCARDIAL HOMOGENATES USING [^{3}H]PIRENZEPINE AND [^{3}H](-)QUINUCLIDINYL BENZILATE. M. Watson*, W.R. Roeske* and H.I. Yamamura (SPON: J.J. O'Neill). Depts. of Pharmacology and Int. Med., Univ. of Az., Tucson, AZ 85724.

Functional data indicate the anti-ulcer agent pirenzepine (PZ) distinguishes putative M_1 and M_2 muscarinic receptor (MCHR) subtypes. Our studies of [^{3}H]pirenzepine ([^{3}H]PZ) show this nonclassical antimuscarinic drug selectively identifies a subpopulation of putative M_1 MCHR as compared to [^{3}H](-)quinuclidinyl benzilate ([^{3}H](-)QNB) in the rat cerebral cortex (Life Sci., 31:2019, 1982). Ions, but not guanine nucleotides, exert potent effects on high affinity [^{3}H]PZ binding and similar high affinity K_d's for [^{3}H]PZ (5 nM) may be obtained in various rat tissues (Life Sci., 32:3011, 1983) and human stellate ganglia (Brain Res., 290:179, 1984). We now further characterize the binding and regulation to MCHR subtypes in the rat cerebral cortex (M_1) and myocardium (M_2). Using our rapid filtration assay, parallel assays were made of inhibition by selected compounds of [^{3}H]PZ and [^{3}H](-)QNB labeled membranes. We examined muscarinic agonists and antagonists in the presence and absence of the non-hydrolyable GTP analog, guanyl--5'-yl imidodiphosphate [Gpp(NH)p] at 25°C in 10mM Na-K, 50mM Na-K, and modified Krebs phosphate buffer. As we previously reported, Gpp(NH)p produces little effect on K_d or B_{max} (receptor density) values in saturation studies of either tissue, and high ionic strength lowers both [^{3}H]PZ and [^{3}H](-)QNB affinity. Agonists versus [^{3}H](-)QNB or [^{3}H]PZ generally had Hill values (n_H)<1, are better fit to a 2-site model, are Gpp(NH)p regulated and show lower affinity in higher ionic strength. Antagonists also show this lower affinity, but are insensitive to Gpp(NH)p with n_H=1. Myocardial PZ/[^{3}H]QNB curves are steep. Although cortical PZ/[^{3}H]PZ curves are also steep, PZ/[^{3}H](-)QNB curves have n_H<1 as do [^{3}H]PZ saturation curves in low ionic strength which promotes both PZ binding and heterogeneity. Thus, direct and indirect studies show PZ labels M_1 sites with high affinity and M_2 sites with low affinity. Carbachol=acetylcholine>oxotremorine reflect agonists relative M_2 selectivity, unlike pilocarpine=McN-A-343. Antagonists are M_1 selective: PZ>dexetimide=scopolamine=atropine>levetimide=(-)QNB. Orders of potency and selectivity for muscarinic drugs emphasize differences between agonist heterogeneity and PZ heterogeneity, and support the concept of putative M_1 and M_2 subtypes.

168.7 BINDING OF SELECTIVE AGONISTS AND ANTAGONISTS TO TWO INTERCONVERTIBLE STATES OF M1 MUSCARINE RECEPTORS. J. Luber-Narod, D.D. Flynn and L.T. Potter, Department of Pharmacology, University of Miami School of Medicine, P.O. Box 016189, Miami, FL 33101.

Pirenzepine has been widely described as an antagonist which distinguishes M1 and M2 muscarine receptors, even though several laboratories have clearly documented three populations of binding sites for this anti-ulcer drug in a variety of tissues. Here we show that this situation is due to the fact that M1 receptors (like M2) have interconvertible affinity states: pirenzepine binds with "superhigh" (SH) affinity to sites on a coupled form of M1, with "high" (H) affinity to sites on uncoupled M1, and with "low" (L) affinity to sites on coupled and uncoupled M2 receptors.

Three lines of evidence show two states of M1 receptors in membranes from the rat or rabbit hippocampus. (1) In media containing Mn^{++} or Mg^{++} ions, competition curves between carbachol and ^{3}H-quinuclidinyl benzilate are flattened, and do not steepen in 0.1 mM GppNHp or NEM, each of which uncouples higher affinity M2 states. Removal of divalent ions with EDTA removes the higher affinity binding to M1 (TIPS Suppl. 22-34, 1984). (2) Pirenzepine-QNB competition curves in physiological media show SH and H affinity binding to hippocampal receptors, as well as L sites on M2 receptors; apparent Kd values are approximately 0.2, 5 and 80 nM. Those who use pirenzepine to block M1 receptors can take advantage of its selective action on SH sites, and autoradiographers who wish only to localize M1 can label only SH sites. SH sites are converted to H sites when divalent ions are removed. (3) ^{3}H-pirenzepine dissociates biphasically from SH and H states of M1 receptors in media containing Mg or Mn ions and from H affinity sites alone in EDTA.

Knowledge of the multiple affinity states of M1 and M2 receptors is obviously important for their accurate distinction, labelling, quantitation, activation or blockade.

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168.8 PIRENZEPINE DOES NOT DISTINGUISH AUTO- FROM POSTSYNAPTIC MUSCARINIC RECEPTORS IN RAT HIPPOCAMPUS. Andrew B. Norman*, George Battaglia and Ian Creese. Dept. Neuroscience, Univ. of Calif., San Diego, Sch. of Med., La Jolla, CA 92093.

The novel muscarinic cholinergic antagonist pirenzepine has been reported to distinguish two subtypes of muscarinic receptors which possess equal affinity for QNB. Lesions of the cholinergic afferents to the hippocampus have been reported to have no effect on hippocampal ^{3}H-QNB binding. However, one of the muscarinic receptor subtypes identified by pirenzepine may have a discrete presynaptic localization or undergo compensatory postsynaptic adaptations following denervation. These possibilities were investigated by the competition of pirenzepine for ^{3}H-QNB binding in hippocampus 21 days following fimbrial transection.

Lesions which produced 50-90% reductions in CAT activity produced no significant change in ^{3}H-QNB binding. Control Bmax: 62.9±1.1; lesion: 62.4±1.6 pmoles/g original wet weight tissue. Pirenzapine competition for ^{3}H-QNB in 50mM NaPhosphate buffer (pH 7.4, 37°C) was analyzed by LIGAND program and fitted best to a model assuming two binding sites. Neither the proportions nor the affinities of the two sites were significantly changed by the lesion. Control: K_H=21.1±1nM, R_H=93%; K_L=425.7±24nM, R_L=7%; Lesioned: K_H=21.7±2.2nM, R_H=95%; K_L=534±108nM, R_H=5%.

Analysis of carbachol competition for ^{3}H-QNB in the presence of 4mM $MgSO_4$ also fitted better to a model assuming two rather than one or three sites. Neither the proportions nor the affinities of these sites were appreciably altered following the lesion. Control: K_H=2.15±0.13µM, R_H=44%, K_L=93.25±11.45µM; Lesioned: K_H=2.54±0.02µM, K_L=100.13±3.75µM, R_H=46%. Furthermore 10µM Gpp(NH)p reduced proportions of K_H for carbachol similarly in control and deafferented hippocampus. Control: K_H=2.23±0.2µM, R_H=27%, K_L=113.25±2.44µM; Lesioned: K_H=2.33±0.04µM, R_H=25%, K_L=114.62±2.43µM. These data provide no evidence for a discrete presynaptic localization of a subtype of muscarinic receptors identified by pirenzepine on CAT containing neurons. Furthermore there are no apparent adaptations of agonist or antagonist binding to postsynaptic muscarinic receptors following cholinergic deafferentation of the hippocampus. Supported by PHS NS17860.

168.9 COMPARISON OF CARDIAC AND CENTRAL MUSCARINIC RECEPTORS. J.W. Wells, K.M. Nguyen,* H.-M.S. Wong* and M.J. Sole*. Faculty of Pharmacy and Department of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 1A1.

(-)-N-[^{3}H]Methylscopolamine (NMS) has been used to compare muscarinic receptors in crude homogenates from the left ventricle plus interventricular septum and in synaptosomal preparations from the cerebral cortex of Syrian golden hamsters. Binding was measured at equilibrium and 30° in Krebs-Henseleit buffer (pH 7.4); bound radioligand was separated by microcentrifugation. Non-specific binding was determined in the presence of excess, unlabelled drug and is the same for all drugs tested. In both preparations, the specific binding of free [^{3}H]NMS is well described by a rectangular hyperbola. Competitive experiments with muscarinic drugs yield Hill coefficients (nH) near or equal to one for antagonists and markedly lower for agonists. Among antagonists, estimates of the dissociation constant correlate well in heart and cortex (P=0.0043, n=5), but values are two- to threefold higher in the former. The binding patterns of agonists are well described as a mixture of sites differing in affinity for the drug; two or three classes are required for a good fit, depending upon the agonist and the tissue. When a third class is found only in the heart, the apparent dissociation constant (K_{SH}) is lower than anything found in the cortex; when a third class is found only in the cortex, the dissociation constant (K_{SL}) exceeds anything found in the heart. All agonists reveal two classes of sites (K_H and K_L) apparently common to both regions, with good correlations for K_H (P=0.013, n=6) and K_L (P=0.0013, n=6). For each agonist, K_H is either numerically similar in both regions or smaller (<5-fold) in the cortex; K_L is similar in both regions or smaller (<5-fold) in the heart. The correlation for K_H and K_L taken together is excellent (P<0.00001, n=12). GMP-PNP at 0.1 mM is without effect on [^{3}H]NMS in either tissue, and essentially without effect on agonists in the cortex; its effect on agonists in the heart is to increase nH to near one with a concomitant increase in IC50. The action of GMP-PNP in the heart suggests that an interaction between the receptor and a G/F-complex accounts, at least in part, for the low values of nH in that tissue. The similarities in K_H and K_L suggest, however, that a similar interaction underlies the low values of nH in the cortex. It remains speculative as to why this similarity does not extend to the sensitivity of that interaction to GMP-PNP. (Supported by the MRC and the Ontario Heart Foundation)

168.10 EFFECT OF ZINC ON ^{3}H-QNB AND ^{3}H-PIRENZEPINE DISPLACEMENT BY MUSCARINIC AGONISTS AND ANTAGONISTS. C.P. Smith and F.P. Huger. Department of Biochemistry, Hoechst-Roussel Pharmaceuticals, Inc. Somerville, N.J. 08876.

Muscarinic receptors can be labeled by either ^{3}H-QNB or ^{3}H-pirenzepine, the latter commonly referred to as M_1 receptors. These binding sites differ in distribution, affinity, density, sensitivity to guanine nucleotides, and may be distinct. Previous work, showing that zinc increases ^{3}H-oxotremorine binding and the affinity of muscarinic agonists for ^{3}H-QNB sites, prompted us to investigate the effect of zinc and GppNHp on ^{3}H-pirenzepine and ^{3}H-QNB binding in rat frontal cortex and cerebellum.

Bound ^{3}H-pirenzepine or ^{3}H-QNB was captured by filtration on GF/C Whatman filters. The binding parameters observed in frontal cortex agreed with those of other investigators. No specific ^{3}H-pirenzepine binding occurred in cerebellum. ^{3}H-QNB bound with similar affinity as at frontal cortex sites, but to a much lower number of sites.

The results indicate that zinc enhances the ability of certain choline ester muscarinic agonists to displace ^{3}H-pirenzepine but has no effect on antagonist affinities or those of oxotremorine, McN-A-343 or AHR-602. The only effect of zinc on ^{3}H-pirenzepine binding parameters is a slight (two-fold) decrease in affinity.

Since ^{3}H-QNB, but not ^{3}H-pirenzepine, labels sites in the cerebellum, it was of interest to study the effects of GppNHp and zinc on agonist and antagonist affinities in this tissue. The affinities of atropine or pirenzepine are not affected by either agent, but the agonist carbachol and oxotremorine have decreased affinity in the presence of GppNHp and increased affinity in the presence of zinc. However, the affinity of the pirenzepine-selective (M_1) agonist McN-A-343 is unaffected by either GppNHp or zinc.

In conclusion, zinc enhances the affinity of choline esters, but not oxotremorine, McN-A-343 or AHR-602 at ^{3}H-pirenzepine sites in frontal cortex. Although ^{3}H-pirenzepine does not bind in cerebellum pirenzepine is able to completely displace ^{3}H-QNB from its cerebellar receptors, but is nearly 50-fold less potent then in frontal cortex. Finally, GppNHp, which decreases carbachol and oxotremorine affinity, has no effect on the affinity of McN-A-343. Thus far, the evidence supports the hypothesis that there are muscarinic receptor subclasses, each with different conformational states, but different receptor-guanine nucleotide regulatory protein interactions.

168.11 MUSCARINIC AGONISTS DIFFERENTIALLY STIMULATE INOSITOL PHOSPHATE RELEASE FROM GUINEA-PIG STRIATUM. S.K.Fisher and R.T. Bartus.Dept.CNS Research, Lederle Labs.,Pearl River,NY 10965

Regional differences in the characteristics of brain muscarinic receptors have to date been identified through indirect means such as the binding of labeled agonists and antagonists. One direct measure of receptor activation suited to such studies is the release of inositol phosphates (IP) that accompanies the phosphodiesteratic breakdown of inositol phospholipids (IPL). In previous studies of the guinea-pig cerebral cortex,two groups of muscarinic agonists could be distinguished; one (full agonists) whose addition elicited a maximal increase in IPL turnover, e.g. oxotremorine-M (oxo-M) and carbamoylcholine, and another (partial agonists) which elicit much smaller increases in IPL turnover e.g. oxotremorine, bethanechol, pilocarpine and arecoline (J.Biol.Chem.,1983:258, 7358; J.Neurochem., 1984: In Press). To determine whether this pattern of agonist efficacy pertained to other brain regions, slices of guinea-pig striatum, cerebral cortex, hippocampus and pons/medulla were labeled with [^{3}H]inositol in the presence of the indicated muscarinic agonists (10^{-3}M). Addition of McN-A-343, a putative M1-selective agonist, resulted in little or no increase in the release of [^{3}H]IP from any brain region. Oxo-M enhanced the release of [^{3}H]IP, with striatum >cerebral cortex =hippocampus > pons/medulla (725,325,300 and 140% of control, respectively). While addition of oxotremorine resulted in a smaller accumulation of [^{3}H]IP, its effect relative to oxo-M was greater in the striatum than in either the cerebral cortex or hippocampus (325,140 and 125% of control respectively) Examination of the effects of other muscarinic agonists revealed that bethanechol also exhibited a greater relative efficacy in the striatum than in the cerebral cortex,whereas no such tissue differences were observed for pilocarpine. Pirenzepine, a putative M1 antagonist, blocked the stimulatory effect of oxo-M in the striatum but only at relatively high concentrations. Analysis of the labeled inositol phosphates in oxo-M, bethanechol and oxotremorine-stimulated striata indicated that for all three agonists, the principal product formed was inositol 1-phosphate with smaller amounts of inositol 1,4-bisphosphate and trace amounts of inositol 1,4,5-trisphosphate. The results indicate quantitative differences in the responses of muscarinic receptors in the striatum and cerebral cortex to selected agonists, and suggest that [^{3}H]IP release may constitute an important means of revealing differences in the coupling characteristics of muscarinic receptors in different brain regions.

168.12 DIFFERENTIAL MUSCARINIC ACETYLCHOLINE RECEPTOR DESENSITIZATION: DEPENDENCY ON PHOSPHOINOSITIDES? J.A.Joseph, D.Critchett*, A.S.Lippa*. American Cyanamid Co., Lederle Labs., Pearl River, NY 10965.

Muscarinic acetylcholine receptors (mAChR) appear to be involved in a variety of physiologic functions. Particular interest has focused on mAChR receptors localized on hippocampal pyramidal cells (HPC). ACh and other agonists increase HPC firing rates,an effect blocked by antagonists (e.g., pirenzepine). However, little is known about the coupling mechanisms involved. One line of evidence suggests a functional relationship between mAChR occupation and increased phosphoinositide (PI) turnover. mAChR agonists have been classified as Class A (e.g., carbachol, oxotremorine-M) or Class B (e.g., bethanecol, oxotremorine-1 depending on whether they stimulate PI turnover and possibly induce conformational/orientational mAChR changes (Class A) or not (Class B).(Fisher et al. J.Biol.Chem. 258(12) 1983,7358-7363) The present study attempted to determine if these differences were manifested electrophysiologically. HPC firing rates were examined following microiontophoretic application of ACh (1m; 5-25 nA; 100µM 25-100 nA) carbachol (100µM 2-100nA) oxotremorine-M (.5mM, 2-30 nA) bethanecol(5mM, 10mM 5-30 nA) and oxotremorine-1 (1mM 5-40 nA) to anesthetized (chloral hydrate 400 mg/kg) male Wistar rats. Arg^8 vassopressin(AVP) was also applied (1mM, 25 nA), since it has been shown to be localized on HPC and to increase PI turnover. A total of 302 cells from 93 animals were used. ACh as well as other agonists produced current dependent increases in firing rates (spikes/sec) when delivered over 20 sec. No differences in stimulatory efficacy were seen among the various agonists. During longer applications of ACh (4 min) the stimulating effects were greatly reduced (41-50%)despite continued administration. This "desensitization effect" (DE) was consistent across several current intensities and concentrations of ACh. Class A agonists produced ACh-like DE while Class B agonists produced no DE over 4 min. AVP had no stimulatory effects on HPC firing when applied alone. However, it potentiated ACh-induced DE (%inhibition 41±8% ACh alone; 60±8% ACh=AVP, t (7)±5.03 $p < .01$). Results suggest increases in PI turnover induced by Class A agonists may (possibly through the induction of changes in receptor conformation) mediate a DE on these sites. AVP results indicate that the DE modulatory effects of this neuropeptide on HPC may be mediated through the PI.

168.13 ALTERATION OF MUSCARINIC ACETYLCHOLINE RECEPTOR BINDING IN RAT BRAIN BY PHOSPHOLIPASE C ACTION. N. Parthasarathy* and R.S. Aronstam (SPON: T.M. Nosek). Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912.

Treatment of neural membranes from rat cerebral cortex with phospholipase C from Bacillus cereus inhibited the binding of radiolabelled antagonists ([^{3}H]3-quinuclidinyl benzilate and [^{3}H]methylscopolamine) to muscarinic acetylcholine receptors. This inhibition was incomplete, was not competitive, and did not appear to be related to the production of inhibitory products. Even after prolonged treatment of the membranes with phospholipase C, muscarinic binding was not decreased by more than 65%. The reasons for this limit are not clear, but may involve restricted access of the enzyme to certain lipid domains. The predominant reaction products, phosphorylated headgroups and diacylglycerides, did not inhibit receptor binding when added directly to the incubation media.

The affinity of carbamylcholine for cortical muscarinic receptors was increased by phospholipase C action. The distribution of receptors between states of high and low affinity was not affected by phospholipase C; rather the affinity for carbamylcholine of the lowest affinity receptors was selectively increased. In control membranes, 73% of muscarinic receptors bound carbamylcholine with a dissociation constant of 8.2 uM; the remaining receptors had a carbamylcholine dissociation constant of 0.04 uM. After treatment with phospholipase C, the fractional distribution of receptors between high and low affinity populations was not altered, nor was the affinity of the high affinity receptors for carbamylcholine. The affinity of the low affinity receptors, however, was increased 3.6 fold, to a K_D of 2.3 uM. The affinity of muscarinic receptors for agonists appears to reflect different states of association of the receptor with various effector and regulatory mechanisms. Thus phospholipase C effects may be due to alterations in the interactions of the binding site subunit with other structures in synaptic membrane.

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168.14 CHARACTERIZATION OF PRE- AND POST-SYNAPTIC MUSCARINIC RECEPTORS IN MYENTERIC NEURONS. B.E. Slack* and R.A. North. Neuropharmacology Laboratory, M.I.T., 56-245, Cambridge, MA. 02139, U.S.A.

Muscarinic receptors can be divided into distinct subtypes, an observation which could account for the multiplicity of effects accompanying muscarinic activation in many tissues. It has been shown that muscarinic agonists depolarize the cell soma and inhibit the fast excitatory postsynaptic potential (e.p.s.p.) in neurons of the guinea-pig myenteric plexus (Morita et al., J. Physiol., 333: 125 and 141, 1982). These actions were investigated further using conventional intracellular recording techniques. Muscarine chloride (300 nM to 10 µM) depolarized both S and AH neurons dose-dependently. In the presence of pirenzepine the dose-response curve was shifted to the right in a parallel manner. Estimated pA_2's for pirenzepine ranged from 9.7 to 10.1, suggesting that the depolarization is mediated by M_1 receptors (Hammer et al., Nature, 283: 90, 1980). Slow e.p.s.p.s elicited by single shocks from a focal stimulating electrode (which have previously been shown to result from activation of muscarinic receptors by acetylcholine released from presynaptic fibers) were also reduced by pirenzepine (100 nM). Presynaptic muscarinic receptors were studied by measuring the inhibition of the fast e.p.s.p. by oxotremorine and muscarine. The latter was effective at concentrations which depolarized the cell, while oxotremorine reduced the e.p.s.p. at concentrations subthreshold for membrane depolarization. These inhibitory effects persisted in the presence of pirenzepine. The results suggest that the receptors which mediate presynaptic inhibition by muscarinic agonists differ from those which elicit depolarization of the cell membrane.

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168.15 GUANYL NUCLEOTIDE EFFECT ON [^{3}H]ACETYLCHOLINE BINDING TO M-2 MUSCARINIC RECEPTORS. R.L. Taylor*, D.P. Hall, Jr.,*, A.M. Martino* and K.J. Kellar (SPON: F.G. Standaert). Dept. of Pharmacol., Georgetown Univ. Schools of Med. and Dent., Washington, DC 20007.

[^{3}H]Acetylcholine ([^{3}H]ACh) binds with high affinity to muscarinic cholinergic sites in brain and heart (see Kellar et al., these abstracts). The binding site has the characteristics of an M-2 subtype of muscarinic receptor. That is, the ratio of the [^{3}H]ACh sites to total muscarinic binding sites labeled by [^{3}H]QNB is highest in the medulla, pons, cerebellum and heart atrium; muscarinic agonists and most antagonists have high affinity for the site, but the affinity of the M-1 selective antagonist pirenzipine is 130-200 times lower than that of nonselective antagonists; and the binding of [^{3}H]ACh is decreased by guanyl nucleotides (GTP and GppNHp).

Previous studies have indicated that in most tissues it is the high affinity M-2 subtype of receptor that is sensitive to guanyl nucleotides. However, the use of indirect assays (i.e., unlabeled agonist vs. [^{3}H]antagonist) and nonselective [^{3}H]agonists have imposed limitations on the interpretation of these experiments. We have directly examined the effects of guanyl nucleotides on the binding of [^{3}H]ACh in brain and heart atrium. We have also compared the extent of the guanyl nucleotide effect on [^{3}H]ACh binding in different tissues to the potency of unlabeled ACh in competing for [^{3}H]QNB binding sites. In the medulla, pons, cerebellum and atrium the binding of 6 nM [^{3}H]ACh was decreased by 80-90% in the presence of 10 uM GppNHp. In contrast, in the cerebral cortex, corpus striatum and hippocampus binding was decreased by less than 30%. The potency of ACh in competing for [^{3}H]QNB (50 pM) in these tissues segregated according to the guanyl nucleotide sensitivity. That is, in the medulla, pons and atria the IC_{50} of ACh was 200-600 nM; while in the cortex, striatum and hippocampus the IC_{50} was 6,000-10,000 nM.

Thus, in tissues such as pons, medulla and atrium, in which the [^{3}H]ACh site represents the largest proportion of the total muscarinic sites (defined by [^{3}H]QNB binding sites), the guanyl nucleotide effect is most extensive, and the potency of ACh in competing for [^{3}H]QNB sites is 10-50 times greater than in tissues in which [^{3}H]ACh sites are a small fraction of total muscarinic sites.

This assay should prove to be useful in studying the regulation of muscarinic agonist binding sites by guanyl nucleotides.

168.16 MUSCARINIC ACETYLCHOLINE RECEPTOR BINDING IN DISSOCIATED INTACT RAT BRAIN CELLS. J.H. Lee* and E.E. El-Fakahany*. (SPON: C. Eccles). Department of Pharmacology and Toxicology, Univ. of Maryland School of Pharmacy, Baltimore, MD 21201

The properties of muscarinic acetylcholine receptors in isolated intact rat brain cell aggregates were studied. Intact cells were prepared by mincing whole brains without the cerebellum. The minced tissue was placed in a 210 um mesh soaked in a calcium-free iso-osmotic physiological salt solution and squeezed through with a glass pestle, then the resulting suspension was filtered under gravity through a tighter mesh (130 um). The filtrate was centrifuged at 400 x g at 4°C for 3 min and the resulting pellet was washed twice with an iso-osmotic HEPES-salt physiological buffer solution (pH 7.4), then finally suspended in the same buffer. Muscarinic receptors in these cells were labeled by incubation with [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) or [^{3}H]N-methylscopolamine ([^{3}H]NMS) for 90 min at 15°C, and nonspecific binding was defined in presence of 2 uM atropine. The binding reaction was terminated by filtration under vacuum over GF/B glass fiber filters, followed by 3 x 5 ml rinses with cold 0.9% saline. Specific binding showed tissue linearity and saturability. In addition, muscarinic antagonists displaced [^{3}H]NMS binding according to the law of mass action. However, agonists had apparent Hill slopes of less than unity, suggesting heterogeneity of agonist but not antagonist binding sites. Interestingly, the equilibrium dissociation constant of [^{3}H]QNB-receptor complex was significantly higher in these cells than the reported values obtained in crude synaptic membranes. In addition, the number of receptors labeled with [^{3}H]QNB was about twice that labeled with [^{3}H]NMS. These results suggest that the apparent affinity of [^{3}H]QNB for muscarinic receptors is lower in intact cells than in membranes, and that in intact cells there are receptors which are available only to a lipophilic ligand such as [^{3}H]QNB but not to the hydrophilic ligand [^{3}H]NMS. These receptors might not be located on the cell surface, but might exist in a lipophilic domain.

168.17 MUSCARINIC RECEPTOR BINDING IN BOVINE ADRENAL MEDULLA. B. A. Barron and T. D. Hexum. Dept. of Pharmacol., University of Nebraska Medical Center, Omaha, NE 68105

Catecholamine secretion from the adrenal medulla of the dog or gerbil is mediated primarily by nicotinic cholinergic receptors [Douglas et al, J. Physiol. Lond. 188 107 (1967); Tsujimoto, et al. E.J. Pharmacol. 34 337 (1975)]. However muscarine increases the outflow of catecholamines from guinea-pig and rat adrenal glands [Role & Perlman, Neurosci. 10 979 (1983); Wakade & Wakade, Neurosci. 10 973 (1983)]. The bovine adrenal medulla has been reported to contain only nicotinic receptors due to the inactivity of muscarinic agents on catecholamine secretion [Wilson & Kirshner, J. Neurochem. 28 687 (1977)]. Nevertheless the content of cGMP of bovine adrenal medullary cells has been shown to increase in response to muscarinic agents [Yanagihara et al. FEBS Lett. 105 296 (1979)].

We decided to investigate the presence of muscarinic receptors in the bovine adrenal medulla using the specific muscarinic antagonist ^{3}H-quiniclidinyl benzylate (^{3}H-QNB). Binding studies were performed using the P_3 fraction obtained from differential centrifugation of a homogenate of bovine adrenal medulla [Bartlett & Smith, Meth. Enz. XXXI Part A 379 (1974)]. Membranes (75 ug protein/ml) were incubated in 0.05 M Na/K phosphate buffer, pH 7.4, for 60 min at 37° in the presence of ^{3}H-QNB (0.05-10 nM). Specific binding was determined by filtration through glass fiber filters in the presence and absence of 5×10^{-5} M atropine sulfate. Binding was linear over a protein concentration of 30 to 120 ug/ml. Scatchard analysis of specific ^{3}H-QNB binding revealed two binding sites with K_D's of 2.34 and 57.4 pM and B_{max}'s of 59.0 and 280 fmoles/mg protein, respectively. The K_D and B_{max} of the second binding site is similar to that reported for ^{3}H-QNB binding in the rat adrenal medulla [Kayaalp & Neff, E.J. Pharmacol. 57 255 (1979)]. These results add support to the evidence suggesting the presence of muscarinic receptors in the bovine adrenal gland [Yanighara, ibid.]

168.18 GALLAMINE'S INTERACTION WITH MUSCARINIC RECEPTORS: COMPETITIVE, NONCOMPETITIVE, OR BOTH? J. Ellis* and R.H. Lenox. (SPON: H. Schmidek), Neuroscience Research Unit, Dept. of Psychiatry, Univ. of Vermont Coll. Med., Burlington, VT 05405

Gallamine is a potent neuromuscular blocker that has previously been shown to inhibit the binding of [^{3}H]QNB (quinuclidinyl benzilate) to muscarinic receptors of rat brain. Analysis of these competition curves suggested that gallamine interacted competitively with discrete subpopulations of muscarinic receptors. This suggestion was strengthened by the observation that selective elimination (by antagonist blockade) of muscarinic subpopulation(s) that possess low affinity for the agonist carbachol resulted in a concomitant elimination of sites that possess low affinity for gallamine (Ellis and Hoss, Biochem. Pharmacol. 31:873, 1982). Subsequently, Stockton et al. (Mol. Pharmacol. 23:551, 1983) presented convincing evidence that the inhibition by gallamine of the binding of another muscarinic antagonist, [^{3}H]NMS (N-methyl scopolamine), is not accomplished by a competitive mechanism. The contrast between these reports led us to compare the effects of gallamine on the binding of [^{3}H]NMS and [^{3}H]QNB in a single system (rat brain membranes in 40 mM Na-K phosphate buffer, pH 7.4).

We report here that the presence of 100μM gallamine markedly slows the rate of dissociation of [^{3}H]NMS from the receptor, in agreement with Stockton et al.; as little as 3μM gallamine increases the half-time for association of [^{3}H]NMS with the receptor approximately ten-fold. However, when [^{3}H]QNB is employed as the labeled ligand under the same conditions, gallamine (100μM) does not significantly affect the time course of association, nor that of dissociation. One possible explanation for these findings is that gallamine does bind allosterically, but that the binding of either gallamine or QNB reduces the affinity of the other to such an extent that ternary complexes cannot be demonstrated and, hence, the interaction appears to be competitive. Another possibility is that gallamine may bind both to the classical muscarinic binding site and to an allosteric site, but that QNB is relatively insensitive to the allosteric effect. It seems possible that the interactions of the positively charged NMS with the receptor may differ from those of a lipophilic molecule such as QNB. The relationships between the subpopulations of muscarinic receptors discerned by agonists, gallamine, and pirenzipine will be discussed.

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168.19 MUSCARINIC CHOLINERGIC LIGAND BINDING TO INTACT AtT-20 MOUSE PITUITARY TUMOR CELLS. K. Akiyama*, T.W. Vickroy, M. Watson*, W.R. Roeske*, T.D. Reisine, H.I. Yamamura. Depts. of Pharmacology, Univ. of Arizona, Tucson, AZ 85724 and NIMH, Bethesda, MD 20205.

It has been reported that activation of muscarinic receptors on AtT-20 mouse pituitary tumor cell line inhibits cyclic AMP dependent ACTH secretion stimulated by secretagogues. Thus, muscarinic receptors of this cell line may be inversely coupled to adenylate cyclase stimulation. Muscarinic receptors have been identified in this cell line using [^{3}H](-)QNB in homogenate fractions. In our study, [^{3}H](-)QNB binding studies were done on intact cells and were compared with homogenate fractions. We also determined whether muscarinic receptor subtypes existed in this cell line. AtT-20 cells were grown in flasks, 75 cm^2 or in 25 mm diameter wells in an atmosphere of 90% air and 10% CO_2 at 37°. The growth medium was 90% DMEM and 10% FCS with antibiotics. [^{3}H](-)QNB binding to intact cells or membrane homogenates was done 5-7 days after subculturing. Atropine (1 μM) was used for the determination of nonspecific binding. Saturation isotherms of [^{3}H](-)QNB binding revealed high affinity binding in the intact cells (K_D = 36 pM) and in membrane homogenates (K_D = 32 pM). However, the number of binding sites in the membrane homogenates was less than that in the intact cells (6.1 versus 22.9 fmoles/1 million cells). The rank order of potency of atropine, a classical antagonist and pirenzepine, a nonclassical antagonist with selectivity for M_1 receptors, was similar in intact cells and membrane homogenates in inhibiting 100 pM of [^{3}H](-)QNB binding. The IC_{50} values of atropine were 0.17 nM (homogenates) and 0.42 nM (intact cells) while the IC_{50} values for pirenzepine were 91 nM (homogenates) and 287 nM (intact cells). In summary, these studies show that specific [^{3}H](-)QNB binding can be performed in intact cells and that the K_D values are similar to those obtained from membrane homogenates. However, the number of binding sites are higher in intact cells. These studies also indicate that AtT-20 cells appear to have M_1 subtype of muscarinic receptors.

168.20 MOLECULAR REQUIREMENTS FOR THE MUSCARINIC RECEPTORS OF N4TG1 NEUROBLASTOMA CELLS. Peter K. Chiang, Y. F. Chang*, Richard K. Gordon* and Marvin C. Pankaskie†. Div. Biochem., Walter Reed Army Inst. Res., Washington, DC 20307; †Dept. Biomed. Chem., University Nebraska Med. Center, Omaha, NE 68105.

We reported recently that there are 2 x 10^5 muscarinic receptors/cell with a K_D of 13 nM in N4TG1 neuroblastoma cells by using [^{3}H]QNB (quinuclidinyl benzilate) binding (Biochem. Pharmacol. 1983, 32, 2979). Two groups of compounds were examined for their ability to inhibit QNB binding to the muscarinic receptors of N4TG1 neuroblastoma cells. The first group consists of analogs of S-isobutyladenosine (SIBA), which are novel muscarinic agents. SIBA and S-isobutyl-3-deazaadenosine (3-deazaSIBA) have been shown to have a variety of biological effects, chiefly attributed to the hypothesis that they act as S-adenosylhomocysteine analogs and consequently as inhibitors of methylation. However, it has lately become obvious that 3-deazaSIBA has effects that are unrelated to methylation reactions (Trans. Am. Soc. Neurochem. 1981, 12, 82; Biochem. Pharmacol. 1982, 31, 2111). The most potent inhibitors of QNB binding are 1-deazaSIBA and 3-deazaSIBA (I_{50} values of 5.2 x 10^{-5} M and 3.9 x 10^{-5} M, respectively). The parent compound, SIBA, and the carbocylic derivative, S-isobutyl-3-deazaaristeromycin are less active. There is a requirement for the S-isobutyl side chain and N7 of the purine because 3-deazaadenosine, 5'-methylthioadenosine and S-isobutyltubercidin are inactive. Substitution of the thioether with N (5'-isobutylamino-adenosine) and modification of the ribose to an acyclic (9-[(2-isobutylthioethoxy)methyl]-adenine) resulted in loss of activity. It is conceivable that the ribose ring assumes a conformation similar to the furan ring of muscarine, and the S-isobutyl group acts like the hydrophobic benzilate portion of QNB or atropine. The next type of compounds tested were QNB analogs: The quinuclidine portion can be substituted with diethylamino ethyl ester, e.g. aprophen, benactyzine and adiphenine, and still retain equal potency to inhibit QNB binding. Furthermore, the quinuclidine can also be replaced by either an alkane, H, or pyrrolidine at the N without loosing their ability to inhibit binding. Additions to the quinuclidine increase the bulk and decrease inhibition of binding. The benzilate (diphenyl) portion can be replaced by quinidines or tricyclic structures and still retain inhibitory activity. Similar to the benzilate in QNB or atropine, a hydrophobic structure is apparently required for activity.

168.21 DISTRIBUTION OF MUSCARINIC RECEPTOR SUBCLASSES IN HUMAN FETAL BRAIN. A.M. Marchisio*, M. Palomba*, C. Marcello* & F.Gremo* (SPON:D. Davies) Dept. of Anatomy & Dept. of Obstetrics and Gynecology, School of Medicine, Cagliari, Italy

Muscarinic acetylcholine receptors are implicated in higher brain functions such as sleep, avoidance behaviour, learning and memory. It is a relatively recent acquisition that muscarinic receptors are heterogeneous. Presumably, different subclasses subserve different functions. No data are available on the subtypes of muscarinic receptors which mature in human brain during fetal development. We consequently investigated the presence of muscarinic receptor subclasses in several areas of brains obtained from spontaneous or therapeutic abortions. In a few cases, also post mortem samples obtained from perinatal infants were examined. Brains were removed from fetuses aborted between the 10th and the 25th week of gestation and dissected into frontal and occipital cortex, cerebellum, thalamus, hippocampus and basal nuclei. After homogenization, membranes were prepared by centrifugation and assayed for ^{3}H-QNB binding. Results showed that the distribution of muscarinic receptors changed among ares with age, even if the affinity for the ligand remained substantially unchanged. In all the areas the number of receptors increased with age. Computer analysis of displacement curves of both agonists and antagonists enlightened the presence of two muscarinic cholinergic subclasses in all the areas considered, which differred for their affinities and relative percentages among the total receptor population. This was particularly evident with the use of pirenzepine, a well known antagonist which can distinguish among muscarinic receptor population and with acetylcholine and carbachol. The relative percentage of the two subclasses varied in areas with age. However, in all of them the receptors showed increasing affinity for acetylcholine, which accounts for functional maturation of the receptor. Areas having the same embryological origin had a very similar distribution of the two receptor subclasses.

168.22 **STIMULATION OF SYNAPTOSOMAL ACIDIC PHOSPHOLIPID SYNTHESIS IS MEDIATED BY THE M_1 MUSCARINIC CHOLINERGIC RECEPTOR SUBTYPE.** **Henry Yamamura and Thomas L. Smith, Dept. of Pharmacol., Univ. of Arizona, Tucson, AZ 85724 and Vet. Adm. Med. Ctr., Tucson, AZ 85723.**

It has been demonstrated previously that in synaptosomes, activation of muscarinic cholinergic receptors enhances [^{32}P] incorporation into both phosphatidylinositol (PhI) and phosphatidic acid (PhA). However, physiological and more recently pharmacological data suggest that muscarinic cholinergic receptors are comprised of distinct M_1 and M_2 subtypes. Therefore, it was desirable to distinguish which of these receptor subtypes are associated with receptor-mediated phospholipid turnover in brain.

A synaptosomal fraction was prepared from either rat cerebral cortex or cerebellum and was incubated with [^{32}P] in a Krebs-Ringer HEPES buffer (pH 7.4) for 1 hr. in the presence of indicated drugs. Individual phospholipids were extracted with $CHCl_3$:CH_3OH (2:1), isolated by TLC and counted for [^{32}P] incorporation. Specific radioligand binding of [^{3}H] pirenzepine ([^{3}H]PZ) and [^{3}H]QNB was determined in the same synaptosomal fraction by a rapid filtration assay using a 10mM sodium-potassium phosphate buffer (pH 7.4). Both carbamylcholine (1.0mM) and McNeal A343 (1.0mM), a selective M_1 - muscarinic agonist, enhanced PhA synthesis by 160 and 120%, respectively, in cortex but had no effect in cerebellum. Stimulation by both drugs was substantially inhibited by pirenzepine (1μM). Synaptosomes from cerebellum, however, were metabolically active in that significant stimulation of PhI and PhA labeling (160% of basal values) could be detected in the presence of 1.0mM norepinephrine.

PZ inhibition curves of [^{3}H]QNB in the cortex and cerebellum showed 2 sites in the former and only 1 site in the latter. The cortex had a high affinity K_D value of 50nM and a low affinity K_D value of 680nM. In contrast the cerebellum had a single K_D value of 1200nM. The proportion of high affinity sites in the cortex was about 50%. Thus, the muscarinic receptor population of cortical synaptosomes was composed of about 50% M_1 and 50% M_2 receptor subtypes while that of the cerebellum was predominately the M_2 subtype. We tentatively hypothesize that only the M_1 muscarinic cholinergic receptor subtype is coupled to cholinergically mediated phospholipid turnover. **(Supported by U.S.H.P. Grants and The Veterans Administration).**

168.23 PURIFICATION OF THE CARDIAC MUSCARINIC ACETYLCHOLINE RECEPTOR. G. L. Peterson*, G. S. Herron*, M. Yamaki*, D. S. Fullerton* and M. I. Schimerlik* (SPON: D. Barker). Dept. of Biochem. Biophys., Oregon State Univ., Corvallis, OR 97331.

The cardiac muscarinic acetylcholine receptor has been purified from porcine atria to apparent homogeneity. The receptor was solubilized from receptor-enriched membrane fractions (prepared from 5-7 kg atrial tissue) by a double extraction procedure using the mixed digitonin/cholate detergent system (G. L. Peterson and M. I. Schimerlik (1984) Prep. Biochem., 14:33). The solubilized receptor was then chromatographed sequentially on wheat germ agglutinin agarose, diethylaminoethyl agarose and hydroxylapatite. This gave a preparation which was approximately 10% pure with a yield of 6% of the membrane-bound receptor. Final purification was achieved by specific muscarinic receptor binding and elution from a 3-(2′-aminobenzhydryloxy) tropane agarose affinity resin (K. Haga and T. Haga (1983) J. Biol. Chem. 258:13575). The purified receptor bound 11.1-12.8 nmol [^{3}H]L-quinuclidinyl benzilate per mg protein and represented a 4-5% yield from the membrane fraction and a 100,000 fold purification over atrial homogenates. Silver stained SDS gradient polyacrylamide gels revealed the presence of two bands. The higher molecular weight band (78,400-90,000) represented 86% of the silver staining and was specifically alkylated with [^{3}H]propylbenzilylcholine mustard, whereas the smaller polypeptide (M_r 14,800) was not alkylated, but co-purified with the large polypeptide, and was approximately equimolar according to silver staining. The purified preparation was very stable with respect to the number of binding sites and the protein profile on denaturing gels. Preliminary ligand binding studies indicated that the purified receptor behaved like the membrane-bound receptor with respect to antagonist (L-quinuclidinyl benzilate, hyoscyamine) and agonist (carbamylcholine) binding affinities.

ALCOHOL AND BARBITURATES I

169.1 STRAIN-RELATED DIFFERENCES IN THE EXCITATORY EFFECTS OF ETHANOL: ROLE OF MU AND DELTA OPIATE RECEPTORS. T.S. Shippenberg* and H.L. Altshuler. Neuropsychopharmacology Research Section, TRIMS and Dept. of Pharmacology, Baylor College of Medicine, Texas Medical Center, Houston, Texas 77030.

Ethanol (ETOH) produces transient behavioral excitation in rodents which appears to involve endogenous opiate pathways. We have reported that rats can discriminate between ETOH's excitatory (EX) and sedative (SED) effects in a drug discrimination (DD) paradigm and that naloxone (NLX) pretreatment blocks the ETOH EX phase discriminative stimulus (DS) but not the SED DS. This study used the DD paradigm to examine the contribution of opiate mechanisms to ETOH's EX effects in 4 rat strains that differ in their behavioral response to ETOH: Sprague-Dawley (SD), Wistar (WS), Maudsley Reactive (MR) and Maudsley Non Reactive (MNR).

Twenty male rats from each strain were trained to discriminate ETOH (0.6-1.0 gm/kg; IP) from saline during ETOH's EX phase (6 min post-dose). A double lever, food reinforced, fixed ratio (FR 10) operant paradigm was used. Following acquisition of the discrimination and dose-response testing, DD was tested after NLX (1.0-10.0 mg/kg, SC) pretreatment (15 min prior to ETOH). Significant ($p < .05$) strain-related differences in ETOH's EX DS effects were observed. MR and WS strains exhibited greater excitation than the DS and MNR strains. Although NLX blocked the EX DS in all strains, antagonism was greatest in those rats exhibiting greater ETOH-induced excitation.

Receptor binding of 3H-dihydromorphine and 3H (2-D-Ala,-5-D-Leu) enkephalin (ENK) was examined in the cortex (CTX), hippocampus (HPP) and striatum (STR) of each strain to determine whether differences in ETOH's EX effects resulted from strain-related differences in mu (dihydromorphine) or delta (ENK) opiate receptor function. Scatchard analysis of ENK binding to STR revealed differences between strains in the number of ENK binding sites (per mg protein). DS rats had significantly ($p < .05$) more ENK binding sites than WS rats. There were no differences in receptor affinity for ENK among strains. These data provide additional evidence and support for the hypothesis that opiate pathways are major sites for the EX actions of ETOH and suggest that sensitivity to ETOH EX effects may result from genetic differences in the functional characteristics of opiate receptors. The receptor binding data imply that such differences may be expressed specifically in the delta opiate receptor.

169.2 GABA MEDIATION OF THE CENTRAL EFFECTS OF ACUTE AND CHRONIC ETHANOL IN MICE. M.S. Dar and W.R. Wooles. Department of Pharmacology, School of Medicine, East Carolina University, Greenville, NC 27834.

The possible mediation by GABA in ethanol-induced hypothermia and motor incoordination was investigated in mice treated with acute and chronic ethanol administration using aminooxyacetic acid (AOAA) and bicuculline. In the acute ethanol studies AOAA alone produced a marked hypothermia although a test dose of ethanol was able to produce a further drop in body temperature in AOAA treated mice. Although tolerance to ethanol-induced hypothermia was present in ethanol-dependent mice, AOAA administration was able to produce a further decrease in body temperature. Bicuculline potentiated ethanol-induced hypothermia in the acute studies but the tolerance to hypothermia which had developed in ethanol-dependent mice prevented the bicuculline-induced potentiation of ethanol hypothermia. Aminooxyacetic acid markedly potentiated acute ethanol-induced motor incoordination whereas bicuculline had no effect. Although partial tolerance had developed to ethanol-induced motor incoordination in dependent mice, AOAA potentiated whereas a lower dose of bicuculline antagonized, motor incoordination. In the acute studies ethanol had a biphasic effect on AOAA-induced GABA accumulation in the hypothalamus and corpus striatum: a low dose prevented and a slightly higher dose was without effect on GABA accumulation. Ethanol-dependent mice were unable to respond to an AOAA-induced increase in GABA accumulation although basal levels of GABA were unaffected by chronic ethanol ingestion. The results show that brain GABA or GABA-mediated central mechanisms may be involved in the mediation of ethanol-induced motor incoordination but not hypothermia.

169.3 EFFECTS OF ACUTE AND REPEATED EXPOSURE TO LOW-LEVEL MICROWAVES ON ETHANOL-INDUCED HYPOTHERMIA IN THE RAT. H. Lai*, A. Horita*, C.K. Chou* and A.W. Guy* (SPON:K. Chan) Departments of Pharmacology, Psychiatry & Behavioral Sciences, and Center for Bioengineering, University of Washington, Seattle, WA 98195, USA.

Effects of acute and repeated exposure to 2450 MHz low-level pulsed microwaves (2 μs, 500 pps, 1 mW/cm^2, SAR = 0.6 W/kg) on ethanol-induced hypothermia were investigated in the rat.

In the acute experiment, rats were exposed for 45 min to microwaves in waveguides and then immediately injected with ethanol (3 g/kg, i.p., in a 25% V/V water solution).Control animals were sham-irradiated simultaneously in similar waveguides and then injected with ethanol.There was no significant difference in body temperature between the microwave- and sham-irradiated rats immediately after exposure.However, the initial rate of fall in body temperature induced by ethanol was significantly retarded in the microwave-irradiated rats.There was no significant difference in the maximal extent of hypothermia between the microwave- and sham-irradiated animals.Furthermore, the effect of microwaves was blocked by pretreating the animals with naloxone (1 mg/kg,i.p.).

In another experiment, rats were subjected to ten daily sessions of microwave exposure (45 min/session, irradiation parameters same as above).On day 11, ethanol-induced hypothermia was studied in the animals immediately after a session of either microwave or sham exposure.Similar to the acute effect, the rate of initial fall in body temperature induced by ethanol was attenuated in the rats irradiated with microwaves (unconditioned effect).A conditioned effect was observed in the sham-irradiated rats.In these animals, the rate of fall in body temperature was significantly enhanced.Thus, the conditioned effect(enhancement) was opposite in direction to the unconditioned effect(attenuation).No tolerance developed to the unconditioned effect after repeated exposure.

169.4 PRENATAL EXPOSURE TO ETHANOL RETARDS KINDLING DEVELOPMENT IN ADULT RATS. D.D.Savage and E.REYES. Dept. Pharmacology, U. New Mexico Sch. Medicine, Albuquerque, New Mexico 87131.

Long term learning disabilities have been described in children exposed to ethanol (EtOH) in utero. Disorganization of certain neuronal elements has been observed in brain of Fetal Alcohol Syndrome (FAS) children and animal models of FAS. The hippocampal formation (HPF), a brain area associated with learning and memory, is quite sensitive to the effects of EtOH pre- and postnatally. Kindling, a phenomenon in which repeated administration to brain of a subconvulsive dose of electrical current leads to a progressive intensification of motor seizures, has been proposed as an animal model of epilepsy, neuronal plasticity and learning. Given the learning deficits of FAS children, we examined the effect of prenatal exposure to EtOH on kindling development in adult rats.

Pregnant Wistar rats were fed a liquid diet containing 6% EtOH (v/v) or pair fed an isocaloric carbohydrate equivalent. At birth, the litters were culled to six and cross fostered to nontreated surrogate mothers. At 80 days of age, a bipolar electrode was implanted into either the right basolateral nucleus of the amygdala or the right angular bundle of the entorhinal cortex. Kindling stimulations were administered three times a day and the motor seizure Class score (Racine, 1972) recorded. The scorer was blinded to experimental condition of the animals. After animals had exhibited a total of three Class 5 motor seizures, histological verification of electrode placements were performed and then the identity of the animals revealed.

The total number of stimulations required to exhibit three seizures of each Class was determined for pair fed controls and 6% EtOH animals. The total number of stimulations required to reach Classes 1 through 5 was significantly higher in 6% EtOH animals. Further, the data indicated that kindling retardation was due entirely to a slower progression from Class 0 to Class 1 seizures. Similar results were obtained for amygdala and angular bundle kindling.

Brain areas involved in the early stages of kindling include amygdala, entorhinal cortex and HPF. Amygdala kindling is retarded by the partial destruction of HPF dentate granule cells. Further, prenatal exposure to EtOH alters the pattern of HPF dentate granule cell axonal projections to HPF CA_{3+4} pyramidal cells. Taken together, these data suggest a possible functional deficit in neuronal communication within the HPF of FAS animals. This proposed defect could, in part, underlie learning deficits in FAS children.

169.5 HIPPOCAMPAL LTP IN THE PRESENCE OF ETHANOL IN SLICES FROM NORMAL AND ETHANOL-DEPENDENT RATS. Deborah L. Lewis and Forrest F. Weight. Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

Acute and chronic ethanol exposure have been associated with cognitive deficits including memory. Long-term potentiation (LTP) of field potentials in hippocampus after brief tetanic stimulation of orthodromic inputs has been proposed as a possible physiological correlate of memory formation (Swanson et al., Neuro. Res. Prog. Bull., 20: 617, 1982). Abraham et al. (Brain Res., 221: 271, 1981) have reported that a 20 wk period of chronic ethanol treatment has no effect on LTP in the CA1 region of hippocampus. On the other hand, Durand et al. (Neurosci. Abst., 6: 89, 1980) have found an impairment of LTP in area CA1 of hippocampal slices from rats treated with ethanol for 8-9 mo. In the present study, we examined the effect of ethanol on the induction of LTP in area CA1 of the hippocampal slice in normal and ethanol-dependent rats. Slices (450um) were obtained from the hippocampus of male Sprague-Dawley rats (250-350g) not exposed to ethanol and from dependent rats undergoing the ethanol withdrawal syndrome. Physical dependence was induced by intragastric intubation with 20% (w/v) ethanol in Sustacal at 9-11 g/kg in fractional doses for 4 days to maintain intoxication (Majchrowicz, Psychopharmacologia, 43: 245, 1975). Rats were withdrawn from ethanol on day 5 and behaviorally assessed for ethanol withdrawal symptoms of hyperactivity, tail rigidity, and general tremors. Field potentials were evoked by stimulation of the Schaffer collateral/commissural fibers in the stratum radiatum and recorded with microelectrodes (1-3 megohms, 3M NaCl) in the stratum pyramidale layer of area CA1. Ethanol (60 and 100mM) was added to the artificial CSF perfusion fluid in a flow-through chamber maintained at 34-35°C. Input/output relationships of field potential population spike amplitude were obtained before and after tetanus (100Hz for 999ms) in the presence of bath applied ethanol. We found that LTP developed in the presence of ethanol in both normal and ethanol-dependent rats. The results indicate that ethanol does not block the induction of LTP in either normal or ethanol-dependent animals.

169.6 ABSENCE OF REINFORCEMENT WITH LOW DOSE INTRAVENOUS ETHANOL SELF-ADMINISTRATION IN RATS. R. Numan. Dept. Psychology, Univ. Santa Clara, Santa Clara, CA 95053

Male hooded rats were implanted with intravenous cannulas and housed in operant chambers supplied with 2 levers and enclosed in sound-attenuating cubicles. In Experiment 1, seven rats received a 1.0 mg/kg infusion of ethanol for each press on the previously determined non-preferred lever. The other lever served to count 'activity lever presses'. An additional 7 rats served as controls and were treated identically except that each press on the non-preferred lever led to an infusion of saline, isovolumetric to the ethanol infused in the experimental subjects. The rats were tested under these conditions of continuous reinforcement for 9 days. Throughout this period, self-infusions and 'activity lever presses' did not differ between the groups, suggesting that ethanol was not reinforcing at a dose of 1.0 mg/kg. These results were replicated, and extended to other low doses of ethanol in Experiment 2. Here, we employed a design where depression of either lever, under conditions of continuous reinforcement, led to the infusion of a solution. Fifteen rats were randomly assigned to one of three groups (5 rats/groups). In one group, depression of the previously determined non-preferred lever led to an infusion of 16.0 mg/kg of ethanol, while depression of the other lever led to an infusion of isocaloric glucose. For the other two groups, depression of the non-preferred lever led to an infusion of 4.0 and 1.0 mg/kg ethanol respectively, and depression of the other lever led to a glucose infusion. The animals were tested for 9 days, and in each case, ethanol self-infusions did not differ significantly from glucose self-infusions. These data confirm the absence of reinforcement with low doses of ethanol. Additional data are presented to support these findings, and we conclude that previous reports of reinforcing effects for low doses of ethanol self-administered intravenously by rats were probably due to the non-specific effects of ethanol. (Supported by NIAAA Grant AA05666)

169.7 EFFECTS OF ALCOHOL ON THE MONKEY'S OCULAR MOTILITY. J.M. Fuster, T.J. Willey and D.M. Riley*. Dept. Psychiatry and Brain Res. Inst., Sch. of Med., Univ. California, Los Angeles, CA 90024.

The purpose of this study was to delineate eye-movement changes produced by alcohol in the monkey engaged in visual cognitive performance. Rhesus monkeys with implanted EOG electrodes (Ag/AgCl) were tested in a visual memory task, delayed matching-to-sample, using colors as memoranda. The experimental animal, with head fixed, faced a panel with three translucid stimulus-response discs (8° each) forming an isosceles triangle with vertex up. On every trial, the top disc was briefly illuminated with a colored light--i.e., the sample. After a period of delay (about 10 sec.), the two lower discs were simultaneously illuminated, one with the sample color and the other with another color. The animal was then rewarded for pressing the disc with the sample color. The color of the sample and its position in the lower discs were changed at random from trial to trial.

In normal conditions the colored stimuli elicited short-latency gaze-directing saccades. At the sample, the eyes were fixated on the top disc and, at the choice, eye position alternated between the two lower discs before the manual response. During the intertrial periods and the intratrial delays, the animals executed regular and self-paced eye movements between the three discs. Ethyl alcohol, administered by remotely controlled intravenous infusion (dose range 0.25-2.00 g/Kg, two rates of infusion), induced the following dose-related changes in ocular motility: 1) slower and shorter fixations; 2) sluggish and wide-ranging saccades; 3) periods of ocular immobility (especially after higher doses). These changes were accompanied by longer reaction time and increased performance errors. Both ocular motility and performance returned slowly to normality as blood-alcohol level descended.

The results give evidence that alcohol, at doses above 0.25 g/Kg, alters markedly the normal patterns of ocular motility. Although the mechanism by which they occur is not clear, it appears that those changes by themselves were sufficient to impair the perceptual processes supporting mnemonic performance.

Supported by NIAAA Grant AA3513.

169.8 THE INTERACTION OF ETHANOL AND STRESS ON BODY TEMPERATURE IN RATS. J. Peris* and C L. Cunningham* (SPON: J. O'Brien). Dept. of Medical Psychology, Oregon Health Sciences Univ., Portland, OR 97201.

Previous research has suggested that stress may counteract the intoxicating (i.e., motor-debilitating) effects of ethanol. It has recently been suggested that stress may not always antagonize the effects of ethanol but may instead produce a synergistic effect when physiological responses are studied. The acute and chronic effects of ethanol were studied in rats surgically implanted with biotelemetric temperature sensors. This technique eliminated the necessity of repeated handling for temperature measurement, a procedure that has been shown to raise body temperature in rats and can be characterized as stressful. Temperatures of two groups of rats were monitored for 3 hr every other day for 28 days. One group was tested while stressed (the stressful stimulation consisted of repeated handling). The other group was left undisturbed after injection and placement in the test chambers. One half of each group received 3.0 g/kg of ethanol during injections while the other half received an equivalent volume of saline.

Ethanol in non-stressed rats induced hypothermic responses that peaked after 2 hr. Handling stress exacerbated the hypothermic effect of ethanol even though stress alone produced hyperthermia. Tolerance to the hypothermic effect of ethanol (as evidenced by a decrease in the magnitude of hypothermia after repeated ethanol exposure) developed at a faster rate in the stressed group relative to the non-stressed group. In addition, when both ethanol-experienced and ethanol-naive rats were later given an ethanol injection, only ethanol-experienced rats that had been stressed during previous ethanol exposures exhibited a decreased hypothermic response to the drug. Ethanol-experienced rats that had not been stressed during previous ethanol exposure were as hypothermic after the test injection of ethanol as ethanol-naive rats. Thus, stress exacerbated both the acute effect of ethanol on body temperature and the development of tolerance to this effect.

169.9 GENETIC SENSITIVITY TO THE ETHANOL WITHDRAWAL SYNDROME: GENETIC CROSS-SENSITIVITY TO A BARBITURATE AND A BENZODIAZEPINE IN MICE. J.K. Belknap, J.C. Crabbe, P.W. Danielson and M. Lame' Dept. of Pharmacology, Univ. of North Dakota School of Medicine, Grand Forks, ND 58202 and VA Med Center, Portland, OR, 97201 (J.C.C.)

Mice selectively-bred for their differential vulnerability to facets of the alcohol withdrawal syndrome have recently been developed by Dr. Crabbe. Animals showing the greatest severity of the alcohol withdrawal syndrome, as indexed by handling-induced convulsions, were interbred to form the alcohol withdrawal seizure prone (WSP) lines. Concurrently, those animals exhibiting the least withdrawal intensity were interbred to form the alcohol withdrawal seizure resistant (WSR) lines. This selective breeding process was repeated in each of five subsequent generations, resulting in a markedly more intense withdrawal syndrome (handling-induced convulsions) in the prone than the resistant lines (strains) after equivalent chronic exposure to the alcohol vapor and equivalent blood alcohol levels. Since all animals were treated identically, these differences between the lines are hereditary in origin.

We sought to determine if the genetic vulnerability differences bred into the WSP and WSR lines with respect to alcohol physical dependence had any counterpart with regard to physical dependence on other drugs. Accordingly, separate groups of WSP and WSR mice were made physically dependent on a barbiturate (phenobarbital), and a benzodiazepine (diazepam or Valium), and the ensuing withdrawal syndromes monitored following several days of chronic intoxication on one of these drugs. Paralleling findings with alcohol, marked differences between the WSP and WSR lines were seen with respect to handling-induced convulsions following withdrawal of either phenobarbital or diazepam. Brain concentrations of phenobarbital did not differ between the lines, so these line differences in vulnerability to the barbiturate withdrawal syndrome, which were similar to those seen with alcohol, occurred despite equivalent exposure to the drug. These data strongly suggest that the mechanisms causing the differential vulnerability between prone and resistant lines with alcohol are similar to those operating with the two sedative-hypnotic drugs, and support the contention that there are commonalities among these three drugs in mechanisms of action with respect to physical dependence. (Supported by PHS Grant DA 02723 and AA 06243)

169.10 CEREBRAL METABOLIC ALTERATIONS IN RATS FOLLOWING PRENATAL ALCOHOL EXPOSURE: A DEOXYGLUCOSE STUDY. R.D. Vingan*, D.L. Dow-Edwards, and E.P. Riley*(SPON:T.H. Milhorat). Laboratory of Cerebral Metabolism, Department of Neurosurgery, SUNY Downstate Medical Center, Brooklyn, N.Y. 11203 and Department of Psychology, SUNY Albany, Albany, N.Y. 12222

Numerous reports dealing with the gross morphological, microanatomical, and behavioral changes occurring with prenatal alcohol exposure in rats have appeared. In order to define the cerebral metabolic correlates of these alterations we chose the autoradiographic ^{14}C deoxyglucose method for measuring in vivo glucose utilization rates in brain (Sokoloff et al., J Neurochemistry 28:897-916, 1977).

Liquid diets containing either 35% or 0% ethanol derived calories (EDC's) were fed to pregnant Long Evans rats during day 6 through 20 of gestation. These liquid diets were isocaloric and a pair-feeding procedure was employed. The offspring were tested using Shuttle box avoidance techniques at 90 days of age and were subjected to the deoxyglucose procedures at 105 days of age. Under halothane anesthesia each male rat (400-450g) received femoral arterial and venous catheters. A loose fitting abdominopelvic cast was applied and the rats allowed to awaken for a minimum of 2 hours. Deoxyglucose (^{14}C labeled) was administered as an intravenous pulse (125 μCi/kg). Timed arterial samples were then taken for determination of the time course of the arterial plasma concentrations of [^{14}C] deoxyglucose and glucose. At 45 minutes the animals were sacrificed with sodium pentobarbital. The brains were removed, frozen and later sectioned for autoradiography as described by Sokoloff et al.(1977). Areas of interest were analyzed densitometrically to determine the local concentrations of ^{14}C in the tissues. From the local tissue $^{14}C_4$ concentrations and the time courses of the plasma [^{14}C] deoxyglucose and glucose concentrations, local rates of glucose incorporation were calculated by the operational equation of the method. Preliminary analysis suggests that alterations in glucose metabolism occur in the posterior pituitary, supraoptic nucleus of the hypothalamus, ventral caudal nucleus of the lateral lemniscus, median raphe nucleus and sensory-motor cortex.

169.11 DIFFERENTIAL SENSITIVITY OF CEREBELLAR NEURONS TO THE ELECTROPHYSIOLOGICAL EFFECTS OF ADENOSINE IN MOUSE LINES BRED FOR DIFFERENTIAL RESPONSIVENESS TO ETHANOL. Michael R. Palmer, Julie S. Promisel* and Thomas V. Dunwiddie. Dept. of Pharmacology, Univ. of Colorado Health Sci. Cntr., Denver, CO 80262.

Long sleep (LS) and short sleep mice (SS) were bred for their differential sensitivity to the soporific effects of ethanol. More recently, we have shown that the cerebellar Purkinje neurons from these two mouse lines express a differential sensitivity to locally applied ethanol. In this study, we investigated the electrophysiological sensitivity of Purkinje neurons to adenosine, a second agent to which LS and SS mice show a behavioral sensitivity difference. The effects of adenosine on 1) spontaneous Purkinje neuron activity and on 2) Purkinje cell activity evoked by parallel fiber stimulation were investigated in urethane-anesthetized LS and SS mice. Single unit activity was recorded from 1 or 2-barrelled glass micropipettes while adenosine was applied *in situ* both by surface superfusion and by micro-pressure ejection from 2-barrelled micropipettes. Adenosine caused potent depressions of Purkinje neuron firing rates after local pressure applications, and depressed the size of parallel fiber-evoked excitations of Purkinje cells when superfused in the 50 uM range. These effects were pharmacologically specific since they could be blocked by theophylline, an adenosine receptor antagonist. In addition, we found that LS mice were much more sensitive to the depressant effects of locally applied adenosine than were SS mice. We conclude that adenosine causes pharmacologically specific depressions of both the spontaneous and parallel fiber-evoked single unit activity of cerebellar Purkinje neurons, and that these neurons express a differential sensitivity to the depressant electrophysiological effects of adenosine in LS and SS mice. (Supported by USPHS grants AA05915 and AA03527, and VA 394463116. J. Promisel is a NIGMS predoctoral fellow.)

169.12 THE EFFECTS OF ETHANOL ON REWARDING INTRACRANIAL STIMULATION: RATE AND THRESHOLD MEASURES. E.M. Unterwald, J.A. Clark,* G. Bain* and C. Kornetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118

Multiple factors contribute to the use and abuse of alcohol. Many of these factors are probably unrelated or only indirectly related to its pharmacological action and include such things as social convention, peer pressure, taste, and psychopathology. Although these are contributing factors, it is likely that the ethanol effect itself must be reinforcing for there to be such extensive and continued use and abuse.

We, as well as others, have suggested that the facilatory effect of many abuse substances on rewarding brain stimulation is a relevant animal model for their reported euphoria. Previous studies have shown that the general effect of many abused drugs including morphine-like compounds, amphetamine, and cocaine is to facilitate rate of response or lower the threshold for self-stimulation to the brain. We tested the effects of ethanol on both rate of response for rewarding brain stimulation and on the threshold for rewarding brain stimulation in the rat.

Male CDF rats were stereotaxically implanted with bipolar electrodes aimed at the medial forebrain bundle at the level of the lateral hypothalamus (MFB-LH). One group of rats was trained to lever press for rewarding intracranial stimulation on a FR 2 schedule either with only initial priming or with regular intra-session priming. The other group was tested on a rate-free procedure utilizing a modification of the psychophysical method of limits to determine their threshold for rewarding brain stimulation. The acute effects of ethanol (0.025-1.6 gm/kg, i.p.) was studied with both procedures. At the higher doses, attenuation of response rate was found, particularly when rats were primed only immediately after injection. However, ethanol caused no significant change in the threshold procedure for determining brain-stimulation reward at any dose. The results of this study suggest that the reinforcing effects of ethanol is not mediated through activation of this major reward pathway.
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169.13 THE EFFECTS OF ETHANOL ON EMBRYONIC, HATCHING AND EARLY POST-HATCH BEHAVIOR IN THE CHICK. L. W. Means, J.L. Henson* and M. Burnette*. Psychology Dept., East Carolina University Greenville, NC 27834.

Three experiments were conducted to examine the behavioral effects of injection of ethanol into the air space of Black Sex-linked Eggs immediately prior to incubation. In experiment 1, which examined embryonic behaviors, 30 eggs were randomly assigned to each of 3 groups and injected with 200 µl of a 37.5%, 12.5%, or 0% ethanol solution. Observations of heart rate and gross body movements for 5 minutes on days 3.5, 4.0, 4.5, 5.0 and 6.0 of incubation revealed only that the low dose group made more gross body movements than the other 2 groups on day 6 ($p<.05$). In experiment 2, which examined hatching behaviors, 20 eggs were assigned to each of 3 groups which received the same dose injections given in experiment 1. The number of hours ($\bar{X}\pm SE$) to both pipping and hatching is shown in the table.

	Group (% ethanol)		
Dependent Variable	0.0	12.5	37.5
Hours to pipping	508.8±4.3	504.2±3.4	522.7±4.3*
Hours to hatching	523.0±2.2	522.3±2.1	534.3±4.0*
Squares entered day 3	4.0±2.9	1.7±1.3	1.5±1.2
Squares entered day 5	21.1±7.8	10.5±3.3	7.2±3.9
Trials to criterion	2.0±0.3	2.4±0.4	3.7±0.4**

* $p<.05$ ** $p<.01$

On both measures the high dose groups took significantly more hours than the other two groups, which did not differ from one another. In experiment 3, which examined post-hatch behavior, 30 eggs were assigned to each of 4 groups that were injected with 200 µl of a 50%, 37.5%, 12.5% or 0% ethanol solution. Due to low survival rates the 37.5 and 50.0 groups were combined and are listed in the 37.5 column in the table. Open-field tests given on days 3 and 5 after hatching revealed no significant differences on number of squares entered in 5 m among the groups. However, the high dose group (37.5 and 50.0 combined) did require more trials to reach criterion on a detour problem involving 1 trial/day on days 7-10. In summary, pre-incubation injections of alcohol affect relatively complex behaviors (pipping, hatching, detour learning) but do not affect relatively simple behaviors (heart rate, gross body movements, open-field activity).

Supported by an East Carolina University Research Committee Grant.

169.14 ALCOHOL EFFECTS ON AGGRESSIVE BEHAVIOR AND GONADAL HORMONES IN HIGH-STATUS SQUIRREL MONKEYS. J. T. Winslow*, K. A. Miczek, and J. Ellingboe. Dept. of Psychology, Tufts Univ., Medford, MA 02155 and Alcohol and Drug Abuse Research Ctr., McLean Hosp.-Harvard Med. Sch., Belmont, MA 02178

Alcohol's effects on primate social behavior may be mediated by gonadal hormones such as testosterone and luteinizing hormone (LH). We investigated the relationship between endocrine processes, sensitivity to alcohol, and social behavior within groups of squirrel monkeys, and quantitatively evaluated the impact of social status within a group on the behavioral and hormonal effects of alcohol. Continuous measurement of monkey behavior in groups with computerized recording devices revealed consistent behavioral differences between dominant and subordinate animals; dominant monkeys displayed aggressively, displaced and grasped others more frequently than subordinates. The amount of time allocated to social behavior in stable groups accounted for less than 10% of an individual's behavior, but was a significant determinant of the entire behavioral repertoire and gonadal activity. Blood samples revealed 267 ng/ml of testosterone in dominant monkeys (n=5) and 57.9 ng/ml testosterone in subordinate monkeys. Alcohol produced dose-related biphasic changes in the number of threats, grasps, and displacements exhibited only by dominant, but not by subordinate monkeys. Low doses of alcohol (0.1, 0.3, 0.6 g/kg) in dominant monkeys increased the frequency of aggressive behavior, and high doses (1.0 g/kg) decreased these behaviors. The largest change in behavior was evident in the first 20-40 min after injection, with a return to baseline 60-120 later. Low levels of aggressive and associative behavior in subordinate monkeys were relatively unaffected at any dose of alcohol. We are studying alcohol effects on testosterone and LH in order to assess how these hormones are related to the concurrently assessed status-dependent behavioral effects.

169.15 THE EFFECT OF ETHANOL ON CEREBELLAR PURKINJE NEURONS IN UNANESTHETIZED, FREELY MOVING RATS S.M. Sorensen, J.K. Chapin, M.O. West and D.J. Woodward Dept. of Cell Biology, Univ. of Texas Hlth. Sci. Ctr. at Dallas, Texas 75235.

The cerebellum has been implicated in the ataxia that is characteristic of ethanol (Et) intoxication. Electrophysiological studies have also shown that moderate Et doses can produce changes in the activity of cerebellar Purkinje (P) neurons. Unfortunately, since these studies involved recording neural activity in anesthetized rats, the observed changes could have been due to the combined effects of both Et and the anesthetic. To alleviate this problem we have developed techniques for measuring the dose and time dependent effects of Et on single P neurons recorded in the awake, freely moving rat. At least one week prior to the experiment animals were anesthetized and surgically implanted with a microelectrode drive apparatus. On experimental days animals were placed in a behavioral chamber and a tungsten microelectrode was lowered into the cerebellum until P neurons were well isolated. The rat was then allowed to move about freely while a computer was used to simultaneously record neuronal activity, behavioral and electrical stimulation events, and synch pulses from a video camera. P neurons recorded from the anterior intermediate zone in this fashion had firing rates that were highly variable and closely correlated with specific movements that the animal made. Most cells studied in this region showed 3- to 4- fold increases in rate associated with walking, usually because they fired during a particular limb movement. This is in sharp contrast to results obtained in anesthetized preparations where little variance in firing rate is noted. Et at 1 gm/kg had little effect on the overall rate of spontaneous discharge of P neurons in these experiments. Again, this is in contrast to earlier reports where the animals were anesthetized and where Et was observed to produce a biphasic change in the spontaneous discharge of P neurons: an initial increase in rate followed by a decrease. The results suggest that behavioral state of the animal plays an important role in determining the effect of ethanol on P neuron spontaneous activity and that more specific measures of P neuron function in relation to specific components of behavior may need to be investigated in order to understand the effects of Et on this system. Supported by grants AA0390, NS18041 & Biologic Humanics Found.

169.16 SEXUAL DIFFERENTIATION AND THE EFFECTS OF ALCOHOL ON AGGRESSIVE BEHAVIOR IN MICE. C.A. Lisciotto*, J.F. DeBold, K.A. Miczek (SPON: J. Politch). Department of Psychology, Tufts University, Medford, MA 02155

Alcohol differentially affects the aggressive behavior of male and female mice. This difference may be related to the sexually dimorphic sensitivity to testosterone. Male mice show enhanced aggressive behavior in response to moderate doses of alcohol when administered testosterone. Testosterone also makes male mice less sensitive to the aggression-reducing effects of high doses of alcohol. Female mice show enhancement of aggression at doses (0.1 g/kg, PO) which have no effect on males. Administration of testosterone to adult females does not cause the same alcohol response as in males, nor does castrating adult male mice induce a female-like response. To determine if this sex difference in alcohol response is influenced by sexual differentiation during development, this study manipulated the hormonal milieu of neonatal male and female mice.

On the day of birth male mice were castrated or sham-operated. Neonatal female mice were injected with 250 ug of TP or the oil vehicle. At approximately 75 days of age the mice which had not been gonadectomized at birth were gonadectomized. Control males and androgenized female mice then received 7.5 mm silastic capsules containing testosterone, SC. Aggressive behavior toward an intruder was assessed following administration of ethanol (0.1-3.0 g/kg) or water PO.

Neonatally sham-gonadectomized male mice responded as expected, with enhanced aggressive behavior following administration of 1.0 g/kg alcohol, and with no significant suppression of aggression at 3.0 g/kg. Neonatally androgenized female mice did not show the male-typical response to adult testosterone and alcohol, but neither did they show a female-typical response. Alcohol did not enhance aggression at any dose in the androgenized females, and suppression of aggressive behavior occurred at the high dose. Similarly, neonatally gonadectomized males showed dose response curves characteristic of neither males nor females. Their response was similar to that of the androgenized females.

Postnatal testosterone does not appear to completely determine the male- and female-typical responses to alcohol on aggression in mice. However, there did appear to be a small effect of neonatal androgen manipulation. Perhaps, the critical period for this sexually dimorphic response to alcohol and testosterone is primarily prenatal.

169.17 EFFECT OF ENVIRONMENT ON PLASMA CORTICOSTERONE OF MICE INJECTED WITH ETHANOL AFTER PASSIVE AVOIDANCE TRAINING. L. A. Lane[1]*, R. E. Poland[1]*, B. J. Branch[2]*, and D. L. Colbern[2]. (SPON: C. Melchior). [1]Dept. Psychia., Harbor/UCLA Med. Ctr. and [2]Brain Res. Inst., UCLA Sch. of Med., L.A., CA 90024.

Post-training ethanol has been shown to enhance passive avoidance behavior when mice are returned to their (group) homecage after training (Alkana et al., Psychopharmac., 66:117-119, 1979; Colbern et al., Substance and Alcohol Actions/Misuse, 1:181-186, 1980). However, these memory-facilitating effects of ethanol were not observed if mice were individually placed into novel environments for 90 min after training. Post-training stress may have interacted with the effects of ethanol and/or training stimuli to affect the outcome of subsequent avoidance testing. Since there are many stressors in both the home and novel environments, plasma corticosterone at various times after passive avoidance training was measured as an indication of stress experienced by mice in either environment.

Adult, male Swiss-Webster mice were given passive avoidance training with or without 0.1 mA footshock in the late afternoon. Immediately after training, each mouse was injected with 3.0 g/kg ethanol (15% v/v) or an equal volume of 0.9% saline (0.25 cc/10g). Half of the mice were returned to their homecage after training and injection; the other half were placed individually into novel environments (32 oz. drink cups). Mice from both environments were decapitated at 30, 60, 90, and 180 min after training. To simulate earlier behavioral studies, before decapitation at 180 min, mice were returned to their homecage after 90 min in the novel environment.

Corticosterone was determined by radioimmunoassay and blood ethanol levels were measured by gas chromatography. As expected, corticosterone levels were higher in saline-treated mice placed in the novel environment rather than those returned to their homecage. Corticosterone levels were much higher in ethanol-treated mice compared to saline-treated mice returned to their homecage. However, corticosterone levels were the same for both ethanol and saline-treated mice placed in the novel environment. The lack of difference between these two groups was not due to ceiling levels of corticosterone release, nor differences in ethanol metabolism since the time course of blood ethanol concentrations was the same for mice in both environments. Thus, we conclude that ethanol- and saline-treated mice experience environmental stress differently.

169.18 THE CONDITIONED PLACE PREFERENCE AND INTRACRANIAL REWARDING STIMULATION AND INTRAPERITONEAL INJECTION OF ETHANOL. Ph. De Witte, D. Poncin, M. Gewiss, B. Le Bourhis, G. Aufrere. Lab. of Psychobiology, Université Catholique de Louvain, Place Croix du Sud, B-1348 Belgium. and Institut de Recherches Appliquées aux Boissons 94015 Créteil, France.

In order to study the reinforcing property induced by brain stimulations or by drugs, the conditioned place preference (CPP) paradigm does not use goal-directed behavior to obtain the reward. Rats with implanted electrodes into the porterolateral area of the hypothalamus and into cortical region area were divided into four groups. A first group never received brain stimulations, a second received cortical non-rewarding brain stimulation, a third received hypothalamic non-rewarding brain stimulations (i.e. no self-stimulation using bar pressing rate paradigm) and, finally, the last group received rewarding brain stimulations. The conditioned stimuli are distinctive environment in a test chamber. In the morning rats were stimulated by experimenter every 1 min, for 10 brain stimulations of .25 sec during 10 min and in the afternoon rats were located in the other side. During these days, rats could not traverse one to the other compartment. On the following day, rats were allowed to cross the all apparatus from one to the other side and the time spent in each side was computed. Results show absence of statistical difference between the cortical groups and the group that had never been stimulated. On the contrary, the two hypothalamic groups show a strong preference for the side where they were stimulated (250% preference). The absence of statistical difference between the two hypothalamic groups seems to show that the CPP paradigm is more sensitive to estimate the reinforcing property of brain stimulations than the bar pressing rate paradigm.

A second series of experiments were designed to study the reinforcing property of ethanol. Rats were conditioned in the same two compartment box after receiving either .5, 1, 1.5 and 2 g/kg ethanol either saline solutions intraperitoneally given. Statistical analysis showed that .5 and 1 g/kg induced a place preference (i.e. 128% and 134% when compared to saline) while rats receiving 2 g/kg showed aversion for the compartment associated with this dosage. These results suggest thus that using the CPP paradigm, ethanol shows positive reinforcing properties for low dosage and negative reinforcing properties for high dosage.

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170.1 A TECTO-THALAMO-TELENCEPHALIC PATHWAY IN THE RATTLESNAKE: EVIDENCE FOR TRANSMISSION OF INFRARED AND VISUAL SIGNALS TO THE FOREBRAIN. D.M. Berson*, E.A. Newman, E.R. Gruberg and P.H. Hartline, Eye Res. Inst. of Retina Fndn., Boston, MA.

In most non-mammalian vertebrates, the optic tectum transmits visual signals to the forebrain by way of nucleus rotundus of the thalamus. Here, we show that rotundus exists in rattlesnakes, and provide evidence that it conveys infrared as well as visual signals to the forebrain.

After tectal injections of HRP, we observed anterograde labeling in a region of the ipsilateral thalamus that is occupied by rotundus in other species. Collaterals of tectorotundal axons crossed in the ventral supraoptic decussation and appeared to terminate at least sparsely in the contralateral rotundus. In sections stained for acetylcholinesterase, rotundus stood out as a region of relatively low enzyme content.

Tectorotundal cells, identified by retrograde transport from the thalamus, lay not only in the superficial tectal layers receiving retinal afferents, but also in deeper strata known to get input from the infrared system. In recordings made along the tectorotundal pathway or in rotundus itself, single and multiple-unit activity could be evoked by warm stimuli in total darkness as well as by thermo-neutral visual stimuli in the light. Some single units in rotundus had large, bilateral spatial receptive fields in both visual and infrared modalities; units habituated rapidly with repeated stimulation.

Anterograde and retrograde HRP tracing studies demonstrated projections from rotundus to a region of the ipsilateral dorsal ventricular ridge (DVR) of the forebrain. Single and multiple-unit recordings from DVR and from the lateral forebrain bundle, in which rotundo-DVR fibers run, revealed sensory response properties similar in many respects to those in rotundus, including input from both visual and infrared modalities, large, bilateral response fields, and habituation with repeated stimulation.

As yet, we lack direct evidence that the infrared and visual responses of DVR cells originate in rotundus. Nonetheless, our findings constitute strong presumptive evidence that extrageniculate thalamic nuclei transmit non-visual sensory information from the deeper tectal layers to the forebrain. Tecto-thalamo-telencephalic pathways have been implicated in form vision in other speicies, raising the possibility that in the rattlesnake, the infrared modality may participate in pattern recognition, in addition to its well-established role in orientation behavior.

170.2 KINEMATOGRAMS DRIVE DEEP TECTAL CELLS IN PIGEONS. °B.J. Frost, +P. Cavanagh* and °B. Morgan*, °Department of Psychology, Queen's University, Kingston, Ontario, K7L 3N6, and +Department de Psychologie, Universite de Montreal, P.Q. Canada, H3C 3J7.

Kinematograms are the motion domain equivalents of Julesz random dot stereograms. They are typically produced by moving a central region of dots in one direction while the surrounding region of dots are moved in a different direction. Human subjects report vivid figure-ground segregation upon viewing such patterns. If movement of the "figure" and "ground" regions is identical, then complete camouflage occurs. In this study, we have presented computer-generated kinematograms to directional neurons located in the deeper laminae of the pigeon's tectum.

Standard techniques were used to assess cells responses to moving stimuli and kinematograms. Element size, luminance and contrast of these kinematograms could be varied along with the size and shape of the "figure" region and the independent velocities and directions of "figure" and "ground". All units studied responded reliably to optically produced patterns where a light spot was moved in one direction while a background pattern was moved in the opposite direction (Frost and Nakayama, 1983). Additionally these same neurons responded vigorously to kinematograms moved in excitatory directions through their receptive fields. Velocity tuning curves for "figures" on a stationary "ground" showed reasonably tight velocity tuning curves. Similar tuning curves for the "ground" with the "figure" constant at optimal velocity showed massive inhibition for all velocities of "in-phase" motion. Of course when "figure" and "ground" were moving in the same direction, and at the same velocity, the "figure" was completely camouflaged. However, even when figure velocities were different from those of the ground (which makes figure visible again), but both moving in the same direction, no excitatory responses were produced. All cells responded when the "ground" was moved in the opposite direction to the "figure", and at slow ground velocities facilitated responses were often obtained.

Since there was no difference in texture density, contrast or luminance between "figure" and "ground" regions these results indicate that correspondence has been computed by this level, and figure/ground segregation on the basis of common motion vectors has occurred.

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170.3 FUNCTIONAL ANALYSIS OF LMmc OF PIGEON ACCESSORY OPTIC SYSTEM. P.J. Chown*, P. Ramm*, B.Morgan, B. Frost (Spon. J. MacPherson) Dept. of Psychology, Queen's University, Kingston, Ont. Canada. K7L 3N6

We have used 14C-2-deoxyglucose (2-DG) autoradiography to examine functional activity in the pigeon accessory optic system during exposure to moving whole-field visual stimuli. We have previously found that, although functional activity in the nBOR is strongly and selectively enhanced by a range of vertically moving whole-field stimuli, enhanced functional activity is only inconsistently evident in LMmc in response to horizontal motion. We now report that orienting the stimulus motion in alignment with the horizontal semicircular canal and in agreement with mean vector analyses of preferred directions of single cells recorded from this area results in consistent preferential labelling of contralateral LMmc.

Pigeons (n = 16) were anesthetized with urethane and placed in a stereotaxic. They were then monocularly exposed to stimuli consisting of a large random visual noise pattern, moving slowly (2-4 deg/sec) in horizontal (temporal to nasal and nasal to temporal) and vertical (up only or down only) directions. A pulse of 100 uCi/kg of 2-DG was injected into the wing vein after which the animals viewed the stimulus for 45 min. The birds were then sacrificed, sections cut at 20 u and autoradiographs prepared.

Analysis of the first group, which viewed forward-moving stimuli, reveals in every animal (n = 4) obvious preferential labelling of LMmc contralateral to the exposed eye. The enhanced functional activity extends throughout LMmc, but a strong focus is present at the ventral-posterior border of the structure, where it appears to join to the nBOR. The nBOR itself reveals only a very weak enhancement of functional activity contralateral to the exposed eye. Current analyses of the other direction groups will determine whether the LMmc effect is indeed specific to motion in the horizontal plane.

170.4 CONTRIBUTION OF NUCLEUS ISTHMI TO OPTIC TECTUM CHOLINE ACETYLTRANSFERASE ACTIVITY AND TO PREY CATCHING BEHAVIOR IN RANA PIPIENS. A.J. Ricciuti* and E.R. Gruberg. Biology Dept., Temple Univ., Philadelphia, PA 19122.

The superficial tectum of the frog, Rana pipiens, is the principal target of the contralateral retina and the exclusive target, bilaterally, of the nucleus isthmi. The superficial layers contain high levels of the enzymes acetylcholinesterase (AChE) and choline acetyltransferase (CAT). Using CAT as a marker we have investigated the possible cholinergic inputs to the superficial tectum. To do this we ablated retinal and n. isthmal inputs and determined CAT activity using Fonnum's method (J. Neurochem. 24, 407, 1975). For ablation of retinal input we either cut the optic nerve or enucleated the eye. To ablate n. isthmi we first recorded electrical activity in the nucleus and then passed 10 to 20 μamps for 5-10 minutes through the recording electrode. The animals were maintained post-operatively for 10-40 days. The extent of n. isthmi ablation was subsequently assessed by sectioning the tegmentum and staining for AChE activity. In addition, animals with n. isthmi lesions were tested for behavioral changes.

Up to 9 weeks after unilateral eye enucleation CAT activity is not significantly different in the deafferented tectum compared to the intact tectal lobe. However, at 14 weeks CAT activity is approximately 30% higher in the deafferented lobe compared to the intact lobe. Following unilateral n. isthmi lesions there is a significant difference in CAT activity in the tectal lobe ipsilateral to the ablated isthmi compared to the tectal lobe contralateral to the lesion. In cases where n. isthmi lesion is most complete CAT activity is approximately 4 times greater in the lobe contralateral to the lesion compared to the other lobe. When both n. isthmi are ablated there is a 90% reduction in CAT activity in the tectal lobes compared to intact controls. Also, we calculate that 85% of this isthmo-tectal CAT activity is contributed by the ipsilateral isthmo-tectal fibers.

Animals with isthmal lesions show pronounced but specific behavioral deficits of visually guided behavior. Following unilateral lesions the animals display an orienting scotoma contralateral to the ablated n. isthmi. In this region, they fail to orient to and attack prey. In avoiding barriers, unilaterally lesioned animals preferentially jump to the side ipsilateral to the lesion. Bilaterally lesioned animals appear to be blind to prey. Barrier avoidance, scototaxis and optokinetic nystagmus appear normal.

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170.5 A CHOLINERGIC PROJECTION FROM THE NUCLEUS ISTHMI TO THE OPTIC TECTUM IN TURTLE AND FROG. P.H. Desan, E.R. Gruberg and F. Eckenstein*. Dept. of Neurobiology, Harvard Med. Sch., Boston, MA 02115, and Biology Dept., Temple Univ., Philadelphia, PA 19122.

The midbrain of the turtle (Pseudemys scripta elegans) and the frog (Rana pipiens) were examined with an immunohistochemical method for the localization of choline acetyltransferase (CAT). The antiserum used was raised against purified mammalian CAT and reacts with putative cholinergic neurons in mammalian brain (Eckenstein and Thoenen, EMBO J. 1 363, 1982). In the turtle and frog it reacts strongly with the cell bodies and processes of neurons presumed to be cholinergic, such as cranial nerve motoneurons.

Virtually all of the cells of the nucleus isthmi caudalis of the turtle are intensely immunoreactive. A compact bundle of immunoreactive axons from the nucleus enters the tectum at the level of the central gray layer and forms a meshwork of coarse axons at the upper edge of the central gray running over the entire tectum. From these axons fine collaterals run upward to the pial surface. Multiple immunoreactive axon fragments and puncta are present in the superficial tectum and may represent the arborizations of these collaterals. This pattern of staining resembles the arborizations of axons from n. isthmi caudalis described by Sereno in the turtle (Soc. Neurosci. Abstr. 9 818, 1983).

Almost all of the cells of the nucleus isthmi of the frog are also intensely immunoreactive. Scattered reactive axons run from the nucleus into the tectum, principally at the level of the central gray. Reactive axon fragments and puncta, as well as a light diffuse labelling of the background, are present from the central gray to the pial surface. A zone of particularly heavy staining is present from the central gray to layer C of Potter. A narrow zone of heavier staining is also present in the stratum zonale. These zones coincide with the main terminal regions of the ipsilateral and contralateral projections from n. isthmi (Gruberg and Udin, J. Comp. Neurol., 179 487, 1978). Preliminary results show that unilateral lesion of n. isthmi results in a marked decrease in the number of axons stained in the appropriate termination zones of isthmo-tectal fibers.

These observations suggest that the cholinergic innervation of the tectum derives largely from the nucleus isthmi.

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170.6 CHOLINE ACETYLTRANSFERASE (ChAT) ACTIVITY IN LAYERS OF GOLDFISH OPTIC TECTUM FOLLOWING EYE REMOVAL. C. David Ross and Donald A. Godfrey, Dept. of Physiology, Oral Roberts Univ., Tulsa, OK 74171

Ten days after eye removal in goldfish, the ChAT activity in homogenates of the contralateral optic tectum was about 15% lower than in homogenates of the ipsilateral tectum. (The retinotectal projection in goldfish is almost completely crossed.) To see if this small change observed in the entire tectum might be concentrated in particular histological layers, such as those to which the optic axons project, samples of different layers were dissected from freeze-dried sections of tectum and assayed for ChAT activity. Fish were killed 4, 7, 10 and 20 days following enucleation of the right eye. ChAT activities in layers of the left tectum were compared to activities in the right tectum as well as to activities in 4 control animals. ChAT activities in samples from tectal layers were averaged from all lesioned animals so far examined (left tectum: 12, right tectum: 10) because no progressive decrease in activity in the left tectum was seen after 4 days. No significant differences were found in ChAT activities between left and right tecta in the marginal (m), combined optic (so) and superficial gray and white (sgw) (containing the highest density of optic axons and terminals), superficial central gray (scg), central gray (cg), or deep white (dw) layers. A significant difference between left and right tecta was found only in the periventricular (pv) layer, which is reported to receive little if any direct optic projection. ChAT activity in the pv layer was lower in the left tectum in all 10 lesioned animals in which both left and right tecta have been examined. These data indicate that the retinotectal projection in goldfish is not cholinergic, although the very high ChAT activity in the so/sgw layer does suggest a strong cholinergic influence upon processing of the retinal input. The ChAT activity change in the pv layer after enucleation is not enough to account for all the difference seen in the homogenates of whole tectum. (ChAT activity: micromol/kg dry wt/min)

LAYER	CONTROL	LEFT TECTUM	RIGHT TECTUM
m	86 ± 21 (26)	226 ± 34 (41)	363 ± 62 (20)
so/sgw	2971 ± 114 (57)	3027 ± 110 (99)	2901 ± 104 (63)
scg	2100 ± 129 (23)	1709 ± 101 (39)	1903 ± 91 (34)
cg	1136 ± 107 (69)	1210 ± 41 (151)	1261 ± 42 (101)
dw	879 ± 76 (23)	670 ± 36 (49)	773 ± 38 (42)
pv	1813 ± 111 (26)	*1366 ± 60 (59)	2071 ± 100 (40)

aver±S.E.M. (n)=no. samples; *diff. right tectum $p < .001$

SUPPORTED BY NIH GRANT EY 03838

170.7 CHOLINE ACETYLTRANSFERASE IMMUNOREACTIVITY IS LOCATED IN INTRINSIC NEURONS BUT NOT IN RETINAL AFFERENT TERMINALS IN THE GOLDFISH TECTUM. N. Tumosa, W.K. Stell. Dept. of Anatomy and Lions' Sight Centre, Univ. of Calgary, Alberta and F. Eckenstein*. Dept. of Neurobiology, Harvard Univ., Boston MA.

We have demonstrated the presence of putative cholinergic neurons in the optic tectum of the goldfish using the immunocytochemical localization of the acetylcholine synthesizing enzyme, choline acetyltransferase (ChAT), visualized by the indirect peroxidase-antiperoxidase (PAP) method. The stained neurons form a uniform population of cells which have their somata in layer I, the stratum periventriculare, and labeled processes that extend upward through all but the uppermost tectal layer. These neurons are a subpopulation of type XIV cells, which are cells that have their somata in layer I (Meek and Schellart, J. Comp. Neurol. 182:89-122, 1978). Type XIV cells can be either tectal interneurons or tectal efferent neurons. Which of these two classes the putative cholinergic neurons represent remains to be determined.

Despite the consistent staining of cell bodies in the tectum, we failed to find a change in the staining levels of layer 5, the stratum fibrosum et griseum superficiale, the primary zone of retinal input, following deafferentation of that layer by enucleation. This lack of change in staining of retinal terminals suggests that if ganglion cells are cholinergic then the loss of ChAT input into the tectum following enucleation is masked by cholinergic input from other, non-retinal, input.

Supported by the A.H.F.M.R. and the MRC of Canada.

170.8 RESPONSES OF SUPERIOR COLLICULUS CELLS TO ELECTRICAL STIMULATION OF THE PULVINAR IN RABBITS. S. Molotchnikoff, C. Casanova* and E. Sicard*. (Spon: F. Leporé) Dépt de Sciences biologiques; Université de Montréal, Montréal, P.Q., Canada H3C 3J7.

Anatomical studies in rabbits have demonstrated that the dorsal layers (visual) of the superior colliculus project to the lateral posterior nucleus (LP) of the thalamus or the pulvinar. This projection has been confirmed for other mammals, however, the role of this tecto-LP pathway (T-LP) remains obscure. Hence, the following investigations were undertaken to shed some light on the functions of the tecto-LP cells. Tecto-LP units were recognized by the disclosure of antidromic potentials and collision tests elicited from electrical pulses applied to the tecto-recipient zones of the pulvinar. Rabbits were anesthetized with urethane and prepared for single unit recordings from the superior colliculus using the common technique. In addition stimulating electrodes were lowered to the optic chiasma. Visual properties of the collicular cells were assessed with an image generator, the screen of which was positioned in front of the eye. To this point recordings have been made from one hundred and thirty-six cells in the stratum zonale, the superficial gray and the stratum opticum of the colliculus. Forty-eight responded to an electrical pulse of the pulvinar. Two types of responses could be distinguished: 1) trans-synaptic excitation via afferent fibers from the brachium of the superior colliculus or via the striate cortex, $N = 21$, $\bar{X} = 6.7$ ms and; 2) antidromic excitation, $N = 15$, $\bar{X} = 2.93$ ms. The pattern of responses varied considerably from cell to cell. Some units responded with a single antidromic spike, whereas in other cases a burst followed the antidromic action potential. Some units reacted with one post-synaptic action potential while others fired with a burst to a single pulvinar stimulation. One interesting trend seems to emerge from the preliminary data; virtually all T-LP cells failed to respond in a robust fashion to light stimuli when short diffuse flashes or localized images were positioned within the receptive fields.

Supp. CRSNG and FCAC

170.9 PROCESSES CONTRIBUTING TO SPATIO-TEMPORAL RESPONSE SUMMATION IN VISUAL CELLS OF CAT SUPERIOR COLLICULUS. N. Desai*, G. Mandl, and J.S. Outerbridge. Aerospace Medical Res. Unit and Biomedical Eng. Unit, McGill Univ., Montreal, Canada. H3G 1Y6

Desai et al (1981)[1] have shown that the discharge pattern of a visual cell in cat superior colliculus, in response to a single flashed slit stimulus, consists of an initial brief high frequency burst of spike potentials, followed by a prolonged period of subthreshold fluctuating depression of excitability. Since the latter observation implicates extensive 'occult' influences as potentially affecting processes of spatial and/or temporal response summation, the present experiments were undertaken to provide further data for a model of the integrative properties of the spike generating mechanism in collicular visual cells of pretrigeminal cat preparations. This was done by examining the relation (a) between stimulus-evoked (flashed slit) spike discharges and simultaneous subthreshold changes in excitability; and (b) between the statistical variability of stimulus-evoked, and 'background', firing. Extracellularly recorded unit discharges were averaged in the form of peristimulus time histograms (PSTHs). Subthreshold excitability was determined using paired conditioning-test (C-T) slit stimuli. Results: (1) There was, as a rule, no correlation between patterns of averaged spike discharges as determined by PSTHs, and the simultaneously evolving patterns of subthreshold excitability changes as revealed by C-T stimulation. (2) 'Spontaneous' fluctuations in the magnitudes of stimulus-evoked spike burst responses were statistically uncorrelated with similar fluctuations in background firing rates. (3) Significantly, there was positive correlation between mean firing rates and standard deviations, both for stimulus-evoked burst responses, and for periods of background firing. Taken together, these results imply that: subthreshold excitability changes; unit spike discharges associated with evoked responses; and spike discharges associated with background firing, represent three separate neural processes related to distinct 'compartments' of the cellular spike generating mechanism. This compartmentalized mechanism can be modelled as a series of linear summing junctions alternating with biased static nonlinearities. Consequently, a cell's overall firing pattern, in response to a sequence of identical flashed stimuli, cannot be inferred by simple summation of spike discharges pertaining to individual stimuli of the sequence. (Supported by the Canadian M.R.C.).

1. Desai, N., Mandl, G. & Outerbridge J.S. (1981). Proc. Can. Fed. Biol. Soc., 24, No. 331.

170.10 CONNECTIONAL ORGANIZATION OF THE CAT SUPERIOR COLLICULUS: A RETROGRADE TRANSPORT STUDY. T. Hashikawa*, M.F. Huerta and J.K. Harting*(SPON: G.J. Royce). Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706.

The retrograde transport of HRP has been used to reveal the cells of origin of particular tectofugal systems in the cat. Our overall goal is to relate the distribution of such cells with the distribution of specific afferent axons.

Our data reveal that several efferent systems of the superficial layers are organized into horizontally-oriented sublaminae within a given layer. For example, cells that project to the dorsal lateral geniculate, ventral lateral geniculate and lateral posterior nucleus are concentrated in sublaminae I, II and III of the SGS, respectively. Such an organization suggests that these three tectofugal systems might be spatially associated with known spatially segregated afferents (i.e., retina, visual cortex, parabigeminal nucleus). Other tectofugal systems arising from the SGS do not arise from specific sublaminae (i.e., the projections to the dorsal lateral pons and the parabigeminal nucleus). Since the cells of origin of these systems are spatially related to several specific afferent input, their axons most likely convey more diverse types of collicular information.

Like the superficial layers, efferent systems of the deeper layers, especially the SGI, arise both from cells which occupy specific subdivisions of that layer, as well as from cells which occupy more extensive regions. For instance, cells which project to the nucleus reticularis gigantocellularis and to the spinal trigeminal nucleus lie primarily in a horizontal tier which occupies the middle of the dorsal-ventral (D-V) axis of the SGI. In contrast, collicular neurons which project to the periaqueductal grey, spinal cord and to the sagulum lie primarily in a more ventral tier of the SGI. A slightly different spatial plan is exhibited by tectoolivary cells, which occupy two tiers, one at the top of the SGI and one at the bottom. Interestingly, many cells in these tiers lie in clusters, and it has been suggested that they might be "linked" with patches of deep collicular afferents, many of which are also tier-like in their distribution. While such specific input-output linkage might exist in several systems, the fact that the majority of deep tectofugal channels arise from cells which are scattered throughout the D-V extent of the SGI suggests that the cells projecting axons into many deep tectofugal channels convey diverse types of collicular information.

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170.11 INTERACTION OF "CLUSTERED" AND "ISOLATED" SPIKE DISCHARGES IN VISUAL CELLS OF CAT SUPERIOR COLLICULUS. G. Mandl (SPON: D. Watt). Aerospace Med. Res. Unit, McGill U., Montreal, Canada.

Mandl (1983)[1] has shown that some visual cells (Groups A and B) in cat superior colliculus respond to small moving slit stimuli by systematic alternation between burst (clustered) and single (isolated) spike discharge patterns; while the majority of units (Group C) yield bimodal interval histograms (IHs) suggestive of the coexistence of 2 patterns of discharge. Newly obtained data from pretrigeminal cat preparations now suggest that cells in Group C can be further reclassified on the basis of stimulus-related modulations of patterned discharges, ie: Subgroup C_1. Cells generate predominantly high frequency rhythmic bursts yielding double-humped IHs. Presentation of a moving slit stimulus results in velocity-related shortening of intervals between bursts, with little modification in the statistical properties of the intra-burst interval distribution. Elimination of short (intra-burst) interval classes from the spike record eliminates both humps of the IH. Subgroup C_2. Spike records consist of both clustered and isolated spikes, similar to those described by Cattaneo et al (1982)[2] for complex cells in cat visual cortex. Semi-log plots of IHs yield bimodal distributions that can be fitted with 2 regression lines, each related to one of the 2 spike processes. Stimulation with different slit velocities results in variations of line slopes, y-intercepts and correlation coefficients suggestive of stimulus-related but independent variations in the statistical properties of the 2 discharge processes, frequently without concomitant modifications in the amplitudes of related peristimulus time histograms (PSTHs). Elimination of short (intra-burst) intervals from spike records eliminates the first, but not the second, limb of the interval distribution. Taken together, these results imply: 1. Temporal patterns of discharge may yield information about stimulus velocity that is not available from a PSTH. 2. While cells of Groups A and B respond to moving slits by OR-type switching of clustered vs. isolated spike patterns, most cells in Group C respond with AND-type modulation of clustered and isolated spike patterns. 3. The simultaneous presence of statistically distinct spike discharge patterns supports the multi-compartment model of the spike generating mechanism suggested by Desai et al (1983)[3]. (Supported by the Canadian MRC).

1. Mandl, G. (1983). Neurosci. Abst. 9: 817.
2. Cattaneo et al. (1981). Exp. Brain Res. 43: 115-118.
3. Desai et al (1984). Neurosci. Abstr. 10.

170.12 GAD IMMUNOREACTIVITY IN THE PRETECTAL COMPLEX OF THE CAT. J.T. Weber and I-li Chen*. Dept. of Anatomy, Tulane University Medical School, New Orleans, LA 70112.

The presence of glutamic acid decarboxylase (GAD) immunoreactivity, as demonstrated by the PAP method, was used to identify the cellular components within the pretectal complex of the cat which presumably use the neurotransmitter GABA. At the light microscopic level, all pretectal nuclei contain both GAD-positive terminals and neurons, but their distribution varies among nuclei. Thus, GAD-positive terminals within the nucleus of the optic tract (NTO) are primarily located within the ventromedial parts of the nucleus and the terminal fields are reticulated in appearance. The GAD-positive terminal fields within the posterior pretectal nucleus (PPN) are also reticulated in appearance, extend the entire medial-lateral length of the nucleus and are continuous with the terminal fields in NTO. The GAD-positive terminals in the olivary pretectal nucleus (ON) and medial pretectal nucleus (MPN) are evenly dispersed. Within the anterior pretectal nucleus (APN), the terminals are more heavily concentrated in the pars reticularis. Samples indicate that GAD-positive neurons comprise about 10-20% of the total population in the NTO, 5-10% in the PPN and APN, 1-2% in the ON and less than 1% in the MPN. As with the terminals, the GAD-positive neurons within the APN are concentrated in the pars reticularis. Comparison of cell body areas reveals that in all pretectal nuclei the mean size of GAD-positive neurons is always smaller than the mean size of Nissl stained neurons. For example, a sample of Nissl stained neurons in the APN has a mean somal area of 228 μ^2 (S.D.=83.6) whereas, in the same region, GAD-positive neurons have a mean somal area of 154 μ^2 (S.D.=77.3). Although the majority of labeled neurons in the pretectum are small, a significant number of large neurons are also GAD-positive especially within the NTO and APN. These results, along with data on the cells of origin of known efferent pathways, allow us to predict that some GAD-positive cells in the pretectal complex are projection neurons. This hypothesis is currently being tested with the combined use of immunocytochemistry and retrograde transport methods.

Appreciation is extended to Dr. Irwin J. Kopin and colleagues for providing antiserum to GAD. Supported by NIH Grant EY03731 and the American Heart Association.

170.13 A DISCRETE POPULATION OF HYPERSTRIATAL NEURONS PROJECTS TO THE AVIAN PRETECTUM. Stefan R. Bodnarenko* and Olivia C. McKenna. Dept. of Biology, City College of the City Univ. of N.Y., New York, N.Y. 10031.

In birds and mammals it has been proposed that the postnatal development of a pathway from the visual telencephalon to a nucleus in the pretectum, which receives retinal slip signals used for horizontal optokinetic nystagmus (OKN), is responsible for changes in OKN response patterns that occur postnatally. In birds this pathway projects from the hyperstriatum or 'Wulst', a multilayered structure in the roof of the rostral telencephalon, to the lentiform nucleus of the mesencephalon (LM) in the pretectum. Before undertaking a developmental study of this projection, we used the HRP tract tracing technique in older birds to identify the hyperstriatal neurons projecting to the LM and to define their location within the hyperstriatum.

After iontophoretic application of 20% HRP in 0.5M Tris-HCl buffer, pH 8.4, into the LM of five week old chicks and a survival period of 24-48 hours, brains were processed for HRP histochemistry using the tetramethylbenzidine chromagen. Small injections confined to the LM resulted in a cluster of HRP labelled neurons in the ipsilateral but not contralateral accessory hyperstriatum (HA), the outermost layer of the hyperstriatum. The neuronal cell bodies were grouped together in a horizontally oriented column which was positioned in the ventrolateral HA and ran along the entire rostrocaudal extent of the HA with the exception of the rostralmost portion, described as somatosensory in other avian species. The ventral portion of the column contained greater numbers of labelled cells than the dorsal portion. All of the labelled neuronal cell bodies appeared either round or stellate in shape and measured between 15-23 μm in diameter. Larger injections, which spread beyond the LM into surrounding areas including the medial optic tectum, resulted in additional labelled cells in more superficial portions of HA. These results suggest that a discrete population of neurons that project to the LM can be localized within the ipsilateral HA. The development of this projection may play a role in the development of horizontal OKN in this species.
(Supported by NIH EY 03613)

170.14 DENDRITIC ARCHITECTURE OF THE NEURONS OF THE TERMINAL ACCESSORY OPTIC NUCLEI IN THE RAT, RABBIT AND CAT K.M. Gregory and R.A. Giolli. Dept. of Biology, CSU Long Beach, CA. 90840 and Dept. of Anatomy, UC Irvine, CA. 92712

The terminal accessory optic nuclei of the rat, rabbit and cat were studied by the Golgi-Cox impregnation method.

The dorsal terminal nucleus of this system (DTN) has been analyzed in the rabbit and cat. In the rabbit the DTN is located along the ventrolateral edge of the superior colliculus external to the brachium. It consists of small multipolar neurons having delicate unbranched dendrites, and medium-sized piriform cells, the somata located deep within the nucleus with their branched dendrites extending laterally. Often one dendrite extended medially into the nucleus of the optic tract (NTO). In turn, NTO neurons are seen to send dendrites into the DTN. In the cat the DTN lies caudolateral to the NTO. Its multipolar neurons range in size from large to small, and morphologically, their dendritic pattern is strikingly similar to NTO neurons. The rat DTN was not adequately impregnated for study.

The lateral terminal nucleus (LTN) in the rat and cat is located on the dorsal shoulder of the cerebral peduncle lateral to the zona incerta. In the rat it is composed of 1) medium-sized bipolar neurons with 1-2 long, primary dendrites oriented mediolaterally, and 2) small multipolar neurons with variably branched dendrites located in the most lateral part of the LTN. In the cat the LTN consists of medium-sized multipolar neurons with dendrites that are long, rarely branched, and oriented in the transverse plane. The LTN in the rabbit is found on the surface of the cerebral peduncle scattered within the superior fascicles, posterior fibers, of the accessory optic system. Like the rat DTN, it was not adequately impregnated for study.

In all three species the medial terminal nucleus (MTN) consists of 1) piriform cells with 2-3 dendrites extending ventromedially, 2) large multipolar neurons each with 2-3 ventromedially directed, radiate dendrites and 1-2 dendrites extending into the dorsal part of the MTN, 3) medium-sized bipolar neurons having long dendrites coursing parallel to the accessory optic axons, and 4) small multipolar neurons with delicate, seldom branching dendrites. Of the three nuclei, the MTN showed the greatest similarity in neuron morphology between the three species.
(Supported in part by N.E.I. grant EY03642 to R.A. Giolli)

170.15 NON-RETINAL AFFERENTS TO THE AVIAN ACCESSORY OPTIC NUCLEUS: AN ANATOMICAL AND ELECTROPHYSIOLOGICAL STUDY . L.R.G. Britto, A. Cukiert* and T.A. Azevedo*. Dept. Physiol. & Biophys., Institute of Biomedical Sciences, U.S.P., São Paulo, S.P., 05508, Brazil.

An interesting aspect of the accessory optic system functional organization is the occurrence of extraretinal projections to its nuclei. The present study aimed at investigating, by both anatomical and electrophysiological methods, such non-retinal inputs to the pigeon's nucleus of the basal optic root (nBOR) of the accessory optic system.

Unilateral deposits of either free HRP or HRP conjugated with wheat germ agglutinin were electrophoretically placed into the nBOR in 6 chloral hydrate-anesthetized pigeons. After a 48 h survival period, the birds were perfused and their brains processed by the tetramethylbenzidine technique . HRP-positive perikarya were found in the contralateral nBOR, the ipsilateral lentiform mesencephalic nucleus of the pretectum (LM), and a few also in the ipsilateral visual Wulst (W). The latter structures are generally considered to be equivalent to the mammalian pretectal nucleus of the optic tract and visual cortex, respectively.

In 12 other chloral hydrate-anesthetized pigeons, unitary and field potential recordings were performed in the nBOR, while electrical stimulation could be applied to the optic tract, LM, W and contralateral nBOR. LM and W stimulation generated clear facilitatory effects upon nBOR spontaneous and optic tract-evoked activity, whereas an inhibitory influence from its contralateral counterpart could be demonstrated.

The above data indicate that the avian nBOR is recipient of LM, W and contralateral nBOR afferents, which could modulate in an important way activity in the accessory optic system. Such an afferent connectivity could represent at least part of the morphofunctional substrate of some reported interactions between those structures in the elaboration of optokinetic nystagmus.

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170.16 DIFFERENT PATTERNS OF CORTICOPONTINE PROJECTIONS FROM DIFFERENT CORTICAL REGIONS WITHIN THE INFERIOR PARIETAL LOBULE AND DORSAL PRELUNATE GYRUS OF THE MONKEY. J.G. May and R.A. Andersen, Smith-Kettlewell Institute of Visual Sciences, San Francisco 94115; The Salk Institute of Biological Sciences, San Diego, CA 92138.

Different patterns of corticopontine projections were seen after injections of a H^3 proline/leucine mixture into each of four clearly distinct cortical areas within the inferior parietal lobule and dorsal prelunate gyrus of nine macaque monkeys (macacca fascicularis).

Injections confined to area 7a (5 cases) produced multiple foci of terminal label along the lateral margin of the lateral and ventral pontine nuclei. Small patches of label were occasionally seen in the rostral dorsolateral and dorsal nuclei. Injection of 7b or both 7a and 7b (2 cases) produced a similar pattern of label along the lateral margin of the pontine nuclei as well as a major terminal field in the medial portion of the ventral nucleus. These cases also exhibited patches of label in the paramedian and penduncular nuclei and more extensive label in the dorsolateral nucleus than seen in injections confined to area 7a.

In one animal the injection was confined to the caudal aspect of the lateral bank of the intraparietal sulcus (lateral intraparietal area, LIP). This injection produced multiple patches of terminal label stretching across the dorso-lateral and dorsal nuclei of rostral and mid pons. The most rostral and most caudal portions of the dorsal nucleus were free of label. There were also restricted patches of label in the lateral nucleus. Multiple injections into the dorsal prelunate gyrus (area DP) resulted in label limited to the dorsolateral nuclear region. As in the lateral intraparietal case there was no label in the ventral or paramedian regions and only sparse label in the dorsal portions of the lateral nucleus.

These results show different termination patterns for pontine afferents from the four cortical areas studied. These patterns are interesting in light of a growing body of data from functional and connectional experiments which have delineated different cortical fields within the inferior parietal region.
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170.17 PROJECTIONS OF VENTRAL MIDBRAIN TEGMENTAL NUCLEI UPON BRAIN STEM CENTERS KNOWN TO BE CONCERNED WITH THE DETECTION AND/OR GENERATION OF VERTICAL EYE MOVEMENTS. R.A. Giolli, R.H.I. Blanks, Y. Torigoe and D.D. Williams. Depts. of Anatomy & Surgery, Univ. of Calif. Coll. Med., Irvine, CA 92717.

Our continuing studies on the neuronal connections of the ventral midbrain tegmental nuclei of the rabbit have shown that the ventral and dorsal divisions of the medial terminal accessory optic nucleus (MTNv, MTNd), the visual tegmental relay zone (VTRZ; Giolli et al. '84, in press), the nuclei parabrachialis pigmentosus and paranigralis (pbp,pn), and the pars compacta and pars reticulata, substantia nigra (SNc, SNr) project variously, and to different degrees, upon certain brain stem nuclei, viz., the deep mesencephalic nucleus, pars medialis (DMNm), the pars oralis of the pontine reticular formation (rpo), the interstitial nucleus of Cajal (INC) and nucleus of Darkschewitsch (D), and the superior vestibular nucleus (vs), all demonstrated by others to be involved with detecting and/or generating vertical eye movements. The distribution and density of labeled neurons within the above-mentioned ventral midbrain nuclei have been determined after injections of horseradish peroxidase (HRP) into the DMNm, rpo, INC/D and vs. Data obtained are provided in the following table in which b, c and i refer to the presence of labeled neurons bilateral, contralateral and ipsilateral to the side of HRP injection and in which the density of labeled somata is indicated by a +1 to +3 with +3 being the greatest value.

HRP Injections into:	Labeled Neurons within: MTNv	MTNd	VTRZ	pbp	pn	SNc	SNr
DMNm	-	i,+3	i,+2	-	i,+1	i,+1	i,+1
rpo	-	i,+2	i,+2	-	i,+1	i,+1	i,+2
INC/D	b,+2	b,+2	i,+2	-	-	-	-
vs	c,+2	c,+2	c,+2	-	-	-	-
	i,+1	i,+1	-	-	-	-	-

The importance of these findings will be considered in terms of the roles ventral midbrain tegmental nuclei may play in the control of eye movements. (Supported by N.E.I. grant EY03642).

SYNAPTOGENESIS I

171.1 NONINVASIVE TECHNIQUES FOR LONG TERM MONITORING OF SYNAPTIC CONNECTIVITY IN CULTURES OF SUPERIOR CERVICAL GANGLION CELLS. H. Rayburn*, J. Gilbert, C.-B. Chien* and J. Pine. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

In order to study the establishment of synaptic connections in developing networks of cultured cells it is desirable to non-invasively monitor the connectivity. We are developing a system applicable to studies of networks formed by cholinergic rat superior cervical ganglion cells, as well as other dissociated cell cultures. Extracellular stimulation can be applied through one or more of an array of electrodes[1] fabricated as part of the bottom of the culture dishes[1]. Non-invasive recording of intracellular voltages can be achieved by using voltage-sensitive fluorescent dyes[2].

To date, two dyes have been found suitable for optical recording in cultures of rat SCG cells (without the need for signal averaging). Observations of cultures for two weeks following repeated staining with 10 μM solutions of these dyes, RH421 or RH237[3], revealed no observable toxic effects or developmental modifications. However, illumination of the cells to obtain optical recordings can generate photodynamic damage. Preliminary data indicate that illumination for several seconds under our normal recording conditions can be done repeatedly, at intervals of a day, with no observable effects on the cells. (This permits many recordings per session.) The illumination source is a mercury arc filtered to select the 540 nm line, and the intensity at the cells is roughly 0.2 μwatts per square μm.

The microcircuit culture dishes utilize platinized electrodes approximately 15 μm in diameter on the bottom of the dish. They have been successfully used to stimulate the cells extracellularly.

[1]Pine, J. and Gilbert J., Abstract 190.9, 12th Annual Meeting, Soc. for Neuroscience, 1982.
[2]Grinvald, A., Hildesheim, R., Farber, I. C. and Anglister, L., Biophys. J. Vol. 39, 301-308, 1982.
[3]The dyes were graciously supplied by A. Grinvald and R. Hildesheim. We are grateful for the invaluable help of Prof. Grinvald in screening a large number of dyes and helping us set up our optical recording system.

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171.2 SYNAPTIC VESICLE ANTIGEN IN PRESYNAPTIC AND APPARENT PRESYNAPTIC ELEMENTS.
Richard W. Burry, Department of Anatomy and Neuroscience Research Laboratory, Ohio State University, Columbus, Ohio 43210

The presence of a synaptic vesicle protein that binds monoclonal antibody #48 has been reported in nerve terminals from several adult tissues (Matthew et al., J. Cell Biol. 91 (1981) 257-269). The results reported here show the presence of the #48 antibody binding protein in cultures of developing rat cerebellum.

Cultures were examined after paraformaldehyde fixation and incubations with the #48 antibody and a secondary antibody labeled with rhodamine or horseradish peroxidase (HRP). In the light microscope, label was seen in aggregates of neuronal cells and in bundles of neurites between the aggregates. Thin sections from these cultures showed that presynaptic elements containing synaptic vesicles were labeled while the non-neuronal cells and neuronal somas did not label. In addition, many of the axons were labeled indicating that they were probably carrying the protein that reacts with the #48 antibody. The results confirm the localization of the #48 antibody in presynaptic elements and show that this antibody can be used with cultured neurons.

When polylysine coated large diameter sepharose beads are added to cultures of rat cerebellum, neurites grow up onto the beads and make contact with the charged surface of the beads. These neurites develop a swelling which contains an accumulation of vesicles and a membrane thickening at the site of contact with the bead (Burry, Brain Res. 247 (1982) 1-16). The swellings are called apparent presynaptic elements with the bead in the position of the postsynaptic element, and have been used as a model to study the formation of presynaptic elements.

With cultures incubated for electron microscopy as described above, most all of the profiles that could be identified as apparent presynaptic elements were labeled with the #48 antibody. Thus, apparent presynaptic vesicles contain the antigen that reacts with the #48 antibody suggesting that the vesicles are synaptic vesicles. In addition, these results suggest that the synaptic vesicle antigen to #48 antibody is present in vesicles prior to their arrival at the forming presynaptic element.

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171.3 SPINE FORMATION AND SYNAPTOGENESIS IN RAT VISUAL CORTEX: A SERIAL SECTION DEVELOPMENTAL STUDY. H. M. Hwang and W. T. Greenough. Neur. & Behav. Biol. Prog., and Depts. Psychol. and Anat. Sci., Univ. Illinois, Champaign, IL, 61820.

A recent quantitative study showed polyribosomes to be preferentially located at the base of dendritic spines of granule cells in adult rat dentate gyrus and that this association was altered reversibly during reinnervation (Steward and Fass, Prog. Brain Res. 59: 131-136, 1984). It was suggested that polyribosome carries out local protein synthesis to regulate synaptic function. The present study attempts to describe postsynaptic structural features associated with synaptogenesis during development, by assessing the relationship of polyribosome location with spine shape and presence of contacts in developing rat visual cortex. Two sets of four littermates aged P13, P15, P20, and P25, and two adult rats aged P120 were used. For each animal, one set of montages, composed of 4 to 6 electron micrographs, covering segments of 2 large parallel (presumed to be apical) dendrites in lower layer IV of visual cortex, taken from 20 serial thin (70nm) sections were used to trace the dendritic profile and note polyribosome location on transparencies. Using several cytological landmarks, each tracing was aligned so as to reconstruct the dendritic segments 3-dimensionally. A computer program digitized each tracing and calculated the surface area, volume, and ratios of these values between dendritic shaft and spines. Results, below, show a dramatic shape change in spine development. Younger animals have more nubby or "sessile" (I) and club-shaped (II) spines. Chubby (III) and big-headed (IV) were more frequent in older animals. A majority of spines (criteria included cisternae oriented toward apex of a protrusion in the dendritic surface with no microtubules or mitochondria present) in younger animals was "empty", i.e. without an obvious presynaptic contact. After day 25, most spines had obvious contacts. Polyribosome distribution in spines appeared to shift from head to base with increasing age.

Age	Spine Type (%)				Polyribosome Location			% With Synapse
	I	II	III	IV	Head	Stem	Base	
P13	38	40	13	9	32	6	62	21
P15	29	41	12	18	41	6	53	22
P20	28	46	3	23	40	3	57	22
P25	6	27	4	63	15	7	78	81
Adu.	16	14	16	54	11	0	89	91

Supported by NIMH 35321 and NSF BNS 82 16916.

171.4 FUNCTIONAL CORRELATES OF DIFFERENTIAL REARING IN RAT DENTATE GYRUS. E. J. Green* and W. T. Greenough (Spon: C. L. Prosser). Neural and Behavioral Biol. Prog., and Depts. Psychol. and Anat. Sci., Univ. Illinois, Champaign, IL 61820

While numerous studies have examined structural correlates of relative environmental complexity, there have been few reports concerning neurophysiological consequences of such treatments (e.g. Sharp et al., Soc. Neurosci. Abstr., 9(2), 1983) and no attempts at combined structural-functional analyses. In the present study, in vitro field potential analyses of entorhinal-dentate function were conducted as part of a larger effort to characterize the hippocampal response to experience.

Rats in the environmental complexity condition (EC) were group reared from weaning in wire mesh cages containing a three dimensional lattice of wooden, metal and plastic objects which were changed daily, and were allowed free exploration in a separate play area containing a similar set of objects for 30 to 60 minutes each day. Rats in the isolated condition (IC) were housed singly in standard laboratory tubs. Following 25 - 34 days of differential rearing, hippocampal slices from littermate pairs were prepared for physiological investigation. Population responses were recorded in the stratum granulosum (ventral blade) of the dentate in response to stimulation of afferents in the middle molecular layer. Slices from EC rats exhibited significantly larger population spikes at the higher stimulus intensities. Measures of granule cell excitability (ps amplitude / epsp slope) failed to reveal group differences. Slices from EC rats also exhibited significantly larger field epsp's across a wide range of stimulus intensities. Thus, the increased activation of granule cells in EC slices appears to reflect an increased synaptic input to those cells.

These results are consistent with the in vivo observations of Sharp et al., and indicate a significant increase in the functional connectivity between entorhinal cortex and dentate gyrus resulting from exposure to a relatively complex environment. Neurophysiological and anatomical studies in progress are designed to further evaluate these functional changes, and to determine whether the increased functional connectivity is a result of alterations in synaptic number, synaptic strength, or both.

Supported by NIMH 35321, PHS EY07005, System Development Foundation, and Univ. Il. Research Board.

171.5 DENDRITIC VOLUME AND POSTSYNAPTIC ELEMENT CHARACTERISTICS IN THE OCCIPITAL CORTEX OF RATS REARED COMPLEX, SOCIAL OR ISOLATED ENVIRONMENTS. Anita M. Sirevaag and William T. Greenough. Depts. Psychol. & Anat. Sci. and Neur. & Behav. Biol. Prog., Univ. Illinois, Urbana-Champaign, IL 61820.

Quantitative studies of Golgi-stained occipital cortex have indicated that neuronal dendritic fields are more extensive in rats reared in complex environments (EC) than in rats reared in social (SC) or isolated (IC) environments. The present electron microscopic estimates of dendritic volume per neuron confirm these results and suggest that some postsynaptic element characteristics may be fairly stable even though synaptic diameter or PST length may change. Members of 11 littermate triplet sets of male Long Evans Hooded rats were reared in EC, a group of rats in a large toy filled cage with daily exposure to a playbox; SC, pairs in a cage; or IC, individual cage for 30 days postweaning. Micrographs representing 236 μm at a final magnification of 41,786X were randomly taken from cortical layers I, II-III, and IV of area 17 excluding somata and blood vessels. Dendritic volume density and spine measurements were calculated from digitized profile tracings. Dendritic volume per neuron was almost 11% greater in EC than IC rats, as expected from prior Golgi studies. This indicates that quantitative Golgi measurements accurately reflect differences in dendritic field dimensions. Layer IV of EC rats contained a small population of larger synaptic contacts that were absent in IC rats and in 10 of 11 litters PST length was greater in this layer. This was accompanied by an increase in presynaptic area in 9 of 11 litters but not by an increase in postsynaptic area. These differences were not seen in other layers. Thus postsynaptic elements of EC rats do not appear to increase in size comparably to synaptic diameters, PSTs and presynaptic elements.

Supported by NIMH 35321.

	EC	SC	IC	EC vs. IC
Dendritic volume per neuron	4947μm^3	4855	4408	p<.02
Largest synaptic diameter La. IV	1.44μm	1.08	0.96	p<.02
Longest PST La. IV	1.32μm	0.96	0.84	p<.03
Largest presynaptic element La. IV	611μm^2	520	481	p<.05
Largest postsynaptic element La. IV	330μm^2	363	363	NS

171.6 COMPARISON BETWEEN THE DEVELOPMENTAL CALENDARS OF THE CEREBRAL AND CEREBELLAR CORTICES IN A PRECOCIAL AND AN ALTRICIAL RODENT: Almut Schüz* (SPON: V.Braitenberg) MPI für biol. Kybernetik, D-7400 Tübingen, FRG.

The development of dendritic spines and synapses in the cerebral cortex of mice and rats is subject to environmental influences (f.ex. Valverde, 1971). However, in the guinea-pig the majority of spines and synapses develop before birth. This makes us hesitant to connect these changes to processes of learning. Similarly, in the cerebellum of rats and mice the migration of granular cells to the internal granular layer and the connection between parallel fibers and Purkinje cells occur mainly after birth. Again, in the guinea-pig, most of the granular cells have already migrated downwards at birth, the parallel fibers are well developed, the Purkinje cells have an adult shape and are densely covered with spines. The inner granular layer is full of mature granular cells showing the typical claws. The Golgi cells,too, have their adult shape. The stellate and basket cells are also well developed. The only striking pecularity on stellate and basket cells compared to the adult seems to be a higher number of long, spinelike appendages on their dendrites and sometimes also on the soma. Thus, in the cerebellum, too, a developmental calendar seems to be ruling which is not affected by the event of birth happening earlier or later in different species.

In the precocial as well as in the altricial animal the differentiation of the granular layer in the cerebellum is roughly synchronous with the increase in spines in the cerebral cortex, and both processes seem to be completed at about the same time.

We may suspect that not only in the cerebral cortex but also in the cerebellum, a learning machine is set up which is ready to incorporate information when the animal begins to discover the environment. The parallelity in the development of the cerebral and the cerebellar cortices could mean that motor learning in the cerebellum as envisaged by Marr (1968)and Albus (1971) is intimately connected with the other kind of learning.

This investigation has been carried out on Nissl and Golgi preparations of mice and guinea-pigs.

171.7 A STEREOLOGICAL APPROACH TO SYNAPTOGENESIS. H. Newman-Gage* and L.E. Westrum. (SPON: L.M. Halpern), Depts. of Neurological Surgery and Biological Structure, Univ. of Washington, Seattle, WA 98195.

The aim of this study is to quantify the changes in number of synapses and synaptic elements during the initial stages of development in fetal rat pyriform cortex. Our previous studies have shown that synapses first appear at embryonic day 16 (E16) and that the period from E15-21 (birth) exhibits exaggerated synaptogenic activity. We also find profiles which have some synaptic characteristics, such as small submembranous densities and an accumulation of vesicles, but lack the other distinguishing features of synapses. These vesicular puncta may be precursors of immature synapses and as such would be protosynaptic. We are adapting new stereological techniques employed in cell biology for volumetric quantification of particle numbers in an effort to correlate changes between numbers of synapses and numbers of possible protosynaptic elements. In addition to yielding information about critical periods of synaptogenic activity, these techniques may also provide information about how synapses are generated.

The stereological methods we are adapting to synaptogenesis require no special assumptions about size, shape, or orientation of the particles of interest within the tissue other than that they are disjoint and randomly arranged. The method proposed here estimates number per volume (N_V) using measurements from random plane sections (micrographs) and several sets of serial sections containing particles of interest. The serial sections are used to determine a length-weighted size factor used in calculation of N_V.

Using these stereological methods we have shown a statistically significant increase in the number of synapses per unit volume from E15 to E19 ($p<.006$) and from E19 to E21 ($p<.008$). However, while there is a significant increase in the number of vesicular puncta between E15 and E19 ($p<.01$) there is not a similar increase between E19 and E21. Using E19 as a representative age during this synaptogenic spurt, we have compared the numbers of synapses and vesicular puncta between sublaminae (lateral olfactory tract; *LOT* and layer I). There are significantly more synapses in layer I than in the *LOT* ($p<.05$) and more vesicular puncta in the *LOT* than in layer I ($p<.02$). These studies show specific laminae-dependent and age-dependent changes in the density of synaptic and protosynaptic elements. (Supported in part by NIH grants NS 09678, NS 17111, and DE 04942. LEW is an affiliate of the CDMRC.)

171.8 QUANTITATIVE SYNAPTIC MORPHOMETRICS: A COMPARISON OF THE OSMIUM AND EPTA STAINING PROCEDURES. T. L. Petit and J. C. LeBoutillier.*
Division of Life Sciences, University of Toronto, Toronto, Ontario, Canada M1C 1A4.

Neurobiologists have come to rely on morphometric analyses to aid them in understanding anatomical perspectives of the brain. At the electron microscopic level, researchers interested in studying synaptic structure have used both the classic osmium staining technique and the more recently developed ethanol-phosphotungstic acid (EPTA) stain. These two procedures stain different properties of the synapse; therefore, a quantitative comparison of the two techniques is important to determine whether they are interchangeable. This is of particular importance in investigating human tissue from autopsy cases where degeneration may alter the synaptic parameters. Adult male hooded rats were sacrificed (3 per time interval) and tissue from the sensorimotor cortex excised at the following time intervals- 0,1,2,4,6,8,10, 15,24, and 36 hours postmortem. Upon excision, all samples were immediately fixed by immersion in 2% glutaraldehyde in Millonigs buffer for 2 hours with at least three changes of solution. One half of each sample was then placed in 1% osmium tetroxide in Millonigs buffer for 1 hour at 4°C. Following dehydration in a graded series of ethanol and acetone, the tissue was embedded in Spurrs. The remaining one half of each sample was dehydrated in ethanol and stained for one hour in 1% phosphotungstic acid in ethanol (EPTA) and embedded in Spurrs. A minimum of four blocks (two for each staining procedure) from each brain were prepared for electron microscopy. Photomicrographs were systematically taken throughout the molecular layer. Synaptic density was determined using the Leitz Bioquant II Image Analysis System from micrographs at 300,000X. Synaptic structures are remarkably stable postmortem, although differences were found between the osmium and EPTA staining techniques. This research was supported by grants from the Ontario Mental Health Foundation and the Natural Sciences and Engineering Research Council of Canada.

171.9 SPINAL CORD AND CILIARY GANGLIA CELLS INDUCE COLLAGEN FORMATION BY MUSCLE CELLS IN CULTURE. Z.Vogel, D.Duksin* and C.Kalcheim*. Depts. of Neurobiology and Biophysics. The Weizmann Institute of Science, Rehovot 76100, Israel.

We have previously reported the presence of a collagen inducing factor (CIF) in rat embryonic brain which stimulates cultured muscle cells to produce collagen types I,III,IV and V upon the activation of prolyl hydroxylase. (Duksin et al., J. Biol. Chem.,258:14585, 1983; Vogel et al., Neurosci. Abs., 9;843,1983). Here we report that the CIF is an ascorbate-like molecule. It is eluted adjacent to ascorbate when filtered through Biogel P-2 and Sephadex G-10. The peak of biological activity showed properties of a reducing agent and both the biological activity and the reducing activity were abolished upon treatment with ascorbate oxidase.

Applied to muscle cultures, CIF promoted the hydroxylation of prolyl residues in procollagen and its subsequent secretion into the culture medium. The amounts of labeled proline and hydroxyproline incorporated into secreted proteins were determined by TCA precipitation and amino acid analysis of the culture media. A 15-fold increase in the hypro/pro ratio was obtained for secreted proteins in treated cultures as compared to controls. High speed supernatant of homogenized rat pheochromocytoma (PC12) cells induced a 7-fold stimulation in this ratio over control(10.6% and 1.4% respectively). Homogenates of various neuroblastoma and glioma cell lines (NG108-15, N18TG-2, N4TG-3A, N115, C6BU-1) showed no significant activity.

Co-culturing spinal cord explants obtained from 15-18 day -old rat embryos with muscle cells for a period of 6-24 hr induced a 20-25 fold stimulation in hypro/pro compared to muscle cultures grown in absence of nerve. Similarly, a 7.5 -3 fold stimulation was measured in 6-72 hr co-cultures of muscle cells with 8 day-old chick ciliary ganglia. The elevation in the hypro/pro ratio was abolished when ascorbate oxidase (5 units/ml) was added together with the explant.

It is an appealing hypothesis that CIF is released from nerve cells during neuromuscular synapse formation to promote collagen secretion and to facilitate the assembly of basement membrane around muscle. (Supported by a grant from the Muscular Dystrophy Association).

171.10 INDUCTION OF SYNAPTIC DIFFERENTIATION IN CULTURED MUSCLE CELLS BY YOLK. H. Benjamin Peng* and Donald R. Markey* (SPON: C.H. Anderson). Dept. of Anatomy, University of Illinois at Chicago, P.O. Box 6998, Chicago, IL 60680.

The development of the neuromuscular junction involves a differentiation of both the presynaptic and the postsynaptic sites. The postsynaptic differentiation is manifested by a concentration of acetylcholine receptors (AChR) and the formation of both cytoplasmic and extracellular specializations. In cultures of *Xenopus* myotomal muscle cells, yolk platelets released from broken cells often become adherent to intact cells. Since the yolk is a major protein storage of blastomeres, the yolk-muscle contact offers a good system to study the interaction of endogenous proteins and the muscle cell with respect to the formation of the postsynaptic apparatus.

When the cultures were labeled with a rhodamine conjugate of α-bungarotoxin (R-BTX) followed by fluorescence microscopy, 20% of the platelet-muscle contacts exhibited intense fluorescence, thus indicating the concentration of AChR. Since many platelets seemingly colocalized with the muscle under the light microscope were not in actual contact with the cell and many still had intact membranes, the effect of cluster induction by the yolk should be much higher. With electron microscopy, elaborate specializations were observed at nearly all the contacts examined. These included a meshwork of 5-6 nm filaments, which excluded cell organelles except a system of smooth membrane cisternae, periodic dense 11-14 nm particulate structures associated with the cytoplasmic face of the membrane, extensive membrane infoldings and a basement membrane spaced at a uniform distance of about 20 nm from the cell surface. In contrast, contacts with platelets still having intact membranes at the interface showed a total absence of these specializations. They were only observed when the crystalline main body of the yolk was in contact with the cell.

These results show that proteinaceous core of the yolk is a strong inducer for postsynaptic differentiation. Our previous works have shown that similar induction can be effected by polybasic amino acids (Nature 292:831, 1981). It is interesting to note that lysine and arginine constitute more than 13% of the residues of the yolk core proteins. Thus, experiments with defined endogenous proteins such as the yolk may help clarify the molecular basis of the trophic interaction during synaptogenesis. (Supported by NIH grant NS 16259 and MDA).

171.11 DEVELOPMENTAL CHANGES IN THE NUMBER OF INTRACELLULAR ACETYLCHOLINE RECEPTORS IN XENOPUS MYOTOMAL MUSCLE. J. Goldfarb*, C. Cantin* and M.W. Cohen. Dept. of Physiol., McGill Univ., Montreal, Quebec, Canada H3G 1Y6.

Intracellular AChRs in Xenopus myotomal muscle were exposed and labelled according to the procedure described by Fambrough and Devreotes (J. Cell Biol. 76, 237-244, 1978). For each experiment at least eight muscles of the same developmental stage were incubated in α-bungarotoxin (αBT) to saturate surface AChRs, fixed with paraformaldehyde to preserve muscle structure and permeabilized with saponin to provide access to intracellular AChRs. The muscles were then divided into two groups. One group was re-exposed to αBT and then both groups were incubated in ^{125}IαBT. Specific intracellular binding was estimated from the difference in ^{125}IαBT binding between the two groups. Non-specific binding was typically 10-20% of total binding.

In control experiments more than 93% of the surface AChRs retained their binding sites for ^{125}IαBT after fixation, saponin did not extract surface AChRs from fixed muscles and saponin did not inhibit ^{125}IαBT binding to surface AChRs. When carbachol was used instead of αBT to measure non-specific binding in fixed muscles, the estimated number of surface AChRs remained essentially unchanged but the number of specific intracellular binding sites was reduced by 25%. These findings suggest that at least 75% of the specific intracellular binding sites are AChRs.

In muscles from stage 24 animals (26 hr old) the number of specific intracellular binding sites per myotome was ~35 million. This value tripled over the next 24 hr of development and then became more variable but exhibited no further consistent increase. By contrast the number of surface AChRs per myotome continued to increase at a rate of 5-8 million/hr. These results suggest that newly synthesized AChRs which are destined for insertion into the plasma membrane remain intracellularly for less than 7 hr at stage 24, and possibly longer at later stages of development. Considered together with previous observations on myotomal dimensions (J. Physiol. 339, 553-571, 1983) the results also suggest that the number of intracellular AChRs per unit volume of myotome is close to a maximum value by stage 24, when the myotomes are less than 9 hr old, and begins to decline a few days later. Autoradiography should provide more direct information in this regard and indicate the distribution of the intracellular AChRs.

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171.12 RELEASE OF ACETYLCHOLINE FROM GROWING GROWTH CONES OF CULTURED XENOPUS NEURONS: THE INFLUENCE OF EXTERNAL CALCIUM. S. H. Young. Dept. of Physiology and Biophysics, University of Calif. Irvine, Irvine CA 92717.

Two studies of the release of acetylcholine (ACh) from growth cones (Hume, Role, and Fischbach Nature 305:632, 1983; Young and Poo Nature 305:634, 1983) differ in one important aspect: Hume et al. report no spontaneous (unstimulated) release whereas Young and Poo did find spontaneous release. Aside from the difference in neuronal preparations (embryonic chick cilliary ganglion vs. embryonic Xenopus neural tube), the bath concentrations of calcium were not equivalent (3.8mM vs. 0.4mM).

I have investigated the influence of external calcium on spontaneous release of ACh from growth cones of embryonic Xenopus neurons in culture. Culture media and recording salines contained the same calcium concentrations. Release of ACh was monitored within 3-6um of the growth cones with the use of an outside-out piece of muscle membrane attached to a patch clamp recording system. The recording saline was changed frequently, but at very slow rates in order to prevent excessive solution flow across the neurons.

In 10mM calcium, release of ACh is essentially stopped. Only one out of seven growth cones showed even slight evidence of release. In 2mM calcium, 7 out of 13 growth cones showed release, but at rates below those reported previously in 0.4mM calcium. In addition, no rapid bursts of release ('staircases') were observed. Such bursting was seen in 0.4mM calcium.

The influence of calcium on transmitter release from the growth cone appears similar to that reported for 'non-quantal' or 'leak' release from Xenopus (Sun and Poo in press) and mouse (Vyskocil, Nikolsky, and Edwards Neuroscience 9:429, 1983) neuromuscular junctions.

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171.13 DEVELOPMENT OF SYNAPTIC CURRENTS IN IMMOBILIZED XENOPUS MUSCLE. R. Kullberg, J. Owens* and J. Vickers*. Department of Biological Sciences, University of Alaska, Anchorage AK 99508.

We have examined the effect of blocking muscle action potentials on the development of synaptic currents in myotomal muscle of Xenopus laevis. Hatched embryos were bathed continuously in tetradotoxin (TTX) 20 ugm/ml following the onset of muscle contractile activity at stage 24. Within a few hours of exposure to TTX, all spontaneous movement and responsiveness to external mechanical stimulation ceased. Animals survived for up to 5 days in TTX. By external criteria, their development appeared to be entirely normal up to stage 41, at which time they started becoming edematous. As an assesment of synaptic function, miniature endplate currents (MEPCs) were recorded throughout development and their rise times and decay constants were measured. At stage 24, MEPCs had rise times of 2.4 ± 0.5 msec (mean ± s.d.). Most MEPCs decayed as single exponentials having a mean time constant of 5.5 ± 1.7 msec. Exposure to TTX did not alter the subsequent shortening of MEPC time course which normally takes place during development. At stages 44-45, MEPC rise times were 0.6 ± 0.1 msec and decay constants were 1.4 ± 0.4 msec in TTX-reared animals as compared to 0.6 ± 0.1 msec and 1.6 ± 0.5 msec in controls. In both rearing conditions, MEPCs were frequently observed to have double exponential decays. The fast and slow decay constants were 0.7 ± 0.1 msec and 3.0 ± 0.9 msec in TTX-reared animals and 0.9 ± 0.1 msec and 2.5 ± 0.5 msec in controls, at stage 44-45. These values are comparable to the apparent open times of the fast and slow ACh receptor channels present in Xenopus muscle. Application of an anticholinesterase (methanesulfonyl flouride) lengthened the duration of MEPCs in control and TTX animals in a similar fashion. These results indicate that both AChE activity and ACh receptor channel gating kinetics developed normally despite block of muscle and presumably also nerve action potentials by TTX. (Supported by NIH grant NS17875)

171.14 ACETYLCHOLINE-LIKE ACTION OF ATP ON CULTURED CHICK MYOTUBES. Richard I. Hume and Marcia G. Honig, Division of Biological Sciences, University of Michigan, Ann Arbor, MI 48109.

It has been generally assumed that acetylcholine (ACh) is the only synaptic transmitter at developing neuromuscular junctions. However, Kolb and Wakelam (Nature 303:621) recently demonstrated that adenosine triphosphate (ATP) can open membrane channels in immature chick myotubes. These "ATP" channels have a unit conductance similar to that of the channels opened by ACh. Since ATP is present in synaptic vesicles and can be released by nerve stimulation, these findings raise the intriguing possibility that ATP may also act as a neurotransmitter at the developing neuromuscular junction. Transmitter release can be detected with high sensitivity by using isolated patches of receptor-rich membrane (Hume et al. Nature 305:632). To begin to address the role of ATP at newly formed synapses, we have compared the response of outside-out patches of chick myotube membrane to exogenous ACh and ATP.

Our principal results are:

1. ATP responsive patches can be found on both immature myotubes and on mature, striated myofibers (up to at least 12 days in culture).
2. All patches that respond to ATP also respond to ACh. In these patches the single channel conductance and reversal potential are the same for both substances.
3. Some patches respond to ACh, but do not respond to ATP. Such patches can be obtained from myotubes that are responsive to ATP at other sites.
4. The response to ATP is blocked by application of the nicotinic receptor antagonist d-tubocurarine.
5. Application of a desensitizing dose of ACh blocks the response to ATP; conversely, a desensitizing dose of ATP blocks the response of some but not all patches to ACh.

The blockade and desensitization experiments suggest that ATP acts on the nicotinic ACh receptor, rather than on a specific ATP receptor. However, the observation that some patches respond to ACh but not ATP implies that there is more than one type of nicotinic receptor in this muscle.

The use of patches with and without ATP sensitivity should allow us to evaluate the relative roles of ACh and ATP as neurotransmitters at developing neuromuscular junctions.

171.15 APPARENT NEURONAL REFRACTORINESS TO MUSCLE CONTACT AFTER SYNAPTOGENESIS. Ida Chow.Dept. of Physiology and Biophysics,Univ. California,Irvine CA 92717 and Jerry Lewis Neuromuscular Res. Ctr., UCLA School of Medicine, Los Angeles, CA 90024.

Previous studies (Chow & Poo,Abstr. Soc. Neurosci.9:688,1983) have shown that when a spherical muscle cell (myoball) was manipulated into contact with the soma of an isolated neuron MEPP-like depolarizations could be detected within min of contact. These depolarizations were due to ACh released from the soma. If the contact was made with the soma of a neuron which had established functional synapse with other muscle cells, no such activity was recorded for the first 30 min. Presently, the study has been extended to other regions of the neuron,i.e. along the neurite and over the growth cone. In contrast to soma-myoball contacts, MEPP-like depolarizations appeared after a much shorter delay, within seconds of contact. In several cases, the delay was smaller than the experimental time resolution (1 sec). About 70% of the neurites of isolated neurons released ACh after contact was produced, and 63% of these neurons responded within 4 min of contact. The characteristics of these ACh potentials were also similar to MEPPs recorded from neuromuscular junctions formed spontaneouslyin the culture, but with lower amplitude and frequency. When contact was produced between myoball and neurite or growth cone of a neuron innervating other muscle cells, only 58% of these identified cholinergic neurons released ACh during the initial 20-50 min contact. This decreased responsiveness of the neuron to further muscle contact was found both along the neurites and at the growth cones. These results suggest that this neuronal refractoriness may be due to a depletion of: ACh molecules, substances responsible for triggering muscle contact-induced ACh release, surface recognition molecules, or a combination of these factors at extrasynaptic regions of the neuron.

(Supported by grants from NIH and NSF)

OPIATES, ENDORPHINS, AND ENKEPHALINS: RECEPTORS II

172.1 A ROLE FOR THE KAPPA RECEPTOR AND DYNORPHIN IN BOVINE ADRENAL MEDULLA. M. Dumont* and S. Lemaire. Département de Pharmacologie, Faculté de Médecine, Université de Sherbrooke, Sherbrooke, Quebec, Canada, J1H 5N4

Dynorphin (Dyn), a potent endogenous opioid peptide, has already been demonstrated to selectively bind the kappa opioid receptor. Our findings of its presence in bovine adrenal medulla and of its secretion from the adrenal chromaffin cells have conducted us to investigate its binding to adrenal membrane preparations and its modulation of catecholamine secretion from isolated adrenomedullary cells. Characterization of the kappa opioid receptors was obtained at 37°C with [^{3}H]-ethylketocyclazocine ([^{3}H]-EKC) in the presence of [D-Ala^2,Me-Phe^4,Gly-ol^5]- enkephalin (Enk) and [D-Ser^2,Thr^6]-Leu-Enk, two specific ligands for cross-reacting mu and delta opioid receptors, respectively. Saturation studies indicate the presence of two receptor sites for [^{3}H]-EKC: a high affinity binding site (kappa) with a K_D of 0.66 nM and a Bmax of 12 pmoles/g protein and a low affinity binding site (kappa$_2$ or benzomorphan) with a K_D of 11.1 nM and a Bmax of 56 pmoles/g protein. The presence of kappa opioid receptors in the membrane preparation was also supported by competition studies. U-50,488H, EKC and Dyn-(1-13), three specific kappa opioid ligands, were all potent inhibitors of [^{3}H]-EKC binding with Ki (high affinity binding sites) of 2.5, 3.4 and 2.3 nM, respectively. The biological activity of the opioid compounds was also verified by their potency to inhibit the secretion of catecholamines from isolated bovine adrenal chromaffin cells. Among the various ligands tested for each class of opioid receptors (mu, delta, kappa and sigma) and of endogenous opioid peptides, U-50,488H and Dyn-(1-13) were the most potent inhibitors of the acetylcholine-evoked release of catecholamines with IC_{50} of 0.31 and 1.14 µM, respectively. The inhibitory effect of these compounds was partially antagonized by diprenorphine (10^{-7} M). These results suggest a putative modulatory role for Dyn in the adrenal medulla, possibly mediated through the stimulation of high affinity kappa opioid receptors.

(Supported by the Medical Research Council of Canada and the Canadian Heart Foundation. M.D. is a recipient of an F.R.S.Q. studentship).

172.2 SEX DIFFERENCES IN OPIATE RECEPTOR BINDING, AS DETERMINED BY QUANTITATIVE AUTORADIOGRAPHY, IN SEVERAL AREAS OF THE RAT BRAIN. S. Iyengar and J. Rabii. Department of Biological Sciences and the Bureau of Biological Research, Rutgers University, Piscataway, NJ 08854.

Using a receptor autoradiographic technique, opiate antagonist binding was measured in several brain areas in male and female rats at 15 and 75 days of age. H^3-Naloxone (2.5 nM) in the presence of 100 mM NaCl (pH 7.4) was incubated with brain sections. Cold morphine (1 µM) was used to determine non-specific binding. Following incubation, the brain sections were exposed against LKB Ultrofilm. Receptor density (fm/mg protein) was then estimated by computerized densitometry according to Rainbow et al. (J. Neurosci. Meth. 5:127, 1982). Areas that were investigated included anterior, dorsomedial and ventromedial hypothalamus, suprachiasmatic, supraoptic and arcuate nuclei, medial preoptic area, median eminence, mediobasal, ventromedial and laterdorsal thalamus, bed nucleus of stria terminalis, medial forebrain bundle, basal, central and medial amygdala as well as the parietal cortex. Specific naloxone binding sites were present in all of these areas in both sexes, in adults and immature rats. Higher concentrations of receptors were found in the amygdala, thalamus, cortex, ventromedial and dorsomedial hypothalamus and the arcuate nucleus than in the other areas tested. Receptor density in the supraoptic nucleus was higher in adult male rats than in the females. In the female brain, however, receptor concentration was higher in medial preoptic area, anterior hypothalamus, suprachiasmatic nucleus and ventromedial thalamus than that seen in the male brain. In the 15-day old female rats, opiate binding was greater in the median eminence, ventromedial thalamus and the parietal cortex than in males of the same age. In the 15-day old males, however, opiate density in the medial preoptic area, anterior hypothalamus, medial forebrain bundle, stria terminalis and basal amygdala was higher than that seen in the females. We conclude that there are sex differences in H^3-naloxone binding in several areas of the brain of immature and young adult rats. Most of these areas have been implicated in a number of neuroendocrine control systems. The relevance of sex differences in opiate receptors to those reported in neuroendocrine control systems, however, remains to be determined. (Supported in part by NIH Grant DA02227 and grants from the Charles and Johanna Busch Memorial Fund.)

172.3 Distribution of (^{3}H) naloxone binding in the normal and partially deafferented interpeduncular nucleus. R. Artymyshyn,* T.C. Eckenrode,* and M. Murray. (SPON: M.E. Goldberger) Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

The distribution of (^{3}H) naloxone binding was mapped in the Rostral, Central, Lateral, Dorsal, and Intermediate subnuclei (Hamill and Lenn '84) in the normal and partially deafferented interpeduncular nucleus (IPN). In the rostral part of the normal IPN, the Rostral subnucleus contains a band of labeling that follows the dorsal margin of the IPN. Further caudally the band thickens and a gradation of label develops, being densest at the dorsal aspects of the subnucleus and virtually absent ventrally. In the caudal half of the IPN dense labelling is found in the Lateral, Central, and Rostral subnuclei. The Rostral subnucleus, which is now confined to the dorsal aspect of the IPN contains heavy labeling but it is still largely restricted to the dorsal portion. This labeling is continuous with that found in the Dorsal subnucleus. The Lateral subnuclei contain heavy labeling which is densest laterally. The Central subnucleus contains unevenly distributed label most prominant centrally. There is almost no label in the Intermediate subnuclei.

The Fasciculus Retroflexus (FR) is the main afferent to the IPN. FR lesions produced some changes in the distribution of (^{3}H) naloxone binding. After unilateral FR lesion there was an ipsilateral decrease of label in the rostral part of the Rostral subnucleus. Further caudally, labelling was decreased in the rostral, ipsilateral parts of the Lateral subnucleus. At this level, no changes were seen in the Rostral subnucleus. Near the caudal pole of the nucleus an ipsilateral decrease in the lateral part of the Lateral subnucleus was seen. No changes were noted contralateral to the lesion or in the Central or Dorsal subnuclei. Bilateral FR lesions do not eliminate or greatly decrease the labelling in the IPN. These results imply that some of the opiate receptors in the IPN are presynaptic and are consistant with the presence of (^{3}H) naloxone binding in the FR of normal animals. The distribution of opiate receptors overlaps the distribution of SP, especially in the Lateral and Rostral subnuclei. This may represent an area of interaction between the SP and opiatergic systems in the IPN. Supported by NIH NS16556.

172.4 (^{3}H)(+)3-(3-HYDROXYLPHENYL)-N-n-PROPYLPIPERIDINE (3-PPP): A PUTATIVE DOPAMINE AUTORECEPTOR AGONIST-LABELS SIGMA OPIATE RECEPTORS. B.L. Largent, A.L. Gundlach and S.H. Snyder. Johns Hopkins University, Dept. of Neuroscience, Sch. of Med., 725 N. Wolfe Street, Baltimore, MD 21205.

Psychotomimetic actions of certain opiates (cyclazocine and SKF 10,047) suggest the existence of specific psychotomimetic opiate receptors, designated sigma receptors. Putative sigma receptor sites have been labeled in brain membranes with [^{3}H]opiates (Su, J. Pharm. Exp. Ther., 223:284, 1982; Tam, S., Proc. Natl. Acad. Sci. USA, 80:6703, 1983).

Several studies describe 3-(3-hydroxyphenyl)-N-n-propylpiperidine (3-PPP) as a centrally acting dopamine agonist with selectivity for dopamine autoreceptors. However, in vitro studies indicate that 3-PPP is quite weak in assays directly measuring dopamine autoreceptor function.

Binding studies with [^{3}H](+)3-PPP in rat whole brain homogenates demonstrate a saturable binding site with a K_D of 30 nM and a B_{max} of 31 pmole/g wet wt tissue. The drug specificity of these binding sites resembles that of sigma receptors. Potent inhibitors of binding include the psychotomimetic opiates, pentazocine, cyclazocine and SKF 10,047, and the phenothiazines, perphenazine and fluphenazine. The butyrophenone, haloperidol, is the most potent drug at the binding site with an affinity of 2-3 nM, while spiperone, pimozide and (+)butaclamol display IC_{50} values of greater than 0.5 μM. Apomorphine, dopamine and lisuride fail to inhibit binding. The regional distribution of [^{3}H](+)3-PPP binding sites in rat brain does not correlate with known dopaminergic projections. High levels of binding occur in the cerebellum and brainstem, while lower levels are seen in corpus striatum and nucleus accumbens.

Stereoselectivity is seen for the isomers of 3-PPP with the affinity of the (+)enantiomer being 5 fold greater. The isomers of butaclamol show reversed stereoselectivity compared to that at D_2 dopamine receptors. Opposite stereospecificity is also displayed from that at classical opiate receptors, where the (-) isomer is generally more potent. Thus dextrallorphan, (+)cyclazocine and (+)SKF 10,047 are more potent than their corresponding (-) isomers. High concentrations of naloxone, levorphanol, dihydromorphine and [D-ala^2-D-leu^5]-enkephalin do not inhibit binding of [^{3}H](+)3-PPP.

172.5 AUTORADIOGRAPHIC LOCALIZATION OF SIGMA OPIATE RECEPTORS IN RAT BRAIN WITH [^{3}H](+)3-(3-HYDROXYPHENYL)-N-n-PROPYLPIPERIDINE (3-PPP). A.L. Gundlach, B.L. Largent and S.H. Snyder. Dept. of Neuroscience, Johns Hopkins University, School of Medicine, Baltimore, MD 21205.

Psychotomimetic actions of certain opiates have suggested the existence of specific receptors, designated as sigma opiate receptors. Previous studies have suggested that 3-(3-hydroxyphenyl)-N-n-propylpiperidine (3-PPP) acts at dopamine receptors in the central nervous system. However, the pharmacological profile of specific high affinity sites labeled by [^{3}H](+)3-PPP differs from that of known dopamine receptors and resembles that of sigma opiate receptors (Largent, B.L. et al., Neuroscience Abstracts, 1984; Su, T.-P., J. Pharmacol. Exp. Ther., 223:284, 1982). This report describes the localization of specific [^{3}H](+)3-PPP binding sites on slide-mounted brain sections revealed by in vitro autoradiography.

After a 15 min preincubation, 8 μM tissue sections were incubated with 6 nM [^{3}H](+)3-PPP in 50 mM Tris pH 8.0 containing 0.18 M sucrose for 45 min at RT. Nonspecific binding was measured in adjacent sections by including 1 μM haloperidol in the incubation. After 2 consecutive 4 min washes in buffer and rinsing in distilled water, slides were dried and apposed to tritium-sensitive film for 6 weeks at 4°C.

Specific [^{3}H](+)3-PPP binding was localized in many brain regions. These included the pyramidal cell layer of the hippocampus, especially the CA3 and CA4 regions; the granular layer of the dentate gyrus; the pyramidal cell layer of the piriform cortex and olfactory tubercle; superficial layers of the cortex; several hypothalamic nuclei; the zona incerta; medial and lateral septum; the periaqueductal grey; the zona compacta of the substantia nigra; facial and vestibular nuclei in the brainstem; and the cerebellar cortex.

These localizations of [^{3}H](+)3-PPP binding sites may represent the distribution of sigma opiate receptors in rat brain and their presence in important limbic and locomotor regulatory regions may explain the psychotomimetic actions of opiates as well as some of the behavioral effects of 3-PPP.

(A.L.G. is the recipient of a National Health and Medical Research Council (Aust.) C.J. Martin Fellowship).

172.6 REDUCED TYPE I OPIATE RECEPTORS IN GENETICALLY OBESE (OB/OB) MOUSE BRAIN. M. Ferguson-Segall*, D.L. Margules, R.B. Rothman, and C.B. Pert. Psychology Dept., Temple University, Philadephia, Pennsylvania 19122 and the National Institute of Mental Health, Bethesda, Maryland 20205.

Genetically obese mice (ob/ob) have elevated levels of pituitary beta-endorphin (1,2), dynorphin (3), and leu-enkephalin (3) compared to their lean litter mates as well as six fold elevated levels of plasma beta-endorphin at 10 weeks of age (2). Furthermore, obese mice are more sensitive to the appetite suppressant affects of opiate antagonists (1,2,3) than their lean litter mates. Apparently, obese mice are chronically exposed to higher levels of endogenous opiates which, like classical, exogenous opiates, appear to alter their "set point" and render them opiate-tolerant and naloxone sensitive (2). We now report a direct autoradiographic comparison of opiate receptors in brains of 4 lean and 5 obese mice. Coronal brain sections spanning the whole brain were labelled under Type I (Naloxone (H^3), 90 min., 0°C, 50 mM Tris 7.0, 100 mM Na(l, and 1 mg/ml BSA) and Type II (DADLE (H^3), 90 min. room temperature, 50 mM Tris 7.4, 100 mM NaCl, 3 mM Mn acetate, 2 μM GTP, 30 nM oxymorphine and 1 mg/ml BSA) opiate receptor conditions. While we failed to detect differences in regional distribution between obese and lean mice (which show a nearly identical pattern to rats), the obese mice had a clear (25%) reduction in Type I opiate receptor binding with no detectable differences in Type II opiate receptor binding.

Interestingly, opiate receptors in the obese mouse brain might be down regulated as a consequence of its increased "opiatergic tone". A number of features of the physiology of obese mice is consistent with the notion that this mouse might represent an "endogenously opiated" animal model; the obese mouse has the following basal traits, which are normally induced by opiates: low metabolic rate, hypothermia (4), respiratory depression, constipation, hyperphagia, reduced libido and motor activity (5), enhanced t-lymphocyte mitogenesis, resistence to metastasis of the B16 melanoma (6), and enhanced natural killer cell activity (7). These findings suggest that alterations in sensitivity occur at Type I opiate receptors.

(1) Margules, D.L., et. al., Science 202:988-991 (1978); (2) Recant, et al., Peptides 1:309-313 (1980); (3) Ferguson-Segall, M., et al., Life Sci. 31:2233-2238 (1982); (4) Thurlby, P.L., et al., Br. J. Natr. 39:397-402 (1978); (5) Bray, G.A., et al., Physiol Rev. 59:719-809 (1979); (6) Thompson, C., et al., Science 220:1183-1185 (1983).

172.7 BINDING OF β-ENDORPHIN TO SPECIFIC RECEPTORS IN RAT HEPATIC MEMBRANES STIMULATES ADENYLATE CYCLASE ACTIVITY. J.R. Dave* and R.L. Eskay* (SPON: S.L. Sabol). Laboratory of Clinical Studies, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD-20205.

The objective of this study was to determine if rat peripheral tissues possessed specific binding sites for β-endorphin (BE). Using ^{125}I-Acetyl-human β-endorphin (Ac-B_HE), specific binding sites for BE were identified in liver, kidney, adrenal, spleen and testis of adult male SD rats, whereas, ventral prostate and pancreas did not exhibit specific BE binding sites. In those tissues containing specific BE binding sites, microsomal membranes (x 15,000 - 100,000 g pellet) exhibited higher BE binding capacity than the crude homogenate (x 125 - 100,000 g pellet). The binding of BE was saturable and time- and temperature-dependent, with a maximal binding achieved at 60 min and 22°C. Furthermore, the BE binding was dependent on the presence of magnesium chloride and was independent of the presence of sodium chloride. Scatchard analysis of the BE binding data revealed the existence of two classes of binding sites. One class had an apparent K_a of 0.19 nM and lower number of binding sites (9.1 pmol BE/mg protein), whereas, the other class had a lower affinity (apparent K_a of 0.006 nM) and higher number of binding sites (159 pmol/mg protein). The binding was inhibited by lower concentrations of Acetyl-Camel β-endorphin (1-27, 1-31) and by higher concentrations of Camel β-endorphin (1-31), but was unaffected by morphine and naloxone. At relatively higher peptide concentrations (10^{-5}M), α-MSH, r-CRH, γ-endorphin, Met-enkephalin and Leu-enkephalin exhibited 20-40% inhibition. For studies on adenylate cyclase, the hepatic microsomal-membranes were incubated for 1 hr at 22°C with concentrations of BE ranging from 0.5 x 10^{-12} to 0.5 x 10^{-7}M. The adenylate cyclase activity was estimated by incuhating the membranes for 10 min at 37°C and determining the amount of cAMP produced by RIA. BE induced a dose-related increase in adenylate cyclase activity and 0.5 x 10^{-10}M BE resulted in a maximal enhancement of adenylate cyclase activity of 148% above controls. These data suggest the existence of a specific non-opiate binding site for β-endorphin in rat liver, kidney, adrenal, spleen and testis. The finding that activation of specific BE binding sites in rat liver stimulate the adenylate cyclase/cAMP system, suggests that circulating β-endorphin may have an important regulatory role mediated via the interaction with specific BE receptors rather than through opiate receptors.

172.8 THE EFFECT OF TOLERANCE ON OPIATE DEPENDENCE AS MEASURED BY THE ADENYLATE CYCLASE REBOUND RESPONSE TO NALOXONE IN THE NG108-15 MODEL SYSTEM. D. L. Greenspan* and J. M. Musacchio (Spon. M. Puig). Dept. Pharmacology, N.Y.U. Medical Center. New York, N.Y. 10016, USA.

The dual effects that opiates have on the adenylate cyclase (AC) of the NG108-15 have been proposed as a model system to study the biochemical correlates of opiate tolerance and dependence (Sharma et al., Proc. Natl. Acad. Sci. USA. 72:3092, 1975). However, the AC rebound response described in recent papers is much smaller than that originally described. One of the common elements of the recent reports is that delta agonists were used, etorphine (E), instead of morphine (M), a mu agonist. Since delta agonists produce a marked opioid receptor down regulation, it is tempting to speculate that when the receptors are markedly reduced in number, the rebound response induced by naloxone (Nx) or opiate suppression, would be attenuated.

To test this hypothesis, we treated NG108-15 cells with mu (M, 10 μM), delta (E, 10 nM) agonists, or vehicle for 48 h. The concentration of opiates was 3-4 fold their IC_{50} to inhibit the PGE_1 stimulation of AC. This concentration of E is known to reduce the opioid receptors to 40 percent of the control values, while M does not (Law et al., Mol. Pharmacol. 24:413, 1983). To measure the AC activity, the cells were incubated with [^{3}H]adenine to label intracellular ATP. Cells from each group were then treated with 10 μM PGE_1 or PGE_1 and 10 μM Nx. [^{3}H]cAMP was isolated by a double chromatographic procedure, and the results expressed as cpm/mg protein. The M treated cells incubated with PGE_1 + Nx showed a significant increase when compared with control groups and M tolerant cells treated only with PGE_1. In contrast, Nx did not induce any significant rebound response in E tolerant or in vehicle treated cells.

These results suggest that the opioid receptor down regulation produced by high specific activity agonists markedly attenuates the AC rebound response that is generally considered a biochemical correlate of opiate dependence. (Supported in part by PHS grants DA-02013, MH-29591 and MH-17785).

172.9 HETEROGENEITY OF OPIOID BINDING SITES IN GUINEA PIG SPINAL CORD. Gary Zarr,* Linda L. Werling, and Brian M. Cox. Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

High concentrations of opioid peptides derived from both proenkephalin and prodynorphin are present in spinal cord, and studies of their localization within spinal cord suggest the opioids may be involved in the regulation of both sensory and motor functions. Sensory deficits and disturbed motor function following the local administration of opiates or opioid peptides to spinal cord also suggest a multiplicity of endogenous opioid regulatory functions in this tissue. We have therefore examined the heterogeneity of opioid binding sites in spinal cord.

Studies of radiolabeled opioid ligand binding to spinal cord membrane preparations confirm that saturable high affinity opioid binding sites in this tissue show heterogeneity of binding properties. In analyses of opiate drug effects, it is helpful to determine ligand binding affinities under conditions which parallel the in vivo situation as far as possible. We have measured the saturable binding of [^{3}H]Tyr-D-Ala-Gly-MePhe-NH$(CH_2)_2$OH, [^{3}H]D-Ala^2-D-Leu^5 enkephalin, and [^{3}H]ethylketocyclazocine binding to spinal cord membrane preparations incubated with Na+ and other salts at 37°, in the presence of opioid binding site selective blocking ligands. Initial experiments indicated the ligands we have used are stable under our assay conditions. Binding sites with μ, δ, and κ type characteristics were readily identifiable, although ligand affinities were variably reduced relative to affinities in the absence of Na+ and at lower temperatures. Ligand affinities measured in the presence of Na+ may give more useful indices of opiate drug binding site selectivity than values obtained in the absence of Na+. (Supported by grant DA03102 from the National Institute on Drug Abuse.)

172.10 QUANTITATIVE LOCALIZATION OF SIGMA OPIATE/PHENCYCLIDINE RECEPTORS IN RAT BRAIN BY LIGHT MICROSCOPY AUTORADIOGRAPHY. S.R. Zukin and R. Sircar. Dept. of Psychiatry, Albert Einstein College of Medicine, Bronx, N.Y. 10461

Zukin et al. (1983) reported the distribution of [^{3}H] phencyclidine (PCP)/sigma opiate receptors in rat brain in homogenate binding studies and showed that the subiculum and hippocampus had the highest specific [^{3}H]PCP binding densities with moderate levels in the striatum, frontal cortex and cerebellum and lowest in the corpus callosum. A similar distribution pattern of [^{3}H]PCP binding sites was obtained by Quirion et al. (1981) with qualitative autoradiography in rat brain. In the present study localization of PCP/sigma opiate receptors was determined quantitatively by light microscopy autoradiography.

Twenty micron thick sections of fresh frozen adult Long Evans hooded rat brain were cut and incubated with 10 nM [^{3}H]PCP (49.9 Ci/mM) in 5 mM Tris-HCl (pH 7.4) for 45 min at 4°C in the presence and absence of 10 μM nonradioactive PCP. Incubation was terminated by six sequential washes (20 sec each) of the sections in 5 mM Tris-HCl at 4°C. Following thorough drying, the sections were tightly juxtaposed against tritium-sensitive LKB Ultrafilm for 10 days at room temperature. The films were developed in Kodak D19 for 5 min and fixed for 10 min. A photovolt densitometer with an aperture diameter 0.1 mm (Photovolt Corp., NY) was used to determine the optical densities over different brain regions. Highest optical density of receptors was measured in the hippocampus (0.27±.023). Intermediate levels ranging from 0.22 ±.01 o.d. in the frontal cortex, through 0.21±.025 in ventromedial hypothalamus, 0.20±.015 in nucleus accumbens, 0.20± .012 in periacqueductal grey, 0.20±.006 in amygdala, 0.19± .056 in cerebellum, 0.18±.02 in substantia nigra and 0.17± .03 in the arcuate nucleus were recorded. Lowest optical densities were obtained in various thalamic and hypothalamic nuclei and from white matter regions such as corpus callosum.

Thus, sigma opiate/PCP receptor distribution clearly differs from distribution of other types of opiate receptors. The present quantitative autoradiographic results are in agreement with previously reported qualitative studies as well as with homogenate binding data.

172.11 HETEROGENEITY OF OPIOID RECEPTORS ON DORSAL ROOT GANGLION NEURONS IN CULTURE: A COMBINED AUTORADIOGRAPHIC AND ELECTROPHYSIOLOGICAL STUDY. M.A. Werz, M.E. Lewis, D.S. Grega, S.J. Watson and R.L. Macdonald. Dept. of Neurology and Mental Health Res. Inst., Univ. of Michigan, Ann Arbor, MI 48109

Multiple opioid receptors have been characterized using receptor binding techniques. However, the functional significance of these binding sites remains unclear. One approach to the problem is to apply receptor autoradiography and cellular physiological techniques to single neurons. Such a comparison can be made using mouse spinal cord and dorsal root ganglion (DRG) neurons in cell culture. Cell culture and electrophysiological techniques were as previously described (Werz and Macdonald, JPET 227:394, 1983). Opioid peptides decreased DRG neuron somatic calcium-dependent action potential duration in a subpopulation of neurons. DRG neurons were observed that responded only to [D-Pen^2,L-Pen^5]-enkephalin, [N-MePhe^3,D-Pro^4]-morphiceptin, or dynorphin A, consistent with responses mediated by δ-, μ- and κ-receptors, respectively. Ligands selective for μ- and δ-receptors are likely to act by enhancing potassium conductance, while κ-ligands appear to decrease voltage-dependent calcium conductance. We based these conclusions on observations that responses to dynorphin, in contrast to responses to μ- or δ-ligands, were associated with decreased action potential after-hyperpolarizations and persisted after intracellular injection of the potassium channel blocker cesium. These results indicate that there are multiple opioid receptors on DRG neurons and that these receptors are coupled to different ion channels. To determine whether this heterogeneity could also be demonstrated by ligand binding methods, we carried out receptor autoradiographic studies to demonstrate that DRG neurons that are differentially sensitive to opioids are also labelled by appropriate radioligands. Opioid receptor autoradiography was carried out according to the method of Herkenham and Pert (J.Neurosci. 2:1129,1982) using ^{3}H-naloxone or ^{3}H-DAGO to label μ-sites and ^{3}H-DADLE or ^{3}H-DSLET to label δ-sites. We have observed binding of μ- and δ-ligands to subpopulations of DRG neurons, the specificity of which was indicated by the absence of labelling in the presence of 1 μM levorphanol. We are presently attempting to correlate the sensitivity of DRG neurons to μ-, δ- and κ-opioids with the presence of histochemically demonstrable binding sites for these ligands.

172.12 ESTROGEN-INDUCED INCREASES IN HYPOTHALAMIC OPIATE BINDING SITES IN FEMALE RATS AND MICE. W. Jacobson*, M. Wilkinson and J.R. Brawer*, Dept. of Physiology & Biophysics, Dalhousie University, Halifax, B3H 4H7 and (J.R.B.) Depts. of Obstetrics & Gynaecology/Anatomy, McGill University, Montreal, Canada.

Hypothalamic binding sites for several neurotransmitters appear to be sensitive to estrogen (E) treatment. For example, β-adrenergic binding can be up- or down-regulated depending upon the dose of E. A toxic effect of E has also been demonstrated; e.g. multifocal degeneration of axons and terminals can be induced in the arcuate nucleus following a large injection of estradiol valerate (EV). Similar changes are seen in ovx rats exposed to chronic (>2.5 months) levels of E (silastic capsules). We describe here the results of experiments performed on hypothalamic tissue from E-treated rats and mice. Different groups of animals were treated with E as follows: 1) Ovx females were implanted with a single estradiol (E2) capsule at 7 days post-surgery (plasma E2 approx. 50 pg/ml); 2) Cycling Wistar female rats were injected with EV (2 mg s.c. in oil); 3) Adult female Swiss Webster mice were injected with 200 μg of EV. E-treated animals from groups 2 and 3 demonstrated constant estrus within 4 weeks. Rats were sacrificed 3 months post-treatment; the mice were sacrificed at 4 months. RESULTS: Expt. 1: H homogenates were prepared by standard techniques. E2 treatment significantly elevated NAL binding; C: 112.3+14.2, E2: 146.3+9.4 fmoles/mg ($p<0.05$). The affinity of the binding site did not change (K_D= approx. 3 nM). Expt. 2: EV treatment induced a similar increase in (^{3}H)-NAL binding at a single point on the binding curve (4.4 nM): C: 40.5+1.9; EV: 79.7+1.8 fmoles/mg ($p<0.005$). In a subsequent experiment performed exactly as for #2, we determined NAL binding separately in mediobasal (MBH) and anterior (AH) hypothalamus. Binding was significantly increased in both parts of the H. In contrast, EV treatment of OVX rats had no effect in AH whereas NAL binding was reduced approximately 25% in MBH ($p<0.005$). Expt. 3: In agreement with the results in #2 above, EV significantly elevated NAL binding in mouse H; C: 15.8+2.1 fmoles/mg; EV: 30.0+2.6 ($p<0.005$). K_D was unchanged (approx. 3 nM). In conclusion, opiate binding sites in H appear to be sensitive to high-dose (EV) or long-term (E2 capsule) treatment. Additionally, EV effects appear to be different in the absence of the ovaries.

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172.13 OPIATE BINDING SITES IN THE AVIAN RETINA. Y.Y.T. Su. Cullen Eye Institute, Baylor College of Medicine, Houston, Texas 77030.

We have previously shown that enkephalins are localized, synthesized and released in the chick retina (Su et al. and Watt et al., Soc. Neurosci. Abst., 1983). As a continuation of these studies, we present here the identification of enkephalin receptors in the chick retina.

Retinal sections, each about 1.8 mg wet weight, were used directly to study the binding of enkephalin receptors. Tris-HCl buffer (0.17 M, pH 7.4) containing peptidase inhibitor and ascorbic acid was used as an incubation medium. The tissues were first incubated in 0.17 M Tris-HCl buffer (pH 7.4) for at least 30 min at room temperature. These tissues were then incubated in the incubation medium containing labeled ligand in the absence (total binding) and the presence (nonspecific binding) of unlabeled ligand for 2 hours at 4°C. The tissues were rinsed four times in large volumes of ice-cold incubation medium and then dissolved in 0.2 ml tissue solubilizer. The labeled ligand bound to the tissue was counted in a scintillation counter. A saturable specific binding (total binding minus nonspecific binding) curve was obtained. The specific binding was 30 to 40% of the total binding. The Hill coefficients are 0.86, 0.83 and 0.98 for ^{3}H-leu-enkephalin, ^{3}H-met-enkephalinamide and ^{3}H-naloxone, respectively. Scatchard analysis showed two binding sites for both enkephalin and its analog but only one binding site for naloxone. Estimated binding site affinities for ^{3}H-leu-enkephalin are 0.48 ± 0.08 nM and 4.2 ± 0.62 nm, those for ^{3}H-met-enkephalinamide are 1.76 ± 0.11 nM and 5.25 ± 0.80 nM and that for ^{3}H-naloxone is 1.68 ± 0.12 nM. The effect of Na^+ and GTP on the specific binding of opioid agonists and antagonist are currently under investigation.

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172.14 **CENTRAL δ-OPIOID RECEPTOR ANTAGONISM BY ICI 174,864 AND NALOXONAZINE REVEALED IN THE RAT URINARY BLADDER PREPARATION.** L. Nunan*, W. Wire* and A. Dray (SPON: A. Kaszniak). Department of Pharmacology, University of Arizona, College of Medicine, Tucson, AZ 85724.

Multiple populations of opioid receptors and their subtypes have been characterized in the central nervous system using receptor selective agonists and antagonists. To facilitate these studies ICI 174,864 has been proposed as a potential δ-opioid receptor antagonist (Cotton et al, Eur. J. Pharmacol. 97:331, 1984) while naloxonazine was introduced as a potent irreversible antagonist of μ_1-opioid receptors (Hahn et al, J. Neurosci. 2:572, 1982). In the present study the central activity of these substances was tested against the inhibition of urinary bladder contractions produced by receptor selective opioid agonists. Spontaneous rhythmic bladder contractions were recorded isometrically from the anesthetized rat (urethane 1.2 g/kg, i.p.) using a pressure transducer. These contractions were inhibited by alternate i.c.v. (lateral ventricle) microinjections of submaximal but equipotent doses of the μ-agonist [D-Ala^2-MePhe^4-Gly-(ol)^5] enkephalin (Handa et al, Eur. J. Pharmacol. 70:531, 1981) (DAGO: 0.1-0.2 μg) and the novel δ-agonist [D-Pen^2, D-Pen^5] enkephalin (Mosberg et al, PNAS 80:5871, 1983) (DPDPE: 2-4 μg). ICI 174,864 (1-3 μg,i.c.v.) consistently antagonized the effects of DPDPE without significantly changing those of DAGO. Antagonism was completely reversed within 2-4 hr. Higher doses (6-15 μg, i.c.v.) inhibited bladder contractions and this was attributed to δ-receptor activation. Naloxonazine (1-6.5 μg, i.c.v.) antagonized both DAGO and DPDPE when these agonists were tested 5-20 min after its administration. At longer periods (2-6 hr) only DPDPE antagonism persisted with partial recovery being observed 24-30 hr later in the same animals. β-Endorphin (βE: 1 μg, i.c.v.) inhibited bladder contractions but also produced a prolonged potentiation of the agonistic effect of ICI 174,864 and prevented the antagonism of DPDPE by both ICI 174,864 and naloxonazine. These observations suggest that ICI 174,864 is a selective δ-antagonist but also possesses δ-agonistic properties. Naloxonazine produces short lasting μ-receptor antagonism but prolonged antagonism of δ-opioid receptors. Finally βE may produce a prolonged change in δ-receptor efficacy.

172.15 **IN VIVO ASSESSMENT OF SPINAL AND SUPRA-SPINAL OPIOID ACTIVITY USING THE RAT URINARY BLADDER PREPARATION.** A. Dray. Department of Pharmacology, University of Arizona, College of Medicine, Tucson, AZ 85724.

The urinary retention and antidiuresis observed following the administration of opioids may be produced by actions at spinal and supra-spinal sites. Thus systemic morphine (0.5-2 mg/kg, i.v.) inhibited urinary bladder contractions recorded isometrically in the anesthetized rat (urethane, 1.2 g/kg i.p.) following catheterization of the bladder and the initiation of volume induced reflex contractions. This action of morphine was reversed by naloxone (1-2 μg) administered by intracerebroventricular (i.c.v., lateral ventricle) or subarachnoid (i.t., between L_3-L_6 vertebrae) microinjection. The pharmacology of these actions was characterized using receptor selective opioids. Thus bladder contractions were inhibited in a dose-related manner by the μ-agonists morphiceptin (1-4 μg) and [D-Ala^2-$MePhe^4$, $Gly\text{-}(ol)^5$] enkephalin (0.1-2.0 μg) (Handa et al, Eur. J. Pharmacol. 70:531, 1981), by the δ-agonists [D-Ala^2, D-Leu^5] enkephalin (Kosterlitz et al, Br. J. Pharmacol. 68:333, 1980) (0.5-5.0 μg), the novel bis-pencillamine derivatives [D-Pen^2, L-Cys^5]-, [D-Pen^2, L-Pen^5]- and [D-Pen^2, D-Pen^5] enkephalin (0.5-10 μg) (Mosberg et al, PNAS 80:5871, 1983) but not by the k-agonist U50,488H (Von Voigtlander et al, JPET 224:7, 1983). Therefore the spinal and supra-spinal opioid inhibition appeared to be mediated by μ and δ-receptors. Furthermore the effects of μ-agonists were readily antagonized by naloxone (1-2 μg, i.c.v. or i.t.) while ICI 174,864 or naloxonazine antagonized the effects of δ-agonists. A number of other substances with opioid activity including endorphins, dynorphins and pro-enkephalin A fragments have been characterized using this method. Therefore the inhibition of rat bladder motility can be utilized as a reliable index of central opioid activity.

172.16 PUTATIVE KAPPA OPIOID RECEPTORS IN GUINEA PIG CEREBELLUM DEMONSTRATED BY LABELLING WITH ^{3}H-ETHYL-KETO-CYCLAZINE. L.T. Hamel*, J.I. Stevenson*, and M.H. Perrone* (SPON. R.A. Ferrari) CNS Section, Dept. of Pharmacology. Sterling-Winthrop Research Institute, Rensselaer, N.Y. 12144.

The presence of three subclasses of opiate receptors, μ, δ and κ. has been demonstrated by both in vitro and in vivo methodologies. Further receptor subclasses have been proposed but the μ, δ and κ classifications of opiate receptor enjoys wide acceptance. In this report we describe the presence of a distinct κ-binding site in guinea pig cerebellum using ^{3}H-ethylketocyclazocine (^{3}H-EKC) as the ligand. Binding was determined in a washed 48,000 x g pellet of the whole cerebellum in TRIS, or K_2HPO_4 buffers. In TRIS buffer binding could not be demonstrated at 4°, but at 25° binding of ^{3}H EKC binding was saturable and reversible. Binding of 0.3 nM ^{3}H-EKC was displaced by low nanomolar concentrations of U-50488, Mr 2266, naloxone, and bremazocine, whereas D-ala, D-leu-enkephalin (Dadle) did not significantly inhibit binding at 1 μM. The μ-selective peptide Rx 783030 inhibited binding with a potency 1/1000 that of U-50,488. In contrast to binding in TRIS buffer, saturable and reversible binding of ^{3}H-EKC could be demonstrated K_2HPO_4 buffer at 4°. Displacement of ^{3}H-EKC binding in K_2HPO_4 buffer was qualitatively similar to that determined in TRIS buffer. U-50,488, Mr2266, naloxone, and bremazocine inhibited binding in the low nanomolar range. Dadle (1 μM) did not displace ^{3}HEKC binding whereas Rx 783030 had an IC_{50} value in excess of 20 μM when measured in K_2HPO_4 buffer. Sodium ion decreased binding of ^{3}H-EKC in a dose-dependent manner in either buffer. The presence of 100 mM NaCl produced rightward parallel shifts of inhibition curves for Rx783030, U-50,488, and bremazocine. In contrast, 100 mM NaCl had no effect on the inhibition of ^{3}H-EKC binding by Mr 2266 but shifted the inhibition curves for naloxone sightly to the left when measured in K_2HPO_4 buffer. The results presented here suggest that the binding sites in the guinea pig cerebellum measured by ^{3}H-EKC are predominately κ in nature with less than 20% μ sites and virtually no δ sites.

172.17 IRREVERSIBLE INHIBITION OF RADIOLABELED OPIATE AND OPIOID PEPTIDE BINDING BY β-FUNALTREXAMINE (β-FNA). L.D. Recht*, J. Holaday, N. Johnson* and G.W. Pasternak. The Cotzias Lab. of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021 and Dept. Medical Neuroscience, Walter Reed Army Institute, Washington, D.C. 20012.

β-FNA has been reported to be a potent irreversible mu antagonist and kappa agonist in the guinea pig ileum bioassay (Portoghese et al, J. Med Chem. 23: 233, 1980). Together with β-FNA's prolonged inhibition of morphine analgesia in vivo, these findings suggest that β-FNA reversibly interacts with kappa sites while irreversibly blocking mu binding sites. We have examined both the reversible and irreversible actions of β-FNA on radiolabeled opioid binding in rat brain and compared these effects to naloxonazine, a mu_1 selective irreversible antagonist (Hahn et al, J. Neurosci. 2:572, 1982). β-FNA potently displaces the binding of a number of radiolabeled opioids. In direct competition studies, β-FNA displaces radiolabeled dihydromorphine (mu), ethylketocyclazocine (kappa), SKF10,047 (sigma) and naloxone (antagonist) binding with IC_{50} values between 1 and 2 nM. ^{3}H-D-ala^2-D-leu^5-enkephalin (delta) binding is less sensitive (IC_{50} value approximately 6 nM), suggesting that β-FNA preferentially binds to opiate rather than opioid peptide sites. These results are similar to those previously reported with naloxonazine. We then examined the ability of β-FNA to irreversibly inhibit radiolabeled opioid binding. In these studies, β-FNA is relatively mu-selective, as previously suggested. However, major differences in the reversible and irreversible actions of the compound were noted. In contrast to the potent competitive interactions of β-FNA with opioid binding sites, β-FNA irreversibly inhibits radiolabeled opioid binding relatively weakly, requiring concentrations more than 100-fold greater than those active in standard reversible competition studies. In reversible competition studies, β-FNA at approximately 1-2 nM inhibits ^{3}H-dihydromorphine binding by 50 % while approximately 500 nM is needed to irreversible inhibit binding to a similar extent. Altnough β-FNA and naloxonazine reversibly inhibit radiolabeled opioid binding with similar potencies, naloxonazine irreversibly inhibits ^{3}H-dihydromorphine binding approximately 5-fold more potently than β-FNA.

172.18 OPIOID EPILEPTOGENESIS IN HIPPOCAMPUS: APPARENT δ-RECEPTOR MEDIATION. B. S. Lamishaw* and R. C. A. Frederickson, The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

Convulsant and anticonvulsant actions of opioid compounds have been reported. The convulsant activity of the opioid peptides is apparently restricted to induction of electrographic seizures without overt convulsions and is believed to be delta-receptor mediated. In the present studies, a series of opioids with widely differing selectivities for the mu (μ), delta (δ) and kappa (k) receptors and several opioid antagonists were utilized to assess the receptor types involved in this phenomenon. The minimum effective dose (MED) of the opioids required to induce hippocampal seizure activity after administration into the lateral ventricles of urethane-anesthetized rats was measured. The prototypical δ agonist, DSLET, had a median MED of 3 μg, whereas the prototypical μ and K agonists, morphine and UH-50,488, were inactive up to 100 μg. While some agents with significant μ activity were active, MED's for the series of opioids tested correlated best with activity on δ receptors. Unexpectedly, therefore, pretreatment of rats with β-funaltrexamine (β-FNA), an irreversible selective μ antagonist in isolated tissues, at 16 mg/kg s.c. 48 hours prior to testing elevated the MED's of the opioids. This may indicate some role for μ-activation in epileptogenesis also; or it may indicate that, contrary to its selectivity in peripheral tissues, β-FNA affords long-lasting functional blockade of central δ receptors, as has been reported for analgesic actions. Further studies are in progress with β-FNA and the selective δ antagonist, ICI 174,864.

172.19 EFFECTS OF GTP ON KAPPA OPIOID BINDING. K.J. Mack, M.F. Lee*, and J.A. Weyhenmeyer. College of Medicine and Neural and Behavioral Biology Program, University of Illinois, Urbana, IL, 61801.

GTP effects the binding of ligands that are involved with adenylate cyclase. The effects of GTP on mu and delta opioid binding have previously been reported, but as yet no definitive studies have looked at the effects of GTP on kappa opioid binding.

^{3}H-ethylketocyclazocine (^{3}H-EKC), a prototypic kappa ligand, crossreacts with mu and delta sites. In rat brain homogenates, this radioligand binds to all three opioid sites with an affinity (K_d) of 1.1 ± 0.1 nM and a binding capacity (B_{max}) of 341 ± 54 fm/mg protein. Using a paradigm in which mu and delta sites are blocked by specific cold ligands, ^{3}H-EKC binds to putative kappa sites with a K_d of 0.31 ± 0.04 nM and a B_{max} of 39 ± 4 fm/mg. By using these two paradigms, we have determined the effects of GTP on ^{3}H-EKC binding at both kappa and non-kappa sites.

The binding of ^{3}H-dihydromorphine (mu agonist), ^{3}H--DADL enkephalin (delta agonist), and unblocked ^{3}H-EKC binding showed a decreased affinity and binding capacity. At 1.0 mM GTP, two groups of binding sites were discernible by NLIN analysis of Scatchard plots. One site had similar characteristics to the kappa site with a high K_d of 0.37 ± 0.12 nM and B_{max} of 48 ± 15 fm/mg. The lower affinity site of 5.7 ± 0.7 nM and 231 ± 34 fm/mg is believed to represent the GTP sensitive mu and delta binding sites.

Under conditions where mu and delta sites are blocked, GTP lowers the affinity of ^{3}H-EKC binding to kappa sites without decreasing the binding capacity. At 1.0 mM GTP, the K_d is 0.68 ± 0.14 and B_{max} is 56 ± 9 fm/mg.

Our results are consistent with the hypothesis that kappa binding is less sensitive to the effects of GTP than are mu and delta opioid binding.

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OPIATES, ENDORPHINS, AND ENKEPHALINS: ANATOMICAL LOCALIZATION

173.1 LOCALIZATION AND QUANTITATION OF ENKEPHALINS AND DYNORPHINS IN RAT HIPPOCAMPUS. D.W. Hoffman and N. Zamir, LNO, NINCDS, NIH; and NIMH, Bethesda, MD. 20205.

The presence of both the proenkephalin A derived peptides (enkephalins) and proenkephalin B derived peptides (dynorphins/neo-endorphins) has been reported in the hippocampal formation. Conflicting reports exist regarding the localization of these opioid peptides within the different neuronal structures making up the hippocampus. However, the use of specific chemical and surgical lesions coupled with high performance liquid chromatography and radioimmunoassays for enkephalins and dynorphins has enabled us to determine the systematic localization of these opioids in rat hippocampus. Three types of lesion were performed:

1) microinjections of colchicine into the dentate gyrus to destroy granule cells and the associated mossy fibers,

2) electrolytic lesions of the entorhinal cortex to ablate the lateral perforant pathway, and

3) transection of the fimbria/fornix, to remove the remaining efferent fibers entering the hippocampus.

None of these treatments caused a significant depletion of enkephalin levels in rat hippocampus, even though enkephalin-like immunoreactivity has been localized to the granule cell-mossy fiber system, the lateral perforant pathway and to fibers entering the hippocampus through the fimbria/fornix. Rather, fimbrial transection resulted in an approximate two-fold increase in levels of met- and leu-enkephalin in the hippocampus contralateral to the lesion, suggesting a commissural interaction with an enkephalin-containing system intrinsic to the hippocampus.

Intrahippocampal colchicine injections did cause a dramatic decrease in dynorphin B levels in the hippocampus. The other manipulations did not affect these levels, in which dynorphin B was used as a marker for the proenkephalin B related peptides. The proenkephalin B derived peptides are found in the colchicine-sensitive granule cell-mossy fiber pathway of the rat hippocampus, while the proenkephalin A derived peptides appear to be confined to another intrinsic neuronal system of the hippocampus, the enkephalin-immunoreactive interneurons demonstrated within strata oriens and radiatum.

Acknowledgement: We thank Drs. Daniel Monaghan and Carl Cotman for performing the lesions (NIMH 19691) and Dr. E. Weber for antiserum.

173.2 ON THE ORIGIN OF LEU-ENKEPHALIN AND MET-ENKEPHALIN IN THE RAT POSTERIOR PITUITARY. N. Zamir* and M.J. Brownstein* (Spon. R.M. Kostrzewa). Hypertension and Endocrine Branch, NHLBI, Experimental Therapeutics Branch, NINCDS, and Lab. of Cell Biology, NIMH, NIH, Bethesda, MD 20205 USA

The rat posterior pituitary contains large amounts of Leu-enkephalin (LE) and Met-enkephalin (ME). Pituitary stalk transection results in a marked depletion of ME (82%) and LE (95%) in the posterior pituitary suggesting that most of the enkephalins are in processes of central neurons. The location of the LE or ME containing neurons was sought by examining the effects of hypothalamic lesions or fiber transections on the LE and ME content of the posterior pituitary. Paraventricular nucleus (PVN) lesions cause a 56% fall in ME; as does medial basal hypothalamic (MBH) deafferentation. Monosodium glutamate (MSG) treatment of neonatal rats which causes selective destruction of arcuate nucleus neurons, has no effect on ME concentrations in the posterior pituitary. Therefore, PVN provides somewhat more than half of the ME in posterior pituitary. The remainder may come from the ventromedial nucleus. On the other hand, PVN lesions cause only a 28% fall in LE, while MBH deafferentation causes a 94% decrease. Again, MSG treatment has no effect. Therefore, the PVN only contributes about a quarter of the LE, though most of LE comes from cells outside of the MBH, perhaps from cells in the supraoptic nucleus. Our findings are consistent with the hypothesis that LE and ME are localized in separate populations of nerve endings in the neurohypophysis. Physiological experiments support this notion. Giving animals 2% NaCl to drink for 100 hrs causes a marked depletion of LE (82%) in the posterior pituitary, but has no effect on ME concentration. The abundance of dynorphin-related peptides in the posterior pituitary, the molar excess of LE over ME there, and the similar results of the surgical and physiological manipulations on LE and dynorphin-related peptide levels in the posterior pituitary, suggest that LE in the posterior pituitary comes mainly from prodynorphin, which contains three LE sequences.

173.3 DISTRIBUTION OF SUBSTANCE P AND ENKEPHALIN IMMUNOREACTIVE PROFILES IN THE SUBSTANTIA NIGRA OF RAT, CAT AND MONKEY. S. Inagaki and A. Parent. Lab. of Neurobiology, Fac. Med., Laval Univ., Quebec, Que., Canada.

A comparative study of the distribution of substance P (SP) and enkephalin (ENK) immunoreactivity in the substantia nigra (SN) of the rat, cat and squirrel monkey (Saimiri Sciureus) was undertaken by means of the indirect immunofluorescence technique.

In the rat, dense neuronal networks composed of fine fibers displaying SP immunoreactivity are uniformely distributed throughout the rostrocaudal extent of the substantia nigra pars reticulata (SNr) and in a part of SN pars compacta (SNc). Coarse SP fibers also occur in SNc. In addition, coarse ENK-positive fibers are scattered throughout the neurons of SNc but abound particularly in the caudo-lateral part of SNr.

In the cat, fine SP-reactive fibers are distributed in SNr according to a pattern similar to that found in rat and coarse SP fibers also occur in SN. Moreover, ENK-positive fine fibers are densely packed in the ventromedial part of SNr whereas coarse ENK fibers are scattered in SNc and SNr, but abound especially in the caudo-lateral portion of SNr.

In the squirrel monkey, SP and ENK-reactive fibers of fine type are primarily present in large number in SNr. Although strikingly complex and heterogeneous, the pattern of distribution of the ENK fibers in SNr is surprisingly similar to that of the SP fibers. In addition, coarse fibers displaying either SP or ENK immunoreactivity are scattered among the neurons of the SNc.

The findings of the present study reveal that dense neuronal networks of SP-immunoreactive fibers occur throughout the entire rostrocaudal extent of SN in the rat, cat and monkey. These SP fibers are distributed according to a pattern that is not markedly different in the three species. In contrast, the number of ENK-immunoreactive fibers in SN and the complexity of their organizational feature increase strikingly from rat to monkey. These results, which are in keeping with the recent biochemical and immunohistochemical demonstration of high level of ENK in human SN, and biochemical and physiological studies supporting neurotransmitter or neuromodulator role of ENK suggest that ENK may play a very important and peculiar role in the functional organization of the SN in primate.

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173.4 SPINAL PARASYMPATHETIC ENKEPHALIN FIBERS: PATTERNS AND PROJECTIONS. M.A.Romagnano and R.W. Hamill. Monroe Community Hospital/Univ Roch Med Ctr, Roch, N.Y. 14603

Previous studies indicate that thoracolumbar sympathetic outflow is densely innervated by enkephalin (Enk) fibers. The present studies were designed to examine whether a similar arrangement existed in the sacral parasympathetic system and thus whether spinal autonomic areas are influenced, at least in part by the neuropeptide Enk.

The distribution of Enk was examined in the sacral spinal cord of the rat. Normal and colchicine pretreated rats were perfused with Zamboni's fixative. Spinal cords were removed and 40µ horizontal serial vibratome sections were stained for Enk by the unlabeled antibody method of immunocytochemistry. Sections were reacted for 48 hrs at 4°C in primary antiserum used at a dilution of 1/500-1/1000 (Immunonuclear Inc, 18H2T) or 1/1000-1/2000 (D.S. Sundberg). Dense accumulations of Enk fibers were found in Lissauer's tract (LT), laminae I and II and in the dorsolateral funiculus. Enk fibers extended from LT along the lateral edge of the dorsal horn toward the sacral parasympathetic nucleus (SPN). Labeled fibers were found both within the SPN and projecting laterally from the SPN into the lateral funiculus toward the pial surface. Medially from the SPN Enk fibers projected in laminae VII toward the dorsal gray commissure. These labeled fibers exhibited a periodicity of 100µ. Enk fibers also extended from LT along the medial edge of the dorsal horn toward the dorsal gray commissure where a dense accumulation of labeled fibers was found. A distinct band of rostro-caudally oriented Enk fibers was observed immediately dorsal to the central canal in lower sacral segments. In the ventral horn Enk fibers were found surrounding individual motorneurons and in Onuf's column. Strands of Enk fibers connect Onuf's column with a column of Enk fibers located in the ventral medial most aspects of the ventral horn. Many labeled Enk cells were found in the dorsal gray commissure while an occasional Enk cell was found in the intermediate zone.

It is apparent that the distribution of Enk fibers in the sacral spinal cord parallels the localization of primary visceral afferents found in the pelvic nerve (Nadelhaft and Booth, 1982; Morgan et al., 1981; Nadelhaft et al., 1983). In addition, Enk fibers are found interspersed among preganglionic parasympathetic neurons in the SPN. These findings provide a morphological substrate for enkephalinergic interactions with parasympathetic functions and with primary visceral inputs to the sacral spinal cord.

173.5 EFFECTS OF A SINGLE, SMALL DOSE OF MORPHINE ON LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU) IN CATS (SPON: E.H. Rubinstein). H.T. Chugani,* J.R. Villablanca, C.M. Harris & J.W. Burgess; Mental Ret. Res. Ctr.; Depts. Neurology, Pediatrics, Psychiatry & Anat., UCLA School Med.; Los Angeles, CA 90024.

Our previous studies indicate that small doses of morphine (0.5-3 mg/kg) produce a typical, reliable and long-lasting behavioral response in cats, and that the basal ganglia might be involved in these effects (Brain Res. 258: 159, 1982). In the present experiments, measurements of LCGU using 14C-2-deoxyglucose (2-DG) were made in 8 adult cats in order to delineate brain structures which may become functionally active under morphine administration. Each cat was placed in a sound-attenuated one-way mirror chamber and administered morphine sulphate (2-3 mg/kg) i.p. (N=4) or saline placebo (N=4). Two hr after injection, at the peak of the behavioral effects (aroused, sitting with discrete visually guided type head-paw movements), the cat was removed from the chamber, the rectal temperature measured, and vein and artery catheters (previously implanted) were exposed. Normality of arterial blood gases was verified, and 2-DG (100 µCi/kg, 50-55 mCi/mmol) was injected i.v. over 30 sec. LCGU of 35 brain regions (excluding cerebellum) was determined by quantitative autoradiography, using the method of Sokoloff et al. (1977). Values for individual brain areas were grouped into functional brain systems for analysis of variance. Only basal ganglia related areas (caudate, putamen, g. pallidum-entopeduncular n., s. nigra, thalamus CM-VL, and subthalamus) and pericruciate motor cortex (pre and post cruciate area 4) showed significant ($P<.01$ and $P<.05$ and respectively) main effects increases in LCGU after morphine; the stronger increases, as revealed by significant single effects, were seen in subthalamic n. ($P<.01$), s. nigra pars reticulata ($P<.05$) and pre ($P<.01$) and post ($P<.01$) cruciate motor cortices. No significant effects of morphine on LCGU were found on 8 sampled areas of the limbic system, visual (lat. geniculate n., s. colliculus) or auditory (med. geniculate, inf. colliculus) systems, except for single effect significance for medial habenula ($P<.05$) and the splenium of c. callosum ($P<.05$). Together with previous findings of the blocking of the typical behavioral effects of morphine by neostriatal lesions, our results suggest the mediation of basal ganglia in the expression of neurobehavioral effects of opiates.

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173.6 ENKEPHALIN NEURONS IN THE NUCLEUS OF THE SOLITARY TRACT PROJECT TO THE PARABRACHIAL NUCLEUS OF THE CAT B. MALEY DEPT. ANATOMY, UNIV. KENTUCKY MED. CTR. LEXINGTON, KY 40536

Immunohistochemical studies have indicated that there are numerous enkephalin immunoreactive neurons within the medial and commissural subdivisions of the nucleus of the solitary tract (NTS). These areas are known to have a major projection to the parabrachial nucleus. In order to determine if the enkephalin containing neurons in the NTS were responsible for a portion of the innervation to the parabrachial nucleus a combination of retrograde tract tracing and immunohistochemistry were used in this study. Two days following an unilateral injection of 40% horseradish peroxidase peroxidase (HRP) into the parabrachial nucleus, cats received an intracisternal application of colchicine and were allowed to survive for an additional 24-48 hours. The animals were sacrificed by a vascular perfusion with 4% paraformaldehyde in phosphate buffer, and the NTS was processed for HRP histochemistry and followed by enkephalin immunocytochemistry. To differentiate HRP granules from the brown enkephalin immunoreactivity within suspected double labelled neurons the tissue sections for HRP histochemistry were pretreated with cobalt chloride to yield a black HRP reaction product.

Following the double labelling procedure, three classes of neurons could be distinguished in the medial and commissural subdivisions of the NTS. The first class were neurons labelled only with black HRP granules (25-40 neurons/75 um section), while a second group (20-30 cells per section) possessed only the brown reaction product of enkephalin immunoreactivity. The third class contained both black HRP granules and brown enkephalin immunoreactivity. This was the smallest group and it contained only 3-7 neurons within every section. The lack of HRP labelling in all enkephalinergic NTS neurons suggests that they may project to other neural areas in the central nervous system or it may be involved in intrinsic NTS circuitry.

Results of the present investigation demonstrate an enkephalinergic projection from the NTS to the parabrachial nucleus in the cat. This input to the parabrachial nucleus from the NTS provides a means by which enkephalinergic systems may play a role in the regulation of the autonomic functions such as regulation of the cardiovascular system.

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173.7 EFFECTS OF ISCHEMIA AND RECIRCULATION ON REGIONAL OPIATE PEPTIDE LEVELS IN GERBIL BRAIN. R.L. Fried* and T.S. Nowak, Jr.* (SPON: T.S. Whittingham). Laboratory of Neurochemistry, NINCDS, NIH, Bethesda, MD 20205

Male Mongolian gerbils (age 3 mo) were subjected to bilateral common carotid artery occlusion for 5 min, followed by recirculation for up to 2 hr. Arteries of control animals were exposed but not clamped. Animals were decapitated and tissues dissected as follows: pituitary (neurointermediate (NIL) and anterior (AL) lobes were separated in some experiments), hypothalamus (HYP), cerebellum (CER), cerebral hemispheres (HEMIS) and brain stem (BS). Extracts were prepared and assayed for total immunoreactive β-endorphin (END) and met-enkephalin (ENK) by radioimmunoassay. Assay procedures and materials were provided by Dr. G. P. Mueller, Department of Physiology, USUHS, Bethesda, MD.

Control END levels obtained in a typical assay were: 0.72 $\pm$ 0.21 µg/NIL, 2.07 $\pm$ 0.76 µg/AL, 3.27 $\pm$ 0.90 ng/HYP, 4.48 $\pm$ 0.52 ng/gm BS, and 1.85 $\pm$ 0.15 ng/gm HEMIS. Control ENK values were: 1.32 $\pm$ 0.28 pg/whole pituitary, 1.70 $\pm$ 0.29 pg/HYP, 7.65 $\pm$ 0.65 ng/gm BS, and 4.84 $\pm$ 0.71 ng/gm HEMIS. Peptide levels in CER were at the limit of detection.

Peptide levels were largely unaffected by ischemia and recirculation, with two exceptions. First, there was a consistent 20% decrease in ENK in HEMIS during recirculation, lasting at least 2 hr. More dramatically, in one of two experiments, END levels were elevated 3-fold in HYP at 10 and 30 min recirculation (longer recovery periods were not examined in this experiment). This increase was entirely accounted for by an increase in acetylated END, and was accompanied by an increased level of α-MSH, suggesting an influx of these peptides from pituitary. Although the pituitary circulation of the gerbil has not been studied in detail, that of most species is considered to arise from the carotid arteries, and the pituitary would thus be subject to ischemia in this experimental model. Blood flow to the hypothalamus is largely unimpaired. These factors may give rise to hemodynamic alterations during the period of recirculation which could account for the changes in peptide levels observed. Further experiments are directed toward determining the reasons for the failure to observe this effect in all experiments. The gerbil ischemia model may provide an interesting experimental system for the more detailed study of processing and release of neuropeptides, since overall protein synthesis (and presumably precursor synthesis) is depressed for some time after recirculation in brain regions subject to ischemia.

173.8 COMPARISON OF THE PROJECTIONS OF SUBSTANCE P AND DYNORPHIN TO THE RAT SUBSTANTIA NIGRA: A RADIOIMMUNOCYTOCHEMICAL AND BIOCHEMICAL STUDY. S. McLean*, M. Bannon, N. Zamir* and C.B. Pert (SPON: J. Thomas). Laboratory of Brain Biochemistry, and Laboratory of Cell Biology, National Institute of Mental Health, Bethesda, Maryland 20205.

The use of radiolabeled antibodies combined with tritium sensitive film and computer assisted densitometry allows for the semiquantitative analysis of changes in antigen level, as well as providing detailed neuroanatomical information (McLean, et al., Brain Res. 278:253-257, 1983).

There is in the substantia nigra (SN) a dense distribution of Substance P (SP) and Dynorphin B (Dyn) immunoreactive fibers and terminals originating from the neostriatum (Brownstein, et al., Brain Res. 135:315; Vincent, et al., Eur. J. Pharm. 85:251). Following knife cuts in the striatum, the topography of SP and Dyn projections to the SN were examined in alternate sections and the changes in immunoreactivity as expressed by optical densitometry (OD) were compared with the values obtained by radioimmunoassay (RIA).

Knife cuts extending from the fasciculus retroflexus to the lateral ventricle significantly reduced immunostaining for both SP and Dyn in all areas of the SN with the exception of the medial reticulata (SNr) which was only slightly reduced in intensity. Cuts which separated the anterior striatum but not the posterior striatum from the SN diminished SP and Dyn in the SNr, but now in addition to the medial aspect, staining for both SP and Dyn was visible in the lateral portions of the SNr. Cuts in the anterior striatum resulted in a smaller decrease of both SP and Dyn in the SN.

The values obtained by RIA and by OD were significantly correlated for both SP (r=.95) and Dyn (r=.88). This indicates radioimmunocytochemistry can be used to assess changes in levels of antigens in very discrete areas such as sub nuclei or laminar patterns.

There is a topographical projection of SP and Dyn containing cells with those cells in the lateral posterior striatum projecting to the lateral SN. The SP and Dyn immunoreactivity in the most medial aspect of the SNr that remained following the knife cut transecting the MFB and ansa lenticularis may be fibers from the caudate that travel along the midline to reach the SN or may arise from structures caudal to the SN.

The similarity of changes in patterns of immunostaining and levels as measured by RIA and OD suggest a close association of cell bodies containing Dyn and SP that project to the SN or perhaps co-localization of these peptides.

173.9 OPIOID RECEPTOR-SPECIFIC MEDIATION OF CHANGES IN THALAMIC CALMODULIN IN NARCOTIC-DEPENDENT RAT AND HUMAN. K.A.Bonnet, M.Orbuch*and M.Wilner*. Millhauser Laboratories, New York Univ.Sch.Med., New York, New York 10016.

Opioids and enkephalins bind stereospecifically to receptor subtypes that we have shown to have differential anatomical distributions in rat and human brain. A pervasive effect of opioid agonist encounter is acute displacement of membrane calcium. Chronic agonist encounter results in specific increases in calmodulin levels in the rat thalamus, and periaqueductal grey but not in amygdala or frontal cortex. Moreover, the calmodulin shows higher membrane bound proportions in those regions showing the change. Such changes in calmodulin appear to account for parallel changes in calmodulin-sensitive phosphodiesterase activity in thalamus and periaqueductal grey. These areas are selectively high in proportionate representation of narcotic-preferring receptor subtypes. However, the relevance of these region-specific changes in rat brain remained to be demonstrated in human narcotic dependence. Our studies, directed to this, use postmortem samples from age and sex-matched drug-free and heroin-abusing brain regions that correspond to the regions studied in rat models. Calmodulin was 98% soluble in postmortem tissues. Calmodulin levels were significantly elevated in heroin-abusing thalamus and amygdala compared to levels in drug-free control samples. No change had been seen in rat amygdala. However, we have reported that the rat amygdala has a large proportion of delta receptors and few mu receptors (narcotic-preferring), whereas the human amygdala has a proportionately greater number of mu than delta receptors. This suggests that increased calmodulin activity represents an anatomically specific adaptive response to chronic narcotic use, and is mediated through narcotic-preferring receptors. This may bias catecholamine systems to greater release rates, and to greater post-synaptic efficacy in some systems upon release from the presence of chronic high levels of opioid agonists.

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173.10 IDENTIFICATION OF LEUCINE-ENKEPHALIN AND OTHER NEUROPEPTIDES IN PELVIC AND PUDENDAL AFFERENT PATHWAYS TO THE SPINAL CORD OF THE CAT. M. Kawatani, J. Nagel*, M.B. Houston*, R. Eskay*, I.P. Lowe and W.C. de Groat. Dept. of Pharmacology and Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, Sch. of Medicine, Pittsburgh, PA 15261.

In sacral dorsal root ganglia (DRG) of the cat, substance P (SP), somatostatin (SS) and vasoactive intestinal polypeptide (VIP) are present in small diameter ganglion cells. We have also recently identified leucine-enkephalin immunoreactivity (L-ENK-IR) in considerable numbers of small and medium size DRG cells. The present experiments were undertaken to determine whether these peptides are contained in pelvic and pudendal nerve afferent pathways to the lumbosacral spinal cord. Retrograde transport of fluorescent dye (fast blue) was applied to pelvic and pudendal nerves 7-14 days prior to sacrifice. Colchicine (10-20 µg) was applied to the sacral DRG using microinjection techniques to build up the concentration of peptides.

A large percentage (45%) of sacral dorsal root ganglion cells contained L-ENK-IR, whereas few dynorphin A 1-8 (DYN) neurons and no methionine-enkephalin neurons were observed. Among the population of pelvic nerve afferent neurons (PLAF) L-ENK was present in 60% of the neurons, SP in 30%, VIP in 15%, and a few neurons contained DYN. SS was not detected in PLAF. Among the population of pudendal nerve afferent neurons (PUDAF), L-ENK was present in 50%, SP in 30%, VIP in 3%, and a few neurons contained DYN and SS. Controls in which each antisera was preabsorbed with its respective peptide (0.05-2 µM/ml) exhibited no reaction product. Dye labelled PLAF ranged from 24 to 40 µm in diameter and PUDAF from 24 to 64 µm. L-ENK was present mainly in the small cells less than 36 µm. VIP and SP were also present in small neurons (20 to 32 µm) whereas SS and DYN were present in medium size neurons (32 to 48 µm).

These results indicate that L-ENK and SP may be important transmitters or modulators in pelvic and pudendal afferent pathways. L-ENK released by visceral and somatic primary afferents could provide feedback inhibition by activation of opiate receptors on afferent terminals. On the other hand, since VIP is present in a larger percentage of pelvic as compared to pudendal afferent neurons, this peptide is likely to be a more important transmitter in visceral afferent systems.

173.11 ULTRASTRUCTURE OF LEUCINE ENKEPHALIN TERMINALS ON NEURONS IN THE SACRAL PARASYMPATHETIC NUCLEUS OF THE CAT. C. Morgan, Dept Anat, UCSF Sch Med, San Francisco, CA 94143

Leucine-enkephalin (L-ENK), shown to be inhibitory in the autonomic pathway to the urinary bladder is also present in varicosities within the region of the sacral parasympathetic nucleus (SPN) and has been shown to surround preganglionic neurons within this nucleus (deGroat et al., 1983; Glazer and Basbaum, 1980). The present study was undertaken to determine if these previously reported varicosities seen with the light microscope (LM) represent actual synaptic contacts with the neurons of the SPN.

Adult cats were anesthetized and then perfused with 4% paraformaldehyde - 0.1% glutaraldehyde. Sacral spinal segments were identified, removed and cut into 50 um transverse or horizontal sections using a vibratome. Tissue sections were incubated for 12 hrs. in L-ENK antisera (1:10,000) (Immuno Nuclear) and processed by the PAP method. Following osmication, dehydration and epon embedding selected sections were cut in serial 4 um sections.

At LM, many L-ENK varicosities surrounded cell bodies and dendrites in lateral laminae V and VII. These neurons were similar in size and orientation to preganglionic and spinal projection neurons of the SPN defined in earlier studies. After photographing and making drawings of these sections, they were cut in serial ultrathin sections.

At EM the varicosites were small, 0.3 to 1.5 um dia and made contact with somata, somatic spines, proximal and distal dendrites and with vesicle-containing profiles. Labeled terminals were packed with vesicles and mitochondria. All organelles as well as the internal face of the terminal membrane had a dark flocculent coating of PAP reaction product. Large granular vesicles (LGV), when present, contained darkly stained centers. There were two types of L-ENK terminals - those with a mixture of LGV and clear round vesicles and those with mostly pleomorphic vesicles and only a few LGV. Synaptic specializations were symmetrical and not prominent.

It may be concluded that many of the L-ENK varicosities observed at LM are indeed terminals upon somata and dendrites of neurons within the SPN and that these terminals provide the structural basis for some of the neuropharmacological actions of L-ENK in the pelvic visceral reflex pathway. It is suggested that the different terminal types may originate from separate sources and perform different functions within this system.

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173.12 IMMUNOCYTOCHEMICAL STUDIES OF DYNORPHIN DISTRIBUTION IN THE RHESUS MONKEY CENTRAL NERVOUS SYSTEM. H. Khachaturian, M.E. Lewis, M.D. Fitzsimmons and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan 48109.

Previous studies of dynorphin distribution in the central nervous system have described its widespread neuronal localization in the rat brain and spinal cord (e.g., Khachaturian et al., Peptides, 3:941, 1982; Vincent et al., Neurosci. Lett., 35:185, 1982; Weber et al., Proc. Nat. Acad. Sci. USA, 79:3062, 1982). However, our knowledge of dynorphin anatomy in the primate brain is limited to one previous comparative anatomical study of opioid peptide-opiate receptor distribution in the rhesus monkey brain (Lewis et al., Life Sci., 33(Suppl. I):239, 1983). The present study extends our findings of dynorphin anatomy in the monkey central nervous system.

Adult rhesus monkeys (Macaca mulatta) were anesthetized with sodium pentobarbital (30 mg/kg,i.v.) and perfused via an intra-aortic cannula with normal saline (4 liters), followed by buffered 4% formaldehyde (16 liters). Some animals were treated with colchicine (1-3 mg,ICV) 24-48 hrs prior to perfusion, to enhance perikaryal immunoreactivity. Each brain was blocked, postfixed for 4 hrs, placed in a buffered solution of 15% sucrose overnight (4°C), and frozen in isopentane at -50°C. Cryostat-sectioned tissue were either stored at -80°C or processed for PAP immunocytochemistry using primary rabbit antisera raised against various prodynorphin peptides, including dynorphin A, dynorphin "bridge peptide," dynorphin B, dynorphin(1-8) and alpha-neo-sendorphin.

All dynorphin antisera under study "stained" similar neuronal structures throughtout the monkey brain. Furthermore, each antiserum was tested for specificity and cross-reactivity with other related peptide fragments. Thus far, these antisera have been shown to be highly specific in the rat brain. Dynorphin immunoreactive perikarya were noted in the caudate nucleus and magnocellular supraoptic and paraventricular nuclei. Immunoreactive fibers were additionally localized to the striatum, globus pallidus, preoptic area, bed nucleus of stria terminalis; hypothalamic periventricular, ventromedial and arcuate nuclei; lateral and posterior hypothalamic areas and median eminence; substantia nigra, interpeduncular nucleus, periaqueductal gray; dorsal raphe, parabrachial, tractus solitarius and spinal trigeminal nuclei; and spinal cord dorsal gray.

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173.13 POSTNATAL DEVELOPMENT OF OPIOID SYSTEMS IN RAT BRAIN. S.E. Loughlin, T.R. Massamiri*, H.I. Kornblum* and F.M. Leslie. Dept. of Pharmacology, Univ. of Calif., Irvine, CA 92717.

The present studies sought to address the question of whether the endogenous opioid peptides and their receptors may play a functional role in the developing rat brain which is distinct from that in the adult. Immunocytochemical and receptor autoradiographic techniques were utilized to examine the postnatal development of opioid peptides and receptors. The distribution of representative peptide products of each endorphin system was analysed utilizing a variety of antibodies. The pattern of met-enkephalin-like immunoreactivity was very similar at birth to the adult, increasing only in intensity throughout postnatal development. In contrast, neonatal β-endorphin-like immunoreactive (BELI) fibers were ubiquitous throughout the brain at birth, condensing gradually to the circumscribed adult pattern. Germinal zones, present only in the neonate, also exhibited dense BELI in cell bodies and terminals. The distribution of dynorphin-B-like immunoreactivity at birth was intermediate in its similarity to that of adult. Certain terminal fields were present, others developed postnatally, and others disappeared with age.

In parallel studies, autoradiographic maps of μ and δ opioid receptor subtypes were generated to determine whether any correspondences existed between the developmental distribution of opioids and their receptors. The μ receptor distribution was defined as the binding pattern of [^{3}H]D-ala^2-met-enkephalin-gly^5-ol. The δ receptor distribution was determined by incubation in [^{3}H]D-ala^2-D-leu^5-enkephalin in the presence of D-pro^4-morphiceptin, a μ selective ligand. Specific binding was defined in the absence or presence of levallorphan. Significant overlap was observed between the distributions of these receptor subtypes and the opioid peptides.

The differential localization of the opioid receptors and peptides in neonatal and adult brain suggests that these may subserve distinct functions in the neonate. In particular, the presence of BELI in the germinal zones, where postnatal neurogenesis occurs, implicates opioid systems in regulation of neuronal cell division. This is consistent with the recent demonstration of changes in brain size following early postnatal naltrexone treatment (Zagon, I. and McLaughlin, P. Science, 221, 1179-1180, 1983). Experiments are currently being undertaken to address this hypothesis.

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173.14 SPECIES DIFFERENCES IN REGIONAL DISTRIBUTION OF OPIOID RECEPTOR SUBTYPES. F.M. Leslie, R.P.Burgoon*, and S.E. Loughlin. Department of Pharmacology, University of California, Irvine, CA 92717

The anatomical localization of opioid receptor subtypes may be an important reflection of functional significance. Previous membrane binding studies have suggested species differences in the ratios of μ, δ and k receptors in rodent brain. The present study therefore addresses the question of whether these differences reflect differential distributions of opioid receptor subtypes. Using highly selective labeling conditions ([^{3}H]D-ala^2-met-enkephalin-gly^5-ol (1.6 nM) or [^{3}H]D-ala^2-met-enkephalinamide (2 nM) + D-pro^4-morphiceptin (300 nM)), tritium film autoradiograms were generated for μ and δ receptors in mouse, guinea pig and rat brain sections. Specific binding, defined in the absence and presence of levallorphan (1 μM), was > 95%. The overall distribution and density of μ receptors was similar in many regions, including nucleus accumbens, anterior thalamus, hippocampus, interpeduncular nucleus and superior colliculus. In caudate-putamen, cortex and subiculum, similar characteristic patterns of labeling were apparent across species, although the relative density of μ receptors was lower in mouse. In contrast, a differential distribution of μ receptors was noted in certain sensory processing areas including olfactory bulb, olfactory tubercle, piriform cortex and medial geniculate. Striking differences were observed in the density of δ receptor labeling in the three species, with mouse exhibiting an overall higher density. Whereas there were no species differences in the density and distribution of δ receptors in olfactory bulb and nucleus accumbens, there were marked differences in labeling of cortex, olfactory tubercle and medial geniculate. The pattern of labeling in caudate-putamen was similar across species, but exhibited a higher density in mouse. Immunocytochemical studies are currently in progress to address the question of whether these differences in receptor localization are correlated with differences in the distribution of opioid peptides.

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173.15 THE LOCALIZATION OF PROOPIOMELANOCORTIN mRNA CONTAINING CELLS IN THE RAT BRAIN BY IN SITU cDNA:mRNA HYBRIDIZATION. J.N. Wilcox* and J.L. Roberts (SPON: M. Mawe). Center for Reproductive Sciences, Columbia Univ., New York, NY 10032

Immunocytochemical data suggests that proopiomelanocortin (POMC) containing cell bodies in the rat brain are primarily confined to the peri-arcuate region of the hypothalamus. However, Northern blot analysis indicates that there might be another population of POMC cells in the amygdala and cortex. Herbert and co-workers found an mRNA in these brain regions that hybridized to a POMC cDNA probe which was smaller than POMC mRNA found in the rat hypothalamus and pituitary. In order to resolve this controversy we set out to localize POMC neurons in the brain using in situ cDNA:mRNA hybridization. The in situ cDNA:mRNA hybridization technique is a procedure that has been developed for the visualization of specific mRNA's in individual cells. Previously, use of this technique to visualize mRNA's in the brain has met with limited success. Ribonuclease appears to be very active in brain tissue even after fixation in paraformaldehyde solutions. Rapid processing of the tissue and storage of the brain sections at -70°C with dessicant partially circumvents these problems. Furthermore, thawing neural tissue in the presence of proteinase-K prior to in situ hybridization degrades ribonuclease as it becomes active and enhances the in situ cDNA:mRNA hybridization signal. Thus, with these modifications incorporated into the in situ protocol we are able to reliably use this technique to visualize POMC mRNA in individual cells of the brain. Silver grain accumulation over confirmed POMC cells is on the order of 150 times background as determined by Bioquant II image analysis. Interestingly, silver grain accumulation in POMC cells is either evenly distributed throughout the cytoplasm of the cell or is localized in the cytoplasm just adjacent to the cell nucleus. We have used this technique to plot the distribution of POMC mRNA containing cells in the rat brain. Not surprisingly the distribution we have seen is a combination of the immunocytochemical staining patterns resulting from both beta endorphin and ACTH antibodies. To date we have not seen POMC mRNA positive cells in either the cortex or amygdala in contrast to earlier work using Northern blot analysis that seemed to indicate that POMC mRNA was found in these regions. It is possible that POMC cells are indeed present in these regions but are so few as to be missed in a serial section analysis of the brain or that the POMC mRNA copy number in these cells is too low for detection by this method.

OPIATES, ENDORPHINS, AND ENKEPHALINS: BIOCHEMICAL CHARACTERIZATION

174.1 HUMAN TOOTH PULP CONTAINS METHIONINE ENKEPHALIN AND NO MU RECEPTORS. D. M. Desiderio, H. Onishi*, H. Takeshita*, F. S. Tanzer*, Jay Walker*, Claire Wakelyn*, and G. Fridland* . Dept. of Neurology, Neuroscience Mass Spectrometry Lab., and Depts. of Orthodontics and Oral Diagnosis, Univ. Tenn. Ctr. Hlth. Sci., Memphis, TN, 38163.

The objective of this research program is to determine the molecular basis of the peptidergic pathways which participate in pain mechanisms. One of the major goals is to establish an analytical measurement system that provides quantitative data with no ambiguity. Novel mass spectrometric methodology is the only method that can now provide that type of data at a sensitivity level required to measure picomoles of endogenous enkephalins. In addition to mass spectrometric methods, commercial antibodies are used in a radioimmunoassay, and a canine limbic system synaptosomal preparation is used for radioreceptor assay. In all cases, gradient or isocratic reverse phase high performance liquid chromatography is used before any assay to provide a relatively enriched peptide fraction. It is found that a normal human tooth has a much higher, nearly 100x, amount of methionine enkephalin compared to teeth that were stressed before removal. While the RIA and RRA data do not agree closely (RRA data are always higher than RIA data), the two sets of data parallel each other. Preliminary corroborative MS data indicate that the RRA data are twice as much as the MS data. In any event, in addition to measuring endogenous peptides in the human tooth for the first time, an analytical method is now available for calibration of RRA and RIA methods where that calibration is performed in a fast, facile, and objective manner.

174.2 METHIONINE- AND LEUCINE-ENKEPHALIN IN MONKEY BRAIN AFTER CHRONIC MORPHINE AND NALTREXONE. D.E. Redmond, Jr., J.D. Elsworth*, R.H. Roth. Depts. Pharmacol. & Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06510.

An interaction of opiates with endogenous opioids has been postulated as a basis for acute or chronic opiate withdrawal, but consistent evidence has not been obtained measuring enkephalin levels in rat brain. Several studies have also failed to demonstrate changes during chronic morphine treatment. We recently reported a sensitive and relatively specific method for measuring methionine- (MET) and leucine- (LEU) enkephalin, using reverse-phase HPLC and radioimmunoassay. We report here that differences in MET and LEU concentrations are found in the hippocampus of monkeys after 10 days of morphine treatment.

Cercopithecus aethiops sabaeus were studied in the following groups (with the N): SHAM PELLET (6), SHAM PELLET + NALTREXONE (3), MORPHINE PELLET (4), MORPHINE PELLET + NALTREXONE (4), AND MORPHINE PELLET + NALTREXONE + CLONIDINE (4). Monkeys were treated and sacrificed, and their brains dissected and stored as previously described. MET and LEU were measured as reported elsewhere (in press).

From 27.65 ± 5.57 picograms/milligram tissue in the SHAM PELLET treated monkeys, MORPHINE PELLET administration reduced MET concentration in the hippocampus to 16.37 ± 2.8 pg/mg. A similar effect on LEU was seen in the same groups, with a reduction from 7.9 ± 3.2 in the SHAM PELLET group to 3.45 ± 0.48 in the MORPHINE PELLET group. The NALTREXONE group had MET (27.4 ± 4.6) and LEU (5.97 ± 2.1) concentrations that were not different from the SHAM PELLET group, but the MORPHINE PELLET + NALTREXONE group had concentrations of both peptides that were closer to the SHAM PELLET group values than to the MORPHINE PELLET group's (MET-22.28 ± 2.43; LEU-7.48 ± 2.87). Clonidine pre-treatment failed to prevent the rise in MET (to 21.88 ± 4.69), but did attenuate the increase in LEU (5.17 ± 0.92).

These data support a possible involvement of MET and LEU in feedback regulation of their own activity and in the process of tolerance to morphine administration. (Supported by DA02321)

174.3 BIOCHEMICAL AND IMMUNOCYTOCHEMICAL STUDIES OF THE POSTNATAL DEVELOPMENT OF BETA-ENDORPHIN IN THE RAT MEDULLA OBLONGATA. N. Alessi, H. Khachaturian and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, Mich. 48109.

Neurons containing pro-opiomelanocortin (POMC) peptides, Beta-endorphin and ACTH have been identified in the nucleus tractus solitarius (NTS) of the adult rat medulla (Schwartzberg and Nakane, Brain Res. 276:351, 1983; Joseph et al., Neurosci. Lett., 38:221, 1983). Previous immunohistochemical studies of the development of this system have demonstrated beta-endorphin immunoreactivity (i.r.) in NTS neurons starting at embryonic day E17, and dropping out in the third postnatal week in colchicine-untreated animals (Baetge et al., Soc. Neurosci. Abstr., 8:636, 1982; Khachaturian et al., Life Sci., 33 (Suppl. I.):61, 1983). With the exception of one report of beta-endorphin i.r. in the medulla during development (Bayon et al., Brain Res., 179:93, 1979), no attempts have been made to correlate the biochemical and immunohistochemical ontogeny of this system. In the present study, beta-endorphin i.r. is determined in the NTS during postnatal development using a radioimmunoassay technique. The results are compared and contrasted to the immunocytochemical findings.

Postnatal male Sprague-Dawley rats were sacrificed at weekly intervals (P1, P7 ... P42), either by decapitation, for radioimmunoassay or cardiac perfusion with 4% formaldehyde for immunocytochemistry. For radioimmunoassay, the caudal medulla oblongata was dissected out and homogenized in acid:acetone (1:3). Protein levels were determined using the Lowry technique. Brains from perfused animals were processed for peroxidase-antiperoxidase immunocytochemistry At P1, Beta-endorphin i.r. ($\bar{X} \pm$ S.E.M.) was 77.0 $\pm$ 1.3 fm per medulla. This level progressively increased to 900.0 $\pm$ 31.7 fm at P42. When Beta-endorphin i.r. was determined per unit protein, no significant alteration of Beta-endorphin i.r./unit protein was noted during development. The immunocytochemical findings indicate that with colchicine pretreatment, some neurons in the pars commissuralis of NTS exhibited i.r. at all ages studied. Without colchicine, perikaryal i.r. was faint at P1 and P7, and disappeared by P14.

The stability in i.r. measured by radioimmunoassay, suggests the hypothesis that the "drop-out" noted with immunocytochemistry is due to a simple loss of peptide stores to a level below detection by immunohistochemical techniques. Supported by the Department of Psychiatry Research Fellowship (NEA) and NIDA Grant #DA02265 (S.J.W.).

174.4 PRODUCTION AND CHARACTERIZATION OF A MONOCLONAL ANTIBODY SPECIFIC FOR PRECURSOR FORMS OF DYNORPHIN. R.W. Barrett* and A. Goldstein (SPON: R.I. Cone). Addiction Research Foundation, Palo Alto, CA 94304.

A common property of antisera raised against dynorphin A (DYN A) or dynorphin B (DYN B) is that these antisera also recognize precursor peptides that contain the sequence of DYN A or DYN B. In an attempt to develop an antibody that is specific for precursor peptides that contain both sequences, BALB/c mice were immunized with synthetic 4000-dalton dynorphin (D32) conjugated to thyroglobulin. Mouse sera were screened for the presence of antibodies directed against D32 using an ELISA developed with established rabbit polyclonal antibodies. Spleen cells from the mouse with the highest serum titer were fused with P3x63-Ag 8.653 myeloma cells and hybridomas were selected with HAT medium. Four positive clones were identified by screening hybridoma medium with the D32 ELISA. Initial characterization of one these clones revealed its specificity for D32 as opposed to DYN A or DYN B. This hybridoma was sub-cloned and grown in ascites tumors.

A sensitive solid-phase RIA was developed using iodinated antibody and synthetic D32 attached to microtiter plates. The RIA has a sensitivity of 30 fmol D32/well. Cross-reactivity studies confirmed the high specificity of the antibody for D32; DYN B cross-reacted 0.05%; all other opioid peptides cross-reacted <0.01%. Extract from rat anterior pituitary, previously shown to contain predominantly 7000-dalton dynorphin, gave competition curves parallel to synthetic D32 standard curve. The unique characteristics of this monoclonal antibody should make it useful for immuno-staining of dynorphin precursors and in other investigations of the relationship of precursor and fully processed dynorphin peptides.

174.5 MORPHINE, MET AND LEU-ENKEPHALINS: DIFFERENTIAL EFFECTS ON cGMP METABOLISM. M. Cohn, J. Samora*, G. Fernandez*, J. Larrinaga*, J. A. Smartt*, D. J. Wooten*, and M.L. Cohn. Dept. of Anesthesiology Research, C.R. Drew Med. Sch., Los Angeles, CA 90059

The opioid peptides methionine (Met) and leucine (Leu) enkephalins have been shown to bind to opiate receptors in neural tissue. Like morphine, Met enkephalin binds to the mμ receptor while Leu enkephalin binds to the delta receptor. Both Met and Leu enkephalins have weak analgetic properties, but only Leu-enkephalin has been reported to potentiate morphine analgesia in rodents. We have previously reported that morphine enhances degradation of cGMP in rat brain slices resulting in significantly increased guanosine accumulation. Here, the effects of morphine on cGMP metabolism were compared to those of Met and Leu enkephalins, D-Ala^2,Met^5-enkephalinamide and D-Ala^2,Leu^5-enkephalinamide. Rat brain slices were incubated in a tonometer at 37°C with 6 ml Krebs-Ringer bicarbonate/glucose buffer, pH 7.35, a constant flow of O_2/CO_2 (20:5) and 1.5 x 10^{-1} mM of standard cGMP. Morphine (1.5 x 10^{-2} mM) or equimolar concentrations of either Met or Leu enkephalin or peptidase resistant analogs were added to the incubation mixture. Sequentially withdrawn aliquots were filtered and analyzed by reversed phase high performance liquid chromatography (HPLC). Our apparatus consisted of a Hewlett Packard (HP) HPLC model 1090, a diode array detector, a HP 85 microcomputer, and a hypersil ODS C-18 column 10 cm x 2.1 mm. The mobil phase, consisting of 3% MeOH and 97% 20 mM phosphate buffer, pH 7.35, was eluted isocratically at 0.3 ml/min. This system continuously displays absorbance values over a wide range of wavelengths and facilitates detection of impurities within peaks. Control samples yielded primary metabolic products GMP, guanosine, guanine, xanthine, and inosine. Addition of morphine to the incubation mixture significantly enhanced the rate of cGMP degradation, thus accounting for the increase of guanosine accumulation over control values. In contrast, neither the enkephalins nor their peptidase resistant analogs altered cGMP metabolism in rat brain slices. Though these compounds are believed to be endogenous opiate ligands, our data suggest that receptor binding characteristics are not the sole determinants of morphine-like activity. Supported by NIH Grant RR-08140 DRR/MBRS.

174.6 MORPHINE AND CALCIUM CHANNEL BLOCKERS: EFFECTS ON cGMP METABOLISM IN RAT BRAIN. M.L. Cohn, J. Yakel*, J. Samora*, J. Larrinaga*, G. Fernandez*, and D.J. Wooten*. Dept. of Anesthesiology Research. C.R. Drew Med. Sch., Los Angeles, CA 90059

Previous analytical evidence from our laboratory indicated that morphine, administered to rats or incubated with rat brain slices using cGMP as substrate, significantly increased guanosine accumulation. To identify the locus of this biochemical event, we initially investigated the action of morphine on rate limiting purine nucleoside phosphorylase. Subsequent kinetic studies failed, however, to demonstrate that the opiate alters the catalytic activity of this enzyme. Reports that calcium is involved in regulating opiate-induced analgesia led us, in the present study, to examine the action of morphine on calcium dependent cGMP phosphodiesterase. Brain slices of naive rats were incubated in tonometer at 37°C with Krebs-Ringer bicarbonate/ glucose buffer, pH 7.35, a constant flow of O_2/CO_2 (20:5) and 1.5x10^{-1} mM of standard cGMP. Sequentially withdrawn aliquots of incubation mixture were filtered and analyzed by high performance liquid chromatography. In control samples, cGMP catabolism resulted in appearance of peaks representing the metabolic products GMP, guanosine, guanine, xanthine, and inosine. Guanosine accumulation, probably due to rate limiting activity of the enzyme which catalyzes the conversion of guanosine to guanine, was consistently observed. Addition of morphine (1.5x10^{-2} mM) to identical incubation mixture resulted in 58 to 65 percent increase in guanosine accumulation over control values at 60 min of incubation. To this morphine-treated incubation mixture was then added one of three calcium channel blockers (CCB), diltiazem, verapamil or nifedipine, in 1 to 50 μM concentrations. All three CCB dose dependently decreased conversion of cGMP to GMP. When CCB was first added to incubation mixture, the subsequent addition of morphine failed to elicit guanosine accumulation. Our data suggest that 1) after binding to extracellular receptor, morphine increases calcium influx through receptor mediated channels, thus enhancing cGMP phosphodiesterase activity which results in increased guanosine accumulation; and 2) in rat brain slices, CCB inhibits activity of calcium-calmodulin dependent cGMP phosphodiesterase. Supported by NIH Grant RR-08140 DRR/MBRS.

174.7 CHANGES IN BETA-ENDORPHIN-LIKE IMMUNOREACTIVITY AFTER PRE-TREATMENT WITH COLCHICINE. Cheryl A. Cahill. School of Nursing, The University of Maryland, Baltimore, Maryland 21201

Cahill et al. reported recently that pretreatment of rhesus monkeys with IVC colchicine resulted in decreased total Beta-Endorphin-Like immunoreactivity in plasma. Further characterization of the material by molecular sieving chromatography and multiple RIA's indicated that the portion of N-acetylated B-END was reduced to less than detectable levels. Colchicine has two reported modes of action: 1) interference with the release of neurotransmitters from cells; 2) disruption in energy production at the subcellular level. Interference with energy mechanisms within the cell could theoretically interfere with post-translational processing of POMC. Since colchicine is often used prior to immunocytochemical study of nervous tissues, it is important to determine if the changes previously reported are due to interference with release from pituitary or actual disruption of synthetic processes.

Four groups of seven Sprague-Dawley rats were studied. Each animal was anesthetized and an intraventricular cannulae was surgically implanted. Colchicine was administered in a total volume of 10μL although concentrations varied. Group I, the control group, received 10μL of normal saline. Group II, received 10μG of colchicine; Group III, 50μG; Group IV, 200μG. After 48 hours the animals were sacrificed by decapitation. Three of the seven animals treated with 200μG of colchicine died before sacrifice. The brain was immediately removed and dissected. Hypothalamus was immediately frozen on dry ice. Within 24 hours, the tissue was extracted with the acid: acetone procedure. Extracts were dried and stored at -80°C.

Each sample will be reconstituted in 1% formic acid and aliquots taken for RIA. Additional aliquots will be pooled for molecular sieving chromatography which has been calibrated to separate peptides of molecular weights similar to POMC, B-LPH, B-END (1-31) and B-END (1-26). Samples and column fractions will be assayed with two RIA's: 1) is a midportion directed B-END RIA (gift of H. Akil) which detects POMC, B-LPH, B-END (1-31) and B-END (1-27); and 2) one specific for N-acetylated forms of B-END (kind gift of H. Akil). These studies are currently on-going.

174.8 ANALYSES OF EXTRA-ARCUATE BETA-ENDORPHIN SYSTEMS IN MAMMALIAN AND REPTILIAN CNS. R.M. Dores, H. Khachaturian, S.J. Watson and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan 48109.

In the rodent CNS the major Pro-opiomelanocortin (POMC) cell body group is located in the arcuate nucleus. Recent studies, however, have detected POMC perikarya in the nucleus tractus solitarius (NTS) (Schwartzberg and Nakane, 1983). In order to determine whether the processing of POMC in the NTC cell body group is similar to processing in the arcuate nucleus, acid extracts of the dorsal caudal medulla of the rat were fractionated by gel filtration on a Sephadex G-50 column and aliquots of column fractions were analyzed by radioimmunoassay with antisera specific for the COOH-terminal of beta-endorphin, the amidated COOH-terminal of alpha-MSH, and the middle region of ACTH. These analyses indicated that beta-endorphin-sized material and alpha-MSH-sized material are present in roughly equimolar amounts and represent the major end products of POMC processing in this region of the rat brain. The beta-endorphin-related material could be fractionated into roughly equal peaks of beta-endorphin(1-31)-sized and beta-endorphin(1-27)-sized forms. These results are in contrast to studies on the forms of beta-endorphin in the arcuate region, but are in agreement with previous studies on the forms of beta-endorphin in the hindbrain of the rat (Zakarian and Smyth, 1979).

In order to determine whether extra-arcuate beta-endorphin systems are unique to the rat CNS, the brain of the reptile, Anolis carolinensis was analyzed immunohistochemically with antisera directed against ACTH, beta-endorphin, and alpha-MSH. Following pretreatment with colchicine, two distinct POMC cell body groups were detected in the reptile brain. One cell body group was visualized in the medial basal hypothalamus in a region homologous to the arcuate nucleus of mammals. A second cell body group was detected in the mesencephalic tegmentum. This latter group has not been reported in mammals. Finally, POMC cell bodies were not detected in the dorsal caudal region of the medulla of the reptile. Possible relationships of the reptile mesencephalic POMC cell body group with POMC neurons in the arcuate nucleus mammals are discussed. This research was supported by NIDA Grant #DA02265 to SJW.

174.9 GPP(NH)P PROMOTES THE FORMATION OF A LOW AFFINITY STATE FOR THE DELTA OPIOID AGONIST-RECEPTOR COMPLEX. J.W. Spain*, D.B. Bennett* and C.J. Coscia (SPON: K. Smith). E.A. Doisy Department of Biochemistry, St. Louis University School of Medicine, St. Louis, MO 63104.

Current concepts of ligand-receptor interaction suggest that agonist binding is a multi-step process. We have previously demonstrated an apparent multi-step association of D-ala^2-D-leu^5-enkephalin (DADL), a prototypic delta opioid receptor agonist, to purified bovine hippocampal synaptic plasma membranes (SPM's). The present study examines the effect of the non-hydrolysable GTP analog, GPP(NH)P, or NaCl on DADL dissociation kinetics. After pre-incubation of bovine SPM's for 40 min with 20 nM D-ala^2-mePhe^4-gly-ol^5-enkephalin (DAGO), association of 1 nM ^{3}H-DADL was allowed for variable time intervals. Dissociation was then initiated by the addition of 1 μM unlabeled DADL. The inclusion of 50 μM GPP(NH)P during dissociation of ^{3}H-DADL from an SPM preparation transformed the slowly dissociating state into a more rapidly dissociating form, consequently the rate of dissociation was no longer association time-dependent. This property of GPP(NH)P is a function of dose; increasing concentration produces a proportional shift from the slowly to the rapidly dissociating form. In contrast, the dissociation of ^{3}H-DADL from a purified bovine microsomal preparation is not affected by the presence of GPP(NH)P during dissociation. We had previously reported (Roth et al., J. Biol. Chem. 256:10117, 1981) the reduced sensitivity of rat brain microsomes to the action of GPP(NH)P, suggesting that microsomal receptors are not coupled to GTP binding protein. The effect of GPP(NH)P on bovine microsomes is consistent with this hypothesis.

In previous studies, the presence of 100 mM NaCl during association was shown to prevent the transformation to the high affinity state. We now find that the inclusion of NaCl at the onset of disssociation results in an immediate loss of 60 to 70% of binding with the remainder slowly dissociating in an association time-dependent manner.

These results suggest that binding of GPP(NH)P to the GTP binding protein rapidly promotes the formation of the low affinity state for the delta opioid receptor-ligand complex in a bovine hippocampal SPM preparation. (Supported by NSF Grant BNS 81-14947.)

174.10 CHARACTERIZATION OF OPIATE-STIMULATED GTPase ACTIVITY IN BRAIN. P.H. Franklin* and W. Hoss (SPON: V. Laties) Center for Brain Research, Univ. of Rochester School of Medicine, Rochester, New York 14642.

One neural mechanism regulated by opioids is the receptor-mediated inhibition of adenylate cyclase activity. Transduction of the hormonal/neurotransmitter message through the plasma membrane to the catalytic subunit of adenylate cyclase (C) is mediated by a GTP-binding regulatory protein, N, which exists as a separate species, N_i or N_s, in negatively and postively coupled systems, respectively. The active GTP-bound form of N reverts to the inactive GDP-bound form by the action of a low K_m GTPase; hormone acts by facilitating the rate limiting step of the cycle, exchange of GDP for GTP at N.

Opiate-stimulated GTPase activity has been demonstrated in NG108-15 cells (Koski, G. and Klee, W., Proc. Natl. Acad. Sci. USA 78:4185-9, 1981), and in the rat brain (Franklin, P.H. and Hoss, W., Trans. Amer. Soc. Neurochem. 14:229,1983; Franklin, P.H. and Hoss, W., J. Neurochem., in press). In brain, both the opiate alkaloids and opioid peptides stimulate GTPase in a concentration dependent manner: etorphine (ET)$>$ D-Ala^2-Leu-enkephalinamide (DALA)$>>$ D-Ala^2-N-Me-Phe^4-Gly-ol (DAGO); GTPase stimulation by DALA is attenuated by naloxone, also with concentration dependency.

Muscarinic receptors, which are also coupled negatively to adenylate cyclase in NG108-15 cells and in brain, have been shown to stimulate GTPase in these tissues. Maximal GTPase stimulated by saturating concentrations of carbamylcholine (CCh) and D-Ala^2-D-Leu^5-enkephalin (DADLE) is additive. This suggests the existence of independent receptor domains for GTPase stimulation, and that receptor concentration may be the limiting element in maximal GTPase stimulation.

Maximal opioid-stimulated GTPase activity is differentially distributed across brain regions in a pattern that is parallel neither to the distribution of total opioid receptor binding nor to the distribution of basal (unstimulated) low K_m GTPase activity that is itself non-uniformly distributed in brain. (Supported in part by DA05232).

174.11 **A COMPARISON OF PEPTIDE E AND β-ENDORPHIN PROCESSING AND MOTILITY IN THE CANINE SMALL INTESTINE.** G. Hoyer* and T. P. Davis (SPON: J. Angevine). Dept. of Pharmacol., Univ. Arizona, Coll. of Med., Tucson, AZ 85724.

Peptide E (PE) and β-endorphin (βE) are opioid peptides of similar length that contain met-enkephalin (ME) as the N-terminal sequence. Pro-enkephalin A is the precursor protein for peptide E and pro-opiomelanocortin is the precursor for β-endorphin. Peptide E has been isolated from the chromaffin vesicles of the adrenal medulla, whereas β-endorphin has been isolated in many areas of the CNS including the pituitary. Since the gastrointestinal tract contains a high density of opioid receptors, the motility response of the small intestine was used as an index of opioid peptide activity.

Using an isolated vascularly perfused canine small intestine preparation (Burks and Long, Am. J. Physiol., 211:619, 1966), we measured changes in intraluminal pressure during the perfusion of specific peptides. The peptides investigated were arterially perfused at a concentration of 1 μg/ml, at a flow rate of 20-25 ml/min. Naloxone (Nal) sensitivity of the motility response was determined by perfusion of 1 μg/ml naloxone concomitantly with the specific peptide being studied. Changes in intraluminal pressure (mmHg) over baseline were as follows: (1) βE-44.5±6.0, (2) PE-42.1±6.1, (3) ME-9.3±4.8, (4) βE+Nal-0.0, (5) PE + Nal-37.2 ± 4.0, (6) ME + Nal-4.0 ± 1.0.

The in vitro formation rate of ME from PE and βE was studied with a selective and sensitive HPLC procedure previously described (J. Pharm. Exp. Ther., 227:499, 1983). A segment of mid-jejunum, small intestine was removed and arterially perfused to remove blood. The mucosal layer was then gently dissected away from the muscularis. Using twice washed membrane preparations of mucosa and muscularis, we incubated βE (20 μM) and PE (10 μM) separately at 37°C, pH 7.4. After 10-90 min incubations, enzyme activity was stopped by boiling for 20 min. Samples were then centrifuged for 20 min at 20,000 xg and the supernatant was analyzed for ME formation by HPLC. Met-enkephalin production from βE, after a 40 min incubation with muscularis, was found to be significantly lower than from PE (23.6 vs. 625 ng/mg protein, PTN). This difference was also demonstrated in the mucosa (41.7 vs. 3267 ng/mg PTN). The rate of ME formation from PE was seen to peak after 40-60 min incubation in both muscularis and mucosa, and then declined rapidly. The rate of ME formation from βE in the mucosa was seen to peak at 40 min and remained constant through the 90 min time point, whereas in the muscularis the levels of ME peaked after 90 min incubation (53.8 ng/mg PTN).

These data indicate a significant difference in the motility response and processing of peptide E and β-endorphin in the canine small intestine; they suggest a more specific formation of ME from PE as compared to βE. (Supported by a PMA grant to TPD.)

174.12 PALATABILITY AND THE SUPPRESSION OF FLUID INTAKE BY CHOLECYSTOKININ-OCTAPEPTIDE (CCK-8). J.R. Blackburn*, W.J. Jacobs* and A.G. Phillips. Dept. Psychology, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

The effect of cholecystokinin-octapeptide (CCK-8) on the consumption of several palatable solutions was studied to investigate the mechanism through which this peptide decreases ingestive behaviour. Mildly (5.5h) water deprived rats were injected i.p. with either 2ug/kg CCK-8 or saline and were then presented with a sweet solution for twenty minutes. Consumption of a 32% sucrose solution was decreased 39.4%, while that of a 0.6% saccharin solution was decreased 37.3%, compared to their respective controls. In contrast, neither consumption of 1.0% sucrose solution nor that of 0.1% saccharin solution was decreased although the subjects detected and preferred these solutions to tap water. This outcome is not consistent with an explanation invoking general malaise to account for the suppressant effects of CCK.

These data are the first to show a suppression of saccharin intake by CCK. This effect is unlikely to involve gastric feedback because saccharin is a metabolically inert substance, and has little osmotic pressure at these concentrations. Instead a pregastric factor such as a decrease in the reward value of the solution must be involved. As there is no decrease in the consumption of the weaker solutions, such a decrease in taste reward cannot be a universal effect of CCK. One possible interpretation of these results is that the satiating effect of strong sweet stimulation is mediated or potentiated by CCK.

PEPTIDES: BIOCHEMICAL CHARACTERIZATION

175.1 **POST-TRANSLATIONAL PROCESSING OF PRO-ACTH/ENDORPHIN-DERIVED PEPTIDES IN RAT HYPOTHALAMUS.** R. EMESON* (Spon: R. Mains). Dept. of Neuroscience, Johns Hopkins Univ., Balto., MD 21205

Extracts of adult rat hypothalami were fractionated by gel-filtration chromatography utilizing Sephadex G-75 or Biosil TSK-400, -250 and -125 columns connected in series. Analyses utilizing an antiserum directed against ACTH(1-13) demonstrated four peaks of ACTH-related immunoreactivity corresponding to the molecular weights of pro-ACTH/endorphin (<1% of total), ACTH biosynthetic intermediate (1%), ACTH (3%) and αMSH (>95%). Analyses utilizing an antiserum directed against β-endorphin(10-19) demonstrated three peaks of β-endorphin-related immunoreactivity corresponding to the molecular weights of pro-ACTH/endorphin (<1% of total), β-LPH (3%) and β-endorphin (96%).

The β-endorphin-sized molecules were analyzed by ion-exchange high performance liquid chromatography (IEX-HPLC) on a Biosil TSK CM-2-SW (250 x 4.6 mm) cation-exchange column equilibrated with 44 mM ammonium formate, pH 2.5, in 30% CH_3CN and eluted with a 60 minute linear gradient to 332 mM ammonium formate, pH 2.5, in 30% CH_3CN. The retention times [min] of six synthetic markers were as follows: Ac-β_h-endorphin(1-26)[13]; Ac-β_c-endorphin(1-27)[19]; β_h-endorphin(1-26)[22]; β_c-endorphin(1-27)[28]; Ac-β_c-endorphin(1-31)[35]; β_c-endorphin(1-31)[43]. Analyses of hypothalamic β-endorphin-sized molecules in the IEX-HPLC system have demonstrated three major forms of β-endorphin-derived immunoreactivity corresponding to β-endorphin(1-31) (50% of total), β-endorphin(1-27) (25%) and β-endorphin(1-26) (25%). The α-N-acetylated forms of β-endorphin, primarily Ac-β-endorphin (1-31), represented less than 2% of the total β-endorphin immunoreactivity. SP-Sephadex ion-exchange chromatography confirmed these results.

Analyses of hypothalamic αMSH-sized molecules utilizing reversed-phase or IEX-HPLC demonstrated that ~95% of the αMSH immunoreactivity elutes with the retention time of desacetyl-αMSH [ACTH(1-13)NH_2] or its sulfoxide, while the remaining ~5% elutes with the retention time of αMSH [α-N-acetyl-ACTH(1-13)NH_2] or its sulfoxide.

The size distributions of hypothalamic αMSH- and β-endorphin-related immunoreactivity are similar to those seen in the pars intermedia. The minor extent of α-N-acetylation of αMSH and β-endorphin in the hypothalamus constitutes a major difference between hypothalamic and pituitary intermediate lobe processing in which essentially all of the αMSH and β-endorphin are α-N-acetylated. Supported by Upjohn, DA-00266 and the McKnight Foundation.

175.2 MOLECULAR FORMS OF A BRAIN SPECIFIC POLYPEPTIDE, 1B236. B.Malfroy*, C.Bakhit, F.E.Bloom and R.J.Milner* Research Institute of Scripps Clinic, La Jolla CA 92037.

We have used recombinant DNA techniques to select and characterize cDNA clones of brain specific mRNAs. The nucleotide sequence of one such clone (p1B236) was determined, providing the amino acid sequence of the corresponding protein (1B236). This molecule was of interest because the sequence contained pairs of basic amino acids suggesting that it could be proteolytically processed to generate a number of novel neuropeptides. Antibodies were raised against synthetic peptides, P5 and P6, corresponding to the most likely peptide products. In Western blotting experiments antibodies against both peptides detect a diffuse band of approximately 100,000 daltons. We have further used these antibodies to develop radioimmunoassays against the peptide regions in order to characterize the molecular forms of 1B236 immunoreactivity (IR) in brain extracts. Whole brains of adult male Sprague-Dawley rats were extracted under various conditions and the extracts analysed by gel filtration on Sephadex G-75 to quantitate high (HMW) and low (LMW) molecular weight species (Table 1).

Table 1. 1B236 Immunoreactivity in brain extracts

	P5-IR		P6-IR	
Extraction Condition	HMW	LMW	HMW	LMW
2N Acetic acid	0	0	0	+
2N Acetic acid + 1% Triton	+	+	0	+
10 mM Tris-HCl, 0.15M NaCl	+	0	+	0
Tris-HCl/NaCl + 1% Triton	+	0	+	+

The elution profiles indicate that the detergent extracts may contain at least two HMW peaks reactive to both P5 and P6 antibodies; whereas in the absence of detergent only a single peak was seen, suggesting that there may be several HMW forms of 1B236, corresponding to the diffuse band seen in Western blots. LMW immunoreactive material was more consistently detected by antibodies to P6: the detergent requirement for the solubilization of LMW P6-IR in Tris/NaCl suggests subcellular compartmentalization of this material as indicated by ultrastructural studies. Analysis of LMW P6-IR by HPLC indicates a molecular weight consistent with a 20-30 amino acid peptide. The multiplicity of 1B236 immunoreactive forms suggests that this molecule undergoes extensive post-translational modification, including proteolytic processing to generate potentially bioactive peptides. These studies were supported by NIH grant NS20728 and a grant from McNeil Pharmaceuticals.

175.3 IMMUNOREACTIVE DYNORPHIN IN CASTRATED RATS IS MOSTLY OF THE DYNORPHIN-32 SIZE. C.J. Molineaux, J.G. Rosenberger*, A.H. Hassen and B.M. Cox. Dept. of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814

No information is available as to the function of immunoreactive dynorphin A (ir-Dyn A) in the rat anterior lobe (AL). Ir-Dyn A and ir-Dyn B have been shown to comigrate at approximately 6000 MW by gel filtration chromatography. The size contrasts markedly with that of neurointermediate lobe (NIL) Dyn A and B, which exist primarily as lower molecular weight forms. These results suggest that forms present in the NIL result from processing of prodynorphin to small peptides, while AL Dyn A and B exist as part of a large molecular weight precursor form.

We have examined the possible role of dynorphin peptides in hormonal regulation in the AL. The levels and the sizes of dynorphin A and B were measured in animals which lacked endocrine feedback from respective peripheral organs. Castration produces a marked enhancement of the immunoreactivities of both of these peptides in rat AL. All of the material in castrated rat AL was found to migrate with dynorphin-32, which may indicate that further processing occurs in the rats two weeks after castration. However, the crossreactivities of the antisera for large molecular weight dynorphin are unknown; therefore, it is not possible to determine whether the increased amount of total immunoreactivity results from a conversion to a smaller form with increased immunoreactivity or a real increase in the dynorphin level stored in the AL.

The effects of castration on the levels of Dyn A and B in AL were not reversed by treatments of 200 ug/day of testosterone for 2 weeks. However, a complete dose-response has not been performed. Further studies are necessary to determine whether there is a change in the molecular weight of the dynorphins in testosterone-treated rats following castration.

This work was supported by the Uniformed Services University of the Health Sciences protocol No. RO7542.

175.4 CEREBELLAR OPIOIDS: EVIDENCE FOR A PREDOMINANCE OF PRO-ENKEPHALIN DERIVED PEPTIDES IN THE RABBIT J. Madden IV, C.J. Evans, A.N. Tyler*, J.D. Barchas, F.S. Esch*+, P. Bohlen*+, and E. Weber. Dept. of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305, and +Laboratories for Neuroendocrinology, the Salk Institute for Biological Studies, La Jolla, CA 92037

Early reports by Hughes et al.[1] demonstrated that significant quantities of enkephalin-like material could be detected in extracts from rabbit cerebellum. Subsequently, a series of observations by Meunier et al.[2] indicated a high proportion of mu-opioid binding sites in this tissue. The rabbit cerebellum with its well-characterized cytoarchitectonics may provide a model system for the study of opioids in the CNS and ultimately advance our understanding of the functional roles of opioids in the brain. Therefore, to more precisely identify the endogenous opioids in this system, we have carried out further biochemical analysis aimed at isolating and characterizing the major opioid substances in this tissue.

To this end, we subjected acid acetone extracts of rabbit cerebellum to gel permeation column chromatography and examined the fractions with a chemical/immunological assay directed at the amino-terminal Tyr-Gly-Gly-Phe sequence which is common to all mammalian opioid peptides. The major peaks of activity were purified to homogeneity by reverse phase high performance liquid chromatography and identified as [Met^5]-enkephalin, [Leu^5]-enkephalin, and [Met^5]-enkephalyl-Arg^6-Phe^7 by automated Edman degradation in the gas phase sequenator, by amino acid composition analysis and by fast atom bombardment mass spectrometry. Interestingly, we were unable to detect any appreciable quantities of [Met^5]-enkephalyl-Arg^6-Gly^7-Leu^8 or metorphamide by these methods or by specific radioimmunoassays. Collectively, these results suggest that the vast majority of opioid peptides in rabbit cerebellum are derived from pro-enkephalin and that active fragments from the pro-enkephalin system may act as endogenous ligands for mu-type opioid receptors which predominate in rabbit cerebellum.

1. Hughes, J., Kosterlitz, H.W., & Smith, T.W. (1977) Br. J. Pharmacol., 61, 639-647.
2. Meunier, J.-C., Koukaou, Y., Puget, A., & Moisand, C. (1983) Mol. Pharmacol., 24, 23-29.

175.5 ON THE SPECIFICITY OF DIFFERENT ANTIBODIES DIRECTED AGAINST OVINE AND RAT CRF IN THE RAT CENTRAL NERVOUS SYSTEM. G. Skofitsch* and D.M. Jacobowitz. Laboratory of Clinical Science, NIMH, Bethesda, MD 20205.

The presence of corticotropin releasing factor (CRF) in the rat hypothalamic area was recently demonstrated. However, there are contradictory immunocytochemical studies concerning the occurrence of extrahypothalamic CRF-like immunoreactivity, mainly in the hindbrain and the spinal cord. Recently, we and others reported on CRF-like immunoreactivity in normal rats and in capsaicin sensitive primary sensory neurons in the spinal trigeminal nuclues and tract, the nucleus tractus solitarii, the substantia gelatinosa and in the dorsal horn of the spinal cord; whereas others did not find CRF-like immunoreactive material there. To further study this problem, we compared 4 different antibodies, 2 of them directed against rat CRF, 2 against ovine CRF; one set of ovine and rat CRF was raised in rabbits, the other set was commercially obtained. Indirect immunofluorescence (IF) and radioimmunoassay (RIA) of rat tissue was used. A complete map of CRF-like immunoreactivity in the rat central nervous system was obtained with each of the 4 antisera by IF and RIA. RIA was used to obtain displacement curves with rat and ovine CRF and sauvagine, a peptide closely related to CRF. Minimal cross reactivity between rat and ovine CRF and sauvagine was observed. IF and RIA of different brain regions showed only minor differences in the distribution of CRF-like immunoreactivity in the hypothalamic area using the 4 different antisera, whereas only one antiserum directed against ovine CRF revealed CRF-like material in the hindbrain and the spinal cord. The immunostaining in this area was preabsorbed with 10^{-6}M synthetic ovine CRF, but not with $2x10^{-6}$, substance P, vasoactive intestinal polypeptide and cholecystokinin; these peptides which are capsaicin sensitive are colocalized with ovine CRF-like material in the rat trigeminal complex. RIA displacement curves also failed to show cross reactivity of the antibodies with these peptides. Using high performance liquid chromatography and RIA, further attempts will be made to characterize CRF-like immunoreactivity of the hypothalamic and the hindbrain areas. It appears that the rat hindbrain (e.g., spinal trigeminal nucleus) may contain a peptide that is not rat CRF but is immunoreactive with ovine CRF antisera.

175.6 PURIFICATION AND BIOLOGIC ACTIVITY OF A NEUROTENSIN-LIKE PEPTIDE FROM LOBSTER. S.R. Kirschenbaum*, R.E. Carraway*, and C.H. Price. Dept. of Biol., Boston Univ., Boston, MA 02215 and Dept. of Physiol., Univ. Mass. Med. Ctr., Worcester, MA 01605.

Although antisera towards some vertebrate peptides have been used to demonstrate related substances in invertebrates, the amino acid sequences and function(s) of these substances are not yet known. We have shown that antisera towards the biologically active C-terminal region of mammalian neurotensin (m-NT) recognize material in extracts of invertebrates. Using antiserum HC-8 to survey for immunoreactive NT (iNT) in various lobster tissues, we found iNT to be differentially distributed with high concentrations in heart, hepatopancreas and ganglia. Here we report the immunochemical, chromatographic and biologic character of a NT-related peptide obtained from lobster hepatopancreas.

Acid extracts of hepatopancreatic tissue from adult lobsters were chromatographed on Sephadex G-25 followed by SP-Sephadex. The largest active peak of iNT was purified by sequential HPLC steps (μ-Bondapak C-18) until a single sharp peak of A_{276} was obtained. The partially purified iNT contained mostly the following amino acids: Arg, Glu, Ile, Leu, Lys, Pro, Tyr, and Val. The fact that this composition resembles that of the C-terminal region of m-NT is consistent with immunochemical data obtained with a battery of region-specific antisera towards m-NT.

This material was then tested in a quantitative bioassay which measures the ability of substances to induce a change in vascular permeability when injected intradermally into rats. Lobster iNT gave a dose-dependent effect similiar to that produced by m-NT. In a second bioassay for NT, involving the isolated, perfused, myogenic heart of a mollusc (Aplysia), lobster iNT decreased within seconds, the amplitude and frequency of spontaneous beating for several minutes when applied at concentrations $\leq 10^{-9}$M. The effect was reversible and resembled that of m-NT.

The identification of this NT-like peptide in lobster represents the opportunity to study, for the first time, the counterpart of a peptide native to vertebrates in an invertebrate. The presence of lobster iNT in the heart, hepatopancreas and nervous tissue and its demonstrated biologic effects suggest multiple roles for this peptide.

175.7 AGE-RELATED CHANGES IN REGIONAL CONCENTRATIONS AND STRUCTURAL FORMS OF ENDORPHINS IN THE RAT BRAIN. C.W. Wilkinson* and D.M. Dorsa, GRECC, VA Medical Center, and Depts. of Medicine and Pharmacol., Univ. of Washington, Seattle, WA, 98108

We investigated age-associated changes in regional concentrations and structural forms of β-endorphins (βE) and γ-endorphins (γE) in the brains of rats using a combination of reverse phase high performance liquid chromatography (HPLC) and radioimmunoassay. Ten 8-month old and ten 24-month old male Fischer 344 rats were decapitated. Brains and pituitaries were quickly removed, dissected into 12 areas, and frozen on dry ice. Tissues were boiled in 1 M acetic acid, sonicated, and the peptides were extracted using ODS-silica cartridges using 80% acetonitrile (ACN) containing 0.1% trifluoroacetic acid (TFA). The peptides were separated on a 5 μ, 330 Å pore, C_{18} reverse phase column using a gradient of 80% ACN containing 0.1% TFA against 0.11% TFA in water with detection at 210 nm. Fractions were collected and assayed for γE-like immunoreactivity (γE-LI) and βE-LI. We have previously reported (Abstracts of the 7th International Congress of Endocrinology, 1984) finding α-N-acetylated forms of βE and γE in the hypothalami of 24-month old but not 8-month old rats. In contrast, in the midbrain we find no qualitative differences in forms of endorphins between old and young rats. In approximately one-half of the midbrains no βE-LI was measured in fractions co-eluting with the α-N-acetylated forms of βE 1-31, 1-27, or 1-26. However, γE-LI co-eluting with acetylated γE was found in several of those midbrains that lacked acetylated βE forms. The relative paucity of acetylated βE forms in the midbrain may be related to a specific analgesic role of the endorphins in the periaqueductal gray. Total βE-LI and γE-LI were generally lower in old rats. The data indicate that age-related changes in structural forms of endorphins are region-specific and suggest that selective alterations in post-translational processing of peptides occur as a function of age. Because these alterations in processing can result in major changes in the biological activity of the peptides, they may play an important role in the physiology of the aging process.

Supported by the Veterans Administration.

LIMBIC SYSTEM: HIPPOCAMPUS AND AMYGDALA

176.1 IMMUNOCYTOCHEMICAL CHARACTERIZATION OF GABAERGIC NEURONS IN CA1 OF RABBIT HIPPOCAMPUS. D.D. Kunkel*, P.A. Schwartzkroin and A.E. Hendrickson. Depts. of Neurological Surgery & Ophthalmology, Univ. of Washington, Seattle, WA 98195.

The hippocampus is known to have effective recurrent and feed-forward inhibitory circuits involving local GABAergic inhibitory interneurons. We have examined these neurons, using GAD (glutamic acid decarboxylase) immunocytochemistry (PAP technique), in tissue from immature (8 day) and mature (30 day) rabbits. The GAD antibody was supplied by Dr. J. Y. Wu.

In mature rabbits, immunoreactive GAD (IRGAD) neurons were found in strata oriens, pyramidale and radiatum of CA1, CA2 and CA3 regions. IRGAD neurons were ovoid to polygonal in shape (35-40 μm in diameter) and appeared morphologically similar to the basket or "polygonal" neurons described by Lorente de No (J. Psychol. Neurol. 46:113-177). They were also similar to a basket cell inhibitory interneuron studied morphologically and electrophysiologically in our laboratory (J. Neurosci. 1:318-322, Neurosci. Absts. 8:216).

IRGAD dendrites were found throughout strata oriens, pyramidale and radiatum. They were aspinous and received numerous synaptic contacts (mostly asymmetric). Immunoreactive bands composed of GAD terminals were evident. A distinct immunoreactive band was localized in stratum pyramidale. Another less distinct immunoreactive band was seen in the proximal third of stratum radiatum in CA1, CA2 and partially into CA3.

Upon electron microscopic examination, numerous immunoreactive terminals were seen surrounding and contacting pyramidal cell somata. In concurrence with light microscopic observations some immunoreactive terminals contacted apical dendrites (both primary and secondary branches) and basal dendrites of pyramidal cells. IRGAD terminals were also seen on initial segments. The synaptic contacts made by the immunoreactive profiles appeared to be symmetric. IRGAD terminal profiles were found in synaptic contact with somata and dendrites of other interneurons.

The pattern of GAD immunoreactivity in young rabbits was similar to that found in the adult, with identifiable IRGAD somata, dendrites and axonal processes. Although apparent IRGAD terminals were seen in association with pyramidal cell somata, dendrites and initial segments, the mature synaptic specializations characteristic of inhibitory (symmetric) synapses were not clear in the immature hippocampus.

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176.2 ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERIZATION OF A PUTATIVE SOMATOSTATIN-CONTAINING INTERNEURON IN GUINEA PIG HIPPOCAMPUS. A.L. Mueller, D.D. Kunkel and P.A. Schwartzkroin, Dept. of Neurological Surgery, University of Washington, Seattle, WA 98195.

Previous immunohistochemical studies have demonstrated the existence in the hippocampus of neurons which are immunoreactive to somatostatin (somatotropin release inhibiting factor, SRIF). These neurons are located primarily in a band in the oriens/alveus border of area CA1, as well as in the hilar region of the dentate gyrus (Kunkel et al., Soc. Neurosci. Abst. 9: 218. 1983; Roberts et al., Neurosci. 11: 35-77, 1984). As part of an ongoing study of local circuitry in the hippocampus, we have attempted to characterize putative SRIF-containing neurons with a combined electrophysiological and morphological approach.

Intracellular recordings were obtained from 18 neurons located in the oriens/alveus border of area CA1 in slices of guinea pig hippocampus maintained in vitro. These "oriens/alveus interneurons" were characterized electrophysiologically in terms of the following parameters: spontaneous firing rate 25 ± 6 Hz (mean ± SEM), resting membrane potential -47 ± 4 mV, input resistance 45 ± 6 MΩ, membrane time constant 4.9 ± 0.5 msec, action potential amplitude 36 ± 3 mV, action potential duration 1.1 ± 0.1 msec. A single action potential was followed by a pronounced afterhyperpolarization (AHP) of amplitude -7.9 ± 0.9 mV. Injection of a depolarizing current pulse (0.5 nA, 100 msec) evoked a rapid, nondecrementing train of 12 ± 1 spikes; such a current-evoked burst was followed by an AHP in only 2 cells. Stimulation of the alveus elicited a burst of action potentials in 5/9 cells, and a single spike in the remaining 4 cells. Embedding the stimulus in a hyperpolarizing current pulse allowed an underlying EPSP to be seen in 7/9 neurons. Taken together, these electrophysiological values are clearly different from those of CA1 pyramidal neurons, and are similar to those of the "basket cells" of Schwartzkroin and Mathers (Brain Res. 157: 1-10, 1978).

Seven of these electrophysiologically characterized "oriens/alveus interneurons" were successfully filled with Lucifer Yellow dye. These neurons are clearly non-pyramidal and closely resemble cells that are immunoreactive to SRIF.

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176.3 HIPPOCAMPAL ^{3}H-MUSCIMOL BINDING: DIFFERENCES BETWEEN TWO *IN VITRO* AUTORADIOGRAPHIC TECHNIQUES. J. Franck* and P. Schwartzkroin (SPON: G. Ojemann). Dept. Neurological Surgery, University of Washington, Seattle, WA 98195.

Two *in vitro* autoradiographic techniques were compared in a study examining the distribution of presumed GABA receptors in rat hippocampus. Using the approach of Young and Kuhar (Brain Res. 179:255-270) lightly fixed rat brain was snap frozen and 8 μ slide mounted sections were incubated at 4° C in Tris-citrate buffer containing 15-60 nM ^{3}H-muscimol. Emulsion-coated coverslips were attached and the assemblies exposed for 4-8 weeks. Under these conditions the pattern of binding site distribution was principally dendritic; the pyramidal cell and granule cell layers were only sparsely labelled. This distribution is contrary to much of what is known of the location of GABA containing terminals and inhibitory synapses in the hippocampus, and prompted us to use the technique of Taxt and Storm-Mathisen (Neurosci. 11:79-100). Fresh 100 μ sections of hippocampus were incubated at room temperature in either Tris-citrate or a balanced salt solution containing 1 μM ^{3}H-muscimol. The thick sections were dried on slides and overlaid with a thin formvar film to prevent chemographic effects. The slides were dipped in emulsion and exposed for 1-2 weeks. Using this technique, we found the distribution of ^{3}H-muscimol binding sites to be principally somatic, although dendritic labelling was present. A dense band of silver grains was localized selectively to the granule cell layer and stratum pyramidale.

Neurochemical and electrophysiological studies indicate that several populations of GABA receptors exist. Furthermore, GABAergic neurons and physiologic responses to GABA agonists and antagonists are found in dendritic as well as somatic regions of hippocampus. In our study, the addition of excess unlabelled GABA eliminated the labelling demonstrated with both techniques. It is possible that the different approaches label different sub-populations of GABA binding sites.

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176.4 AN ANALYSIS OF THE INCREASE IN GRANULE CELL EXCITABILITY ACCOMPANYING PERFORANT PATH HABITUATION IN THE DENTATE GYRUS. W.C. Abraham and T.V.P. Bliss*. Dept. Psychology, Univ. Otago, Dunedin, New Zealand.

Low-frequency perforant path stimulation produces a habituation-like decrement in the population EPSP recorded extracellularly in the dentate hilus. The EPSP decrement is accompanied however, by an increase in the population spike height/population EPSP slope relation, suggesting that an increase in granule cell excitability also occurs.[1] The present experiments explored the mechanisms of this apparent increase in excitability using standard field potential recording techniques to assess perforant path input/output curves in rats anesthetized with sodium pentobarbital.

Low-frequency homosynaptic stimulation (512 pulses, 1 Hz) of the perforant path resulted in a decreased spike threshold and overall shift to the left of the function relating population spike height to EPSP slope. These changes occurred very early during the low-frequency train. On the other hand, no changes were observed in either slope or y-intercept of the spike onset latency/EPSP slope function. Low-frequency heterosynaptic (lateral perforant path) or antidromic (mossy fiber) driving of the granule cells only slightly increased the medial path spike/EPSP relation, and did not alter the spike threshold. These latter data, combined with the fact that homosynaptic stimulation did reliably decrease spike threshold even when granule cell discharge was inhibited by conditioning stimulation of the contralateral hilus, suggest that the increased excitability is pathway specific and independent of granule cell discharge.

The most reasonable explanation of our results is that the perforant path makes excitatory synaptic contacts on feed-forward inhibitory interneurons that decrement in strength under conditions that produce habituation of the direct monosynaptic excitation of the granule cells. Increased granule cell excitability would thus accompany perforant path habituation through a process of disinhibition.

(Supported by New Zealand MRC and NIMH grant MH-08793.)

[1]Harris, E.W., Lasher, S.S. and Steward, O. (1979) Brain Res. 162: 21-32.

176.5 MEDIAN RAPHE TO DENTATE GYRUS PATHWAY: ACTIVATION IN THE PARAFASCICULUS REGION. Dennis Dahl* and Jonathan Winson, The Rockefeller University, New York, N. Y. 10021

We have previously reported that, in anesthetized rats, stimulation of the median raphe prior to stimulating the perforant pathway markedly increases the amplitude of the granule cell population spike. In freely moving animals, this increase is present during slow-wave sleep but does not occur when rats are in a still-alert state. We have characterized the pathway activated by median raphe stimulation as a non-serotonergic, polysynaptic circuit arising in the medullary reticular formation and terminating in the dentate gyrus.

In this experiment, stimulation (3 pulses, biphasic, 100μsec per phase, 1 msec interpulse interval) was applied in rats anesthetized with Chloropent at a series of loci in the midbrain rostral to the median raphe, in the attempt to activate the median raphe to dentate gyrus pathway. Enhancement of the granule cell population spike similar to that seen with median raphe stimulation was produced at low intensities of stimulus current (threshold 20μA) in the region of the fasciculus retroflexus (parafasciculus region, PF) medial to the ventral tegmental area.

Rats were implanted with electrodes for stimulating PF and the perforant pathway and recording granule cell field responses, and were subsequently tested in the freely moving state. As has been noted previously, the amplitude of the population spike was significantly greater during slow-wave sleep than during still-alert behavior. Prior stimulation of PF markedly increased the already elevated population spike during slow-wave sleep. In contrast to the lack of effectiveness of median raphe stimulation in increasing the population spike during the still-alert state, prior stimulation of PF increased the population spike during still-alert behavior as effectively as during slow-wave sleep. The data suggest that neurons in the PF region constitute a relay in the median raphe to dentate gyrus pathway, and that neural transmission from the median raphe to the granule cells is restricted at this relay during the still-alert state.

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176.6 QUANTITATIVE ANALYSES OF HRP-FILLED GRANULE CELLS IN THE RAT DENTATE GYRUS. B.J. Claiborne. The Salk Institute, La Jolla, CA 92037.

Studies of Golgi-impregnated dentate granule cells have suggested that certain aspects of dendritic morphology are correlated with cell body location in the dentate gyrus. As the first step in a quantitative study of granule cell development, we have analyzed the three-dimensional structure of HRP-filled granule cells from different locations in the dentate gyrus of young adult rats.

Transverse slices (350 μm thick) from the middle third of the hippocampus of 35 to 50 day-old rats were maintained in vitro. Granule cells were impaled and filled with HRP, and the slices fixed, reacted and cleared, as previously described (Claiborne et al., Neurosci. Abstr. 9:220, 1983). The filled neurons were reconstructed in three dimensions using a microscope interfaced with a PDP 11-03 computer. A total of 38 cells were judged to be completely filled and have been analyzed. The total dendritic lengths (uncorrected) of all cells ranged from 2300 to 4600 μm (3318 ± 90; M ± S.E.). These lengths are 30 to 50% greater than the uncorrected lengths of Golgi-impregnated cells, but are close to the corrected average of 3660 μm reported by Desmond and Levy (J. Comp. Neurol. 212:131, 1982).

Our results indicate that, as previously reported in studies of Golgi-stained granule cells, the more superficial cells in the granule cell layer have from 2 to 4 major dendrites, whereas most of the deeper cells have only 1 primary dendrite. We also found that cells in the suprapyramidal blade (SPB) differ in two respects from those in the infrapyramidal blade (IPB). First the mean total dendritic length of SPB cells (3508 ± 92 μm; n=28) is greater than that of IPB cells (2788 ± 115 μm; n=10). Second, SPB cells have wider dendritic fields in both the transverse (358 ± 15 μm) and longitudinal (178 ± 9 μm) dimensions of the dentate than do IPB cells (290 ± 24 and 154 ± 6 μm, respectively). There are, however, no significant differences between SPB and IPB cells in the numbers of dendritic segments per cell, in the maximum branch order, or in cell body size; nor is there a significant difference in the width of the molecular layer over the two blades. Since cells in the SPB arise, on average, before those in the IPB, the differences in total dendritic length and in dendritic spread reported here may reflect this, and other, developmental differences between the two blades.

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176.7 A GOLGI STUDY OF NON-GRANULE NEURONS IN THE DENTATE GYRUS OF THE RAT. Michael A. King. Dept of Neuroscience, University of Florida College of Medicine, Gainesville Fl 32605.

The classic neuroanatomical descriptions of hippocampal cell types, summarized by Lorente de No, reported 17 types of nonpyramid neuron in Ammon's Horn. In contrast, apparently only basket cells complement the granule cells of the dentate gyrus. Recently, 5 types of putative inhibitory demonstrating glutamic acid decarboxylase (GAD) immunoreactivity have been classified (Seress & Ribak, 1983 Exp. Br. Res. 50:173-182), but only one of these is found in the molecular layer (ML). In the present study, several classes of non-granule neurons were found in the ML of male Long-Evans rats prepared according to several Cox, Kopsch, and rapid Golgi methods. When several dozen of these neurons had been drawn through the microscope, an attempt was made to classify them into morphological classes. At least 8 and possibly as many as 11 types were found, depending on whether certain examples reflect extremes of variation within a type or are in fact different types. The most frequently stained type (>60 examples) is much larger than granule cells, and has very thick, long dendrites densely covered with spines. Dendritic branches often descend from somata in the inner half of the ML, then turn upward, ascend to the pial surface, and are deflected but usually do not terminate for several hundred microns. Axons form basket plexi in the granule cell layer, less so in the inner third of the ML, and may continue into the hilus. An inverted counterpart in the distal ML may be a variant or a distinct type, but appears more rarely. The second most numerous type to stain (Golgi) has a spheroidal soma and appears most frequently in distal ML. Thick straight initial dendrites extend a short distance, then form a dense plexus of fine perisomatic branches, with occasional varicosities but no or only few spines. Other types include "supergranule" cells, which may be variant or ectopic granule cells but appear to have a broader and longer dendritic tree; small multipolar cells with long dendrites and a low spine density; large smooth cells with a chandelier appearance; a spiny type similar to these chandelier cells; aspiny fusiform bipolar cells; large smooth aspiny multipolars in the outer half; and small cells with somata in stratum lacunosum-moleculare, but with one or more main dendrites crossing the ML. These have spines, and may reach the granule cell layer. Drawings and photographs of representitives of each type will be presented with discussions of the functional implications of each.

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176.8 TEMPERATURE SENSITIVITY OF SPONTANEOUS ISOLATED DISCHARGE AND STRUCTURED BURST FIRING OF HIPPOCAMPAL PYRAMIDAL CELLS IN VITRO. R.M. Lebovitz. Dept. Physiology, University of Texas Health Science Center, Dallas, Texas 75235.

The spontaneous activity of presumed pyramidal cells were recorded from 350-400 micron thick sections of juvenile rat hippocampus maintained in vitro, using customary methods and media. Extracellular recordings from the dentate and zones CA1-4 of the hippocampus were obtained with 4-6 Megohm glass microelectrodes filled with 2 M NaCl. The temperature of the media and slice within the 1.5 cc chamber was regulated by a Peltier device capable of rapid (0.5°C per sec) heating or cooling from a nominal 37°C. The influence of simple temperature shifts on mean rate and on structured (superimposable) burst firing parameters (intraburst interval distribution, burst frequency and duration) was determined. A direct dependence of mean firing rate on temperature was found over the range of 20 to 41°C. Spontaneous unit discharge generally ceased, reversibly, at the lower end of this range. Above 42°C, firing rates showed episodic variations that were frequently irreversible. For units showing spontaneous structured bursting, burst frequency and intraburst intervals tended to vary directly, though not linearly, with temperature. The intraburst interval distribution appeared to be distinctly less temperature sensitive than other firing parameters, such as mean interburst period and mean firing rate, For most cells showing spontaneous structured burst firing, the number of spikes per burst decreased with decreasing temperature of the slice so that bursts of 5-8 spikes, at 37°C, reverted to simple superimposable doublet or triplet firing at 25°C. In a distinct class of cells, believed to be CA4 pyramidal cells, temperature variation alone was sufficient to cause reversible alterations in firing mode from burst to single spike discharge.

With the assistance of Ms. Linda King-Breeding; supported by NIH Research Grant RO1-ES-02750.

176.9 PARALLEL RECORDING OF NEURAL ACTIVITY IN THE HIPPOCAMPAL FORMATION: I. MULTICHANNEL UNIT ACTIVITY IN ANESTHETIZED AND WAKING RATS. M. Kuperstein & H. Eichenbaum. Dept. Biology, Wellesley College. Wellesley MA 02181.

A new version of a 24 channel microelectrode system has been fabricated and used in experiments in the rat hippocampal formation. The system called PRONG (Parallel Recording Of Neural Groups) includes removable microelectrodes, a pressure fit connector, a 24 channel hybrid preamplifier, a 3-band 24 channel amplifier, a 1/4 MHz A/D, a parallel 10KHz digital interface and DEC 11/23 computer. In the present design the recording section of the microelectrodes has a 2X12 array of recording sites along both edges spanning 100umX1320um. The electrodes are fabricated in patterned layers totaling 17um thick using photolithography (Kuperstein & Whittington, IEEE Trans. Biomed. Eng. 28:288-293, 1981). Site area can be varied from 20-120 sq-um and each site is plated with platinum black.The connector is designed for a fast change of electrodes and is sufficiently light to allow unrestrained rat behavior.

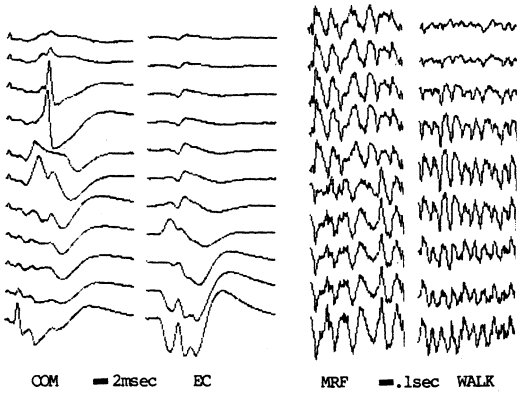
Initial recordings were focused on assessing the performance of the electrode in recording three kinds of extracellular activity in the hippocampal formation of anesthetized and waking rats: (1) action potentials, (2) evoked field potentials, and (3) slow wave activity. Results on the latter are summarized in the next abstract.

Rats were anestheized with urethan or pentobarbitol and implanted with the PRONG. Recording sites extended across all layers of the dorsal CA1 and dorsal blade of the dentate gyrus. Background noise was low, 10-20 uV, presumably due to the high resistance of the thin and narrow gold leads. Action potentials were typically recorded at one-half to two-thirds of the viable recording sites. Large (signal-to-noise 5:1 to 15:1) action potentials were observed in the pyramidal cell layer; many of these exhibited complex spikes. Action potentials recorded in other layers was typically smaller (2:1 to 5:1) and some cells exhibited rhythmic bursting with coincident theta activity. Backgound noise level was sometimes larger in waking rats, but otherwise PRONG performance in recording unit activity was similar to that in anesthetized rats. Some cells had obvious behavioral correlates, or fired in relation to theta activity. Thus the qualities of unit activity recorded with the PRONG is similar to that recorded with conventional microelectrodes in both acute and chronic preparations. (Supported by PHS grant NS18744)

176.10 PARALLEL RECORDING OF NEURAL ACTIVITY IN THE HIPPOCAMPAL FORMATION: II. INSTANT LAMINAR PROFILES OF SPONTANEOUS AND EVOKED FIELD POTENTIALS. H. Eichenbaum & M. Kuperstein. Dept. Biology. Wellesley College. Wellesley MA 02181.

A common procedure for the localization of neural events in cortical structures is the "laminar profile". an accumulation of field potentials sampled at multiple depths. Profiles using successive samples suffer in three ways: (1) Data collection is time-consuming. (2) Important laminar interactions may be obscured when the experiment requires comparison of averages of multiple samples. (3) The experimental preparation may change between samples.

We used the PRONG multichannel microelectrode system. described in the preceeding abstract. to record field potentials across laminae in the hippocampal formation. Rats were implanted for stimulation of the ventral hippocampal commissure (COM), entorhinal cortex (EC). and midbrain reticular formation (MRF). Single simultaneous recordings through CA1 and the dorsal blade of the dentate demonstrated: (1) COM and EC evoked potential profiles indicating characteristic slow potentials and population spikes in the pyramidal and granule layers, and (2) theta profiles indicating separate. phase shifted maxima in CA1 and in the dentate after MRF stimulation and in walking rats. The PRONG is a useful new tool for instant laminar analysis. (Supported by PHS grant NS18744)



176.11 VARIATIONS IN PLACE CELL FIRING WITH THE STATE OF THE HIPPOCAMPAL EEG. J.L. Kubie, S.E. Fox and R.U. Muller*. Dept. of Physiol., Downstate Med. Ctr., Brooklyn, NY 11203.

In freely moving rats, the firing of complex-spike (CS) cells (ie, pyramidal cells) depends on the environment. If recording is done in a small (8x30 cm) apparatus, most CS cells never fire faster than about 1 AP/sec. We will call these slow CS cells. By contrast, in a 76 cm diameter cylinder, many CS cells show bursts of rapid (> 20 AP/sec) firing. Because the rapid firing is confined to a limited part of the cylinder (the "place field"), such cells are called "place" cells (1). Out-of-field firing rates are similar to those of slow CS cells.

The 6-9 Hz hippocampal EEG "theta" pattern occurs mainly during translational motions such as walking or running; Large amplitude, Irregular Activity (LIA) is seen if the rat is immobile, eating, grooming etc (2). Prior work suggests that slow CS cells fire even less during theta than during LIA. Here we ask if either the in-field or out-of-field firing of place cells also covaries with the EEG state.

Single cells are recorded with a micro-wire in the CA1 or CA3/4 layer. Cells are selected for the existence of a well defined place field (ie, NOT randomly). Theta is recorded between a steel tube in the hippocampus and a skull screw. The rat is tracked by a TV system that locates a small light on the rat's head in each TV frame.

We look for clear-cut theta or clear-cut LIA with a device that integrates voltage in the theta and LIA (2-5 Hz) bands (200 ms time constants). The difference in these magnitudes is divided into 3 ranges. The top range is called theta; the bottom range is deemed LIA. The wide middle range is discarded. Spike and location samples are sorted into theta or LIA bins, and time-averaged firing rate maps are printed. Very similar maps are obtained when theta/LIA sorting is done by inspecting the raw EEG.

We find that in-field firing is nearly independent of the EEG state and that out of field firing seems slower during theta than LIA. We conclude: (A) Given that theta is well correlated with behavior, in-field firing is indeed place and not activity dependent (1); (B) Slow CS cells are likely place cells that happen not to have a place field in the current environment; (C) The spatial specificity of place cells is somewhat higher during theta than LIA.

Supported by NS17095 to SEF and NS20686 to JLK and RUM.

(1) O'Keefe and Conway. Exp Brain Res, 31:573-590 (1978).
(2) Vanderwolf. EEG Clin Neurophys, 26:407-418 (1969).

176.12 HEAD DIRECTION CELLS IN THE DEEP CELL LAYER OF DORSAL PRESUBICULUM IN FREELY MOVING RATS. J.B. Ranck, Jr. Dept. of Physiology, Downstate Medical Center, Brooklyn, N.Y. 11203.

The firing of single neurons in freely moving rats was recorded in the dorsal presubiculum (sometimes called area 48 or paradoxically postsubiculum). In the deep cell layer (lamina principalis interna) there are neurons which increase their firing when the rat's head is pointing in a particular direction. Initial qualitative observations show that the firing is independent of whether the rat's head is pointing up or down (pitch); or if the head is rotated left ear up or right ear up (roll): i.e. firing is related to yaw or azimuth, not pitch or roll. (Azimuth is the angle that a projection to the horizontal plane makes with a fixed, arbitrary line in that plane). For a given cell in a stable environment, the azimuth of the head direction during maximal firing is the same at all places. In this sense, the directional specificity of a cell is constant, independent of place. So far this observation has been tested in an area 25 feet across.

The angular range over which firing is greater than background is about 90°, with the maximal firing rate of about 20-30/sec in the middle. Preliminary results show that this firing is independent of behavior (if the rat is awake), and independent of trunk position. The firing seems to be independent of whether head direction is constant or changing and firing does not adapt to a constant direction. The firing is the same if the rat's head points a particular direction when the rat moves himself (active), or if he is held in the experimenter's hand and his head turned (passive).

When the rat is in a familiar box with sides, rotation of the box often affects the direction of maximum firing, but, so far in a variable way. When the rat's eyes are covered in a familiar and stable environment, direction specific firing is lost for a few minutes, but then returns with the same directionality. When the rat is carried to a new environment, cell firing is not directional for a few minutes, but then returns with the same directionality.

Cells with many different preferred directions have been seen. The preferred direction of one cell does not seem to predict the preferred direction of neighboring cells.

In nearby Ammon's horn there are "place cells" whose firing is place specific and often independent of direction. Thus the hippocampal formation processes spatial information about both place and direction, and the two processes are partly separated.

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176.13 DEPTH PROFILES OF THE HIPPOCAMPAL THETA RHYTHM IN RATS, WITH AND WITHOUT ATROPINE OR URETHANE TREATMENT. S.E. Fox. Dept. of Physiol., Downstate Med. Ctr., SUNY, Brooklyn, NY 11203.

Hippocampal theta rhythm occurs in undrugged rats during locomotion. One component of this rhythm is abolished by atropine (1). Theta rhythm also occurs during urethane anesthesia. Recordings were made from undrugged walking rats, from walking rats pretreated with atropine sulfate (25 mg/kg ip) and from rats given anesthetic doses of urethane (1 g/kg iv) to determine the effects of these drugs upon the depth profiles of theta rhythm.

A moveable metal microelectrode and a fixed electrode in the dentate molecular layer were connected to differential amplifiers referred to a stainless steel skull screw. The bandpass was 0.3 Hz (-6dB/octave) to 20 Hz (-24dB/octave, Bessel). The moveable electrode was lowered in 50 um increments through the hippocampus. Ten second epochs of the theta rhythm recorded from these electrodes were digitized at 100 Hz at each depth. The phase and amplitude of the signal from the moveable electrode, relative to the fixed electrode, were computed using Fourier methods.

In undrugged walking rats, the phase of the theta rhythm reversed between the pyramidal cell layer and the hippocampal fissure over a distance of about 400 um and the amplitude profile showed no null (N=9). This is consistent with published data (2). Pretreatment with atropine usually spread the phase shift over a distance of greater than 500 um and produced a null zone (2 of 3 tests). The broader region of phase shift was predicted by Leung (3) by modelling the relative changes in strength of two phasic synaptic inputs to CA1 pyramids. It seems that two lagged inputs are necessary to produce a broad phase shift. In urethane treated rats the phase reversal usually occurred over a distance of only 150 um on the apical side of the pyramidal cell layer and was associated with a clear null (N=15), as expected (4). This sharp phase shift could be produced by a single phasic synaptic input. On the other hand, when the electrode penetrated CA1a (N=5) in urethane treated rats, the profile was similar to that of undrugged rats. In CA1a two phasic, lagged synaptic inputs must therefore be active even during urethane anesthesia.

(Supported by NIH Grant NS17095)

1) Vanderwolf and Leung (1982) Neurosci Lett Suppl. 10 S501.
2) Winson (1974) EEG Clin Neurophys 26: 291-301.
3) Leung (1983) Neurosci Abstr 9: 1193.
4) Green and Rawlins (1979) EEG Clin Neurophys 47: 420-429.

176.14 EFFECTS OF TONE AND DRUGS ON SPONTANEOUS ACTIVITY IN RATS WITH HIPPOCAMPAL SYSTEM LESIONS. R.N. Shull & F.A. Holloway. Univ. Okla. Health Sci. Ctr., Okla. City, OK 73190.

At least some of the behavioral symptoms of hyperactivity can be viewed as a lack of inhibitory control and the proper functioning of the hippocampal system appears essential to the development of response inhibition. One of these inhibitory processes involves stimulus reactivity and habituation. Using stabilimeter apparatus, six lesion groups (pentobarbital anesthesia) of adult male rats (N= 6/group) were tested to see the effect of repeated presentation (between consecutive 3 min measurement intervals) of a 2900 Hz (95 db/2.5 sec) tone on spontaneous activity and how methylphenidate (4.0 mg/kg) and pentobarbital (5.0 mg/kg) might alter this behavior during tone presentation. The six groups were: kainic acid/pyramidal cell lesions (HCK); colchicine/dentate granule cell lesions (HCC); electrolytic lesions of the hippocampus (large/HC); medial septum (small/MS); and ventral mesencephalic tegmentum (small/VMT); and sham neocortical lesions (no current/ CON). Although intergroup differences were noted, no group showed significant differences in overall session activity levels between saline alone and saline plus tone conditions. Methylphenidate plus tone tended to increase these levels above both saline plus tone and methylphenidate alone levels in all groups but these differences were not significant except for the HC group ($p < .05$). Pentobarbital alone lowered these levels in all groups below saline alone levels while tone presentation did not significantly alter the drug alone levels. Separate interval analysis showed that there were no significant differences between activity levels immediately before and after each of the tone presentations for virtually all sessions and groups. There were group differences, however, in activity level patterns plotted across stimulus presentations with the VMT and HCC groups especially showing initially higher than control levels in contrast to the HCK group which showed relatively small pattern change across either within-session intervals or experimental conditions. In general, all groups showed habituation to the tone as within-session testing progressed. Pre-injection baseline measurements showed the HCC group to be more active than any of the other groups in this paradigm; post-injection activity in this group also was higher during the methylphenidate alone treatment. Of the brain loci examined, the integrity of the VMT and HCC would appear to be most necessary for the maintenance of normal activity levels.

176.15 THE EFFERENT PROJECTION OF THE LATERAL DORSAL THALAMIC NUCLEUS OF THE RAT. J.M. Wyss and K. Sripanidkulchai, Department of Anatomy, University of Alabama, Birmingham, AL 35294.

Evidence from past degeneration studies demonstrated that the lateral dorsal nucleus of the thalamus projects to the posterior cingulate and parahippocampal cortices; however, more recent studies have suggested that the lateral dorsal nucleus also projects to the anterior cingulate cortex. In the present study, the fluorescent dye, retrograde labeling technique has been employed to delineate the cortex that receives a projection from the lateral dorsal nucleus. A total of 60 albino rats each received a single injection of 50 nl of 3% Fast Blue which was stereotaxically placed in various segments of the cingulate or subicular cortex via a 0.5 µl Hamilton syringe equipped with a 200 µm tip needle. In 8 other animals, a second tracer (50nl 4% Nuclear Yellow) was injected into a second region of the cingulate cortex three days after the initial injection. All animals were sacrificed 4 days after the initial injection, and the brains were removed, sectioned (30µm) and placed on clear uncoated slides for inspection by fluorescence microscopy. None of the 23 injections into the anterior cingulate cortex labeled neurons in the lateral dorsal nucleus; however, all of these injections labeled neurons in the anteromedial, dorsomedial, ventromedial, ventrolateral or centrolateral thalamic nuclei. In contrast, lateral dorsal nucleus neurons were always labeled by injections of the posterior cingulate and subicular cortices. Further, injections of the most dorso-lateral portion of the posterior cingulate cortex labeled a higher number of neurons than more ventral injections. The lateral dorsal neurons appear to have a prominent topographic organization in that the ventromedial neurons are labeled by injections of the rostral most part of the posterior cingulate cortex, and the lateral neurons are labeled by injections of the caudal pole of the cingulate cortex. Intermediate injections tend to label neurons in intermediate position within the lateral dorsal nucleus. Our autoradiographic data suggest that each segment of the lateral dorsal nucleus receives a reciprocal cortical projection from the area of cortex to which it projects. This pattern contrasts with that which we have found in the anteroventral nucleus, in which the reciprical projection from the cortex ends in a topographically, organized manner, but does not appear to end directly on the cell bodies giving rise to its thalamocortical projection.

176.16 QUANTITATIVE AUTORADIOGRAPHIC MAPPING OF THE ENTORHINO-DENTATE PROJECTION IN THE RAT R.L. Reep, B.E. Hunter, and D.W. Walker. Department of Neuroscience and Alcohol Research Center, University of Florida, and Veteran's Administration Medical Center, Gainesville, Florida, 32610.

Previous studies have demonstrated topographic organization in the projection from entorhinal cortex (ERC) to the dentate gyrus. Particular emphasis has been placed on the laminar distribution of terminal fields originating in the lateral, intermediate, and medial portions of the ERC. In order to define this projection system more precisely, we have developed a procedure, using computer based image analysis, whereby quantified information concerning the cells of origin and terminal field is obtained in all spatial dimensions. This allows for detailed mapping of the system and provides a rigorous method for assessing the effects of experimental treatments such as chronic ethanol exposure.

After injections of 3H-leucine/proline into the lateral ERC of adult Long Evans rats, adjacent coronal sections were processed for autoradiography or Timm's heavy metal stain.

Within the dorsal hippocampus of each brain, ten equally spaced autoradiographic sections were analyzed. In each such section ten M-L points were sampled along the buried blade of the dentate gyrus. At each sampling point were measured: total molecular layer width, labeled band width, labeled band grain density, grain density in the 'unlabeled' inner part of the molecular layer, and grain density in a nearby background region (stratum radiatum of CA1). Thus a 10 x 10 array of terminal field points was sampled per brain. Similar band width measurements were made in adjacent Timm's sections.

Lateral ERC injection sites were also assessed quantitatively. In five equally spaced ERC sections were calculated: the % length of layer II containing labeled cell bodies, the D-V center point of the labeled part of layer II, and the relative density of labeling.

We find a previously unreported medial-lateral topography such that labeling is consistently heavier in the lateral dentate gyrus. Bandwidths are identical in autoradiographic and Timm's sections, indicating that the lateral ERC terminal field occupies the outer 32% of the molecular layer. Finally, this method allows precise correlations to be made between features of the injection site and those of the terminal field.

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176.17 TOPOGRAPHIC PROJECTION OF THE ANTEROVENTRAL THALAMIC NUCLEUS TO THE RETROSPLENIAL CORTEX IN THE RAT. K. Sripanidkulchai and J.M. Wyss. Department of Anatomy, University of Alabama, Birmingham, AL 35294.

Past studies have demonstrated that the anteroventral thalamic nucleus (AV) projects to the retrosplenial (posterior cingulate) and parahippocampal cortices; however, the topographic organization of this projection has not previously been addressed. Using the fluorescent dye retrograde transport method we have determined that this projection does display a precise topographic organization. In each of forty male albino rats a single injection of 50 nl of 3% Fast Blue (FB) was made via a 0.5 µl Hamilton syringe equipped with a 0.2 µm tip needle (Reno, Nevada). The injections were placed at 0.5 - 2.0 mm lateral to the sagittal sinus and at different rostrocaudal and dorsoventral levels of the retrosplenial cortex and postsubiculum. In 5 additional rats, multiple dye injections, were made with a Fast Blue injection made in one location, a 50nl injection of 4% Nuclear Yellow made in a second site and a 50nl injection of 20% Evans Blue made in a third site. The two blue dyes were injected 4 days prior to sacrifice, and the Nuclear Yellow was injected 15 hours prior to sacrifice. We found that the AV neurons project to the retrosplenial and postsubicular cortices, but do not project to the anterior cingulate cortex. Further, the results demonstrated a dorsoventral organization of the neurons in the anteroventral nucleus. Injections of the most rostral part of retrosplenial cortex (2mm posterior to bregma), labeled neurons only in the most ventromedial part of the nucleus. Progressively more caudal injections resulted in the labeling of neurons in progressively more dorsolateral segments of the AV nucleus. Injections placed in the postsubiculum resulted in the labeling of neurons in the most dorsolateral part of the nucleus. This spatial relationship was confirmed by multiple injections of different fluorescent dyes. In addition to the dorsoventral organization, the anteroventral nucleus also displays a slight parvocellular to magnocellular organization. The most medial injections of the retrosplenial cortex resulted in the labelling of neurons in both parvocellular and magnocellular division of the nucleus; however, more lateral injections labeled relatively fewer parvocellular neurons. Thus the neurons of the anteroventral nucleus appear to have a prominent dorsoventral organization and a less prominent parvocellular to magnocellular organization.

176.18 TOPOGRAPHICAL AND LAMINAR ORGANIZATION OF AFFERENT AND EFFERENT CONNECTIONS OF THE PARAHIPPOCAMPAL CORTEX IN THE CAT. M.P.Witter*, P.Room*, H.J.Groenewegen and A.H.M. LOHMAN*. (SPON.European Neuroscience Association). Dept. Anatomy, Vrije Universiteit Amsterdam, The Netherlands.

According to recent insight the parahippocampal cortex (PHC) subserves a role in the connections leading both to and from the hippocampal formation. In a combined anterograde and retrograde tracing study in the cat an attempt was made to clarify the topography and laminar organization of the input/output systems of PHC. The strong PHC projection to the hippocampal formation, arising from layers II and III, is organized such that the septal pole of the hippocampus is related to lateral PHC and its temporal pole to medial PHC (Witter and Groenewegen, J.C.N.224:371,1984).Injections of retrograde tracers in lateral PHC result in labeled cells in meso- and neocortical association areas, lateral amygdaloid nucleus and dorsal claustrum. In contrast, injections in medial PHC result in retrogradely labeled neurons in the hippocampal formation, olfactory structures, mesocortex, septum, basal amygdaloid nucleus, ventral claustrum and brainstem. This topographical organization could be confirmed by means of anterograde tracing experiments which, in addition, show that cortical afferents of PHC terminate predominantly in superficial layers I-III, whereas the projections from the hippocampal formation, septum, amygdala and claustrum distribute to deep layers IV-VI. The results of anterograde tracer injections in PHC indicate that PHC reciprocates most of its afferent connections. Furthermore, all injections result in rather strong labeling in the striatum. Also these efferent projections are topographically organized: lateral PHC projects to neo- and mesocortex and to the lateral amygdaloid nucleus, dorsal claustrum and dorsal striatum, whereas medial PHC projects to olfactory structures, mesocortex, basal amygdaloid nucleus, ventral claustrum and ventral striatum. Retrograde tracer injections in these PHC projection areas indicate that the majority of extra-hippocampal projections arise from deep layers of PHC. Projections to olfactory structures and mesocortex originate mainly in layer IV, whereas projections to neocortex, amygdala and basal ganglia arise from layers V and VI. The present anatomical data underscore the strategic position of PHC. First, PHC may supply the hippocampal formation with information from the association cortex, and in turn may relay this hippocampally processed information back to these cortices and to the striatum. Second, PHC may subserve an intermediairy role between the hippocampal formation on the one hand and the amygdala and claustrum on the other.

176.19 TOPOGRAPHY OF THE FORNIX TRAJECTORY AND MAMMILLARY BODY TERMINATION OF EFFERENTS FROM THE SUBICULUM AND SUPRACALLOSAL SUBICULUM IN THE RHESUS MONKEY. J. M. EKSTEIN & D. L. ROSENE. Dept. of Anatomy, Boston Univ. Med. Cntr., Boston, MA 02118

The projection from the hippocampal formation through the fornix to the mammillary body has been shown to originate in the subicular fields of the hippocampal formation in the rat. We have investigated the origin, course and termination of these fibers in the rhesus monkey. Injections of tritiated amino acids were place into the subiculum in eight rhesus monkeys and involved all levels of the subiculum from the uncus to the posterior hippocampal formation beneath the splenium.

Analysis of subicular efferents revealed that labeled fibers coursed through the alveus and entered the fimbria posterior to the level of the injection. Within the subcallosal fornix these fibers occupy a consistently dorsal position in the fornix, i.e toward the ventricular surface, regardless of the longitudinal (anterior-posterior) location of the subicular injection within the hippocampal formation. However, anterior subicular injections near the uncus labeled fibers in a lateral position in the fornix, while progressively more posterior injections labeled fibers at more medial positions. Within the medial mammillary nucleus (MMN) label from an injection into the anterior subicular field was distributed in the anterior part of MMN, while label from more posterior injections was distributed to progressively more posterior and inferior parts of the MMN. However, none of these injections produced labeling within the posterior-superior quadrant of the MMN.

An HRP injection into the MMN was made to identify afferents to the MMN including possible sources of projections to the unlabeled posterior-superior quadrant. Within the hippocampal formation retrogradely labeled neurons were limited to the subicular subfields throughout the longitudinal extent of the hippocampal formation. However, at the most posterior extent of the subiculum, labeled neurons continued dorsally around the splenium with the induseum griseum and were found in the supracallosal part of the retrosplenial cortex. An injection of tritiated amino acids placed into the retrosplenial cortex confirmed that this supracallosal subiculum does indeed project to the MMN as anterogradely labeled fibers could be traced ventrally through the corpus callosum into the most medial aspect of the fornix and then ventrally to terminate in the posterior-superior quadrant of MMN.

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176.20 NEURAL PROJECTIONS FROM THE HIPPOCAMPUS TO THE VENTRAL PALLIDUM VIA THE NUCLEUS ACCUMBENS: AN ELECTROPHYSIOLOGICAL STUDY. C. R. Yang* and G. J. Mogenson. Department of Physiology, University of Western Ontario, London, Canada, N6A 5C1.

The nucleus accumbens receives afferents from the hippocampus (HIPP) and, in turn, projects to the antero-ventral globus pallidus (VP), and the subpallidal (SP) region which includes the substantia innominata, the lateral preoptic and the lateral hypothalamic area. Accumbens neurons were activated monosynaptically by stimulation of the hippocampus (Yang and Mogenson, Neurosci. Abs., 1983, 277.3) but it is not known whether they are output neurons to the VP and/or SP regions. This possibility was investigated in the present series of experiments in urethane-anaesthetized rats using electrophysiological techniques.

Single pulse stimulation (0.4 - 1.2 mA, 0.2 ms, 1.0 Hz) of the ventral subiculum of the HIPP synaptically activated 170 of 180 neurons throughout the rostro-caudal axis of the medial accumbens. 55 (30%) of these neurons were also antidromically activated by stimulation (0.5 - 1.5 mA, 0.2 ms, 1.0 Hz) of VP, and 14 (9%) by stimulation of the SP. This suggests that HIPP input signals are transmitted preferentially to accumbens output neurons projecting to the VP as compared to SP ($X^2 = 24$, $p < 0.001$). However, some HIPP signals reaching the SP region may be transmitted by accumbens interneurons. This possibility is supported by the long onset latency of the inhibitory responses of the SP neurons to HIPP stimulation (n = 33, mean = 19 ms, range, 14-24 ms). Furthermore, 61 (33%) and 8 (5%) of accumbens neurons, activated by HIPP stimulation, were orthodromically activated by stimulation of the VP and SP, respectively. This may be due to activation of ascending fibres of passage from the basal forebrain to the accumbens.

In conclusion, these observations provide electrophysiological evidence to indicate that HIPP signals reaching the accumbens are transmitted directly to accumbens output neurons projecting to the VP. On the other hand, some HIPP signals appear to reach the SP polysynaptically via the nucleus accumbens.

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176.21 LIGHT AND ELECTRON MICROSCOPY OF GOLGI-IMPREGNATED PYRAMIDAL NEURONS IN AREA 29. Brent A. Vogt and Joanne M. Quealy*. Departments of Anatomy and Physiology, Boston University School of Medicine, Boston, MA 02118.

Pyramidal neurons are categorized into small, medium and large types. Pyramids with inverted apical dendrites or recurrent axonal trees have also been described. However, there are a number of modifications in the morphology of pyramids in cingulate cortex of rodents. These include a bimodal distribution of dendritic spines, concentration of apical tufts in layer Ia and the presence of extraverted pyramids.

The cingulate cortices of 34 rat brains were impregnated with a Rapid Golgi technique. The blocks were either embedded in celloiden for light microscopy or they were prepared according to a Golgi-EM protocal in which the silver chromate deposit is replaced with a gold deposit.

Light Microscopy. Most layer V neurons have a bimodal distribution of spines on their apical dendrites. Spine counts indicate that in layer V peak numbers occur with 35 spines/10 μm length of dendrite. The number falls precipitously in layer II-III to 11 spines, while in layer I there is an increase to 19 spines/10 μm length of dendrite.

The apical tufts of small and fusiform pyramids in layers II-IV are concentrated mainly in layer Ia. Since primary apical dendrites in layers Ia-b have few branches and spines this dendritic tree appears specialized for receiving inputs in layer Ia.

Electron Microscopy. The ultrastructure of seven gold-toned pyramidal neurons was surveyed. In general the distribution of symmetric and asymmetric synapses formed by these cells parallels that noted for neurons in other cortices. Since the extraverted pyramid does not have a single apical dendrite there is some doubt as to whether or not it is actually a pyramid. Symmetric synapses are formed only with the soma and proximal dendrites and asymmetric synapses are formed with spines. Thus, these cells meet one criterion for being classified as pyramids.

Pyramidal cell morphology in cingulate cortex is influenced by certain structural properties such as the extremely dense packing of cell bodies in layer II-III and dense termination of thalamic afferents in layer Ia.

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176.22 PHYSIOLOGICAL EVIDENCE FOR A PATHWAY FROM ENTORHINAL CORTEX TO AMYGDALA IN THE RAT. Leslie A. Brothers*, David M. Finch and Thomas L. Babb (SPON: J. Lieb). Reed Neurological Research Center and Brain Research Institute, University of California, Los Angeles, CA 90024.

This study was undertaken to extend previous work in this laboratory on the anatomy and physiology of retrohippocampal efferents to the subiculum and entorhinal cortex. Anatomical evidence for entorhinal to amygdala connections has been provided by anterograde {Wyss, JCN 199: 495-512 (1981)} and retrograde {Veening, Neurosci. Lett., 8: 191-195 (1978)} studies; this project has provided physiological evidence for such a pathway.

Electrical pulses of 500 uA intensity and 0.2 msec duration were applied at 0.5/sec to sites in the entorhinal cortex of anaesthetized (Equi-Thesin) 100-400 gm Sprague-Dawley rats. Recordings were performed in varying locations within the amygdala which were subsequently marked with fast green dye. Intracellular (spike amplitude > 40 mV) and extracellular data were obtained in the form of action potentials and post-synaptic potentials.

Of seventeen amygdala cells which responded to entorhinal stimulation, three responded with an EPSP alone, one with an EPSP followed by an IPSP, one with an IPSP alone, and twelve with action potentials. Of the latter responses, nine were orthodromic and three antidromic. Orthodromic response latencies ranged from 4 to 10 msec. Increased stimulus frequency to 5/sec resulted in response potentiation in five cells. One cell located adjacent to the amygdala in pyriform cortex responded with orthodromically-activated action potentials. Effective stimulation sites were located in the lateral entorhinal cortex in superficial and deep cell layers. Responsive cells were found throughout the amygdaloid complex and in one case clearly in the cortical nucleus.

Concurrent work in this laboratory shows the presence of antidromically activated cells in entorhinal cortex in response to amygdala stimulation. Together, these studies provide physiological evidence for a pathway from entorhinal cortex to amygdala which has excitatory and inhibitory effects. The basis of the inhibition, i.e. whether recurrent or feed-forward, and its role in information processing in the amygdala, merit further study.

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176.23 NEUROPHYSIOLOGY OF LIMBIC SYSTEM PATHWAYS: PROJECTIONS FROM THE SUBICULAR COMPLEX AND AMYGDALA TO THE ENTORHINAL CORTEX. David M. Finch, Edie L. Derian, Ernest E. Wong, Xue-Huan Chen, Nancy L. Nowlin, Leslie A. Brothers and Thomas L. Babb. Reed Neurological Research Center and Brain Research Institute, University of California, Los Angeles, CA 90024.

A variety of anatomical studies has shown that both the subicular complex and the amygdala project to the entorhinal cortex. We studied the physiological action of these pathways in the adult albino rat using electrical stimulation and intracellular (spike amplitude > 40 mV) and "quasi-intracellular" (spike amplitude 20-40 mV) recordings. Most recordings (N=61) were obtained from cells in layer II (layer of star cells, after Lorente de Nó) and layer III (superficial pyramids) of the entorhinal cortex and adjacent perirhinal cortex. Stimulation of either the amygdala or subicular complex evoked large inhibitory postsynaptic potentials (IPSPs) in the vast majority of both layer II and III entorhinal neurons. Many individual cells responded to both stimulating loci. The IPSP latencies ranged from 2-10 msec, amplitudes from 5-20 mV, and durations from 100-500 msec. Stimulation of either the amygdala or subiculum also evoked excitatory postsynaptic potentials (EPSPs) in about 1/3-1/2 of both layer II and III entorhinal neurons. The EPSPs usually just preceded the IPSPs. In many cases the EPSPs were sufficiently large to trigger action potentials only when responses were potentiated by higher frequency (10/sec) stimulation. Antidromic responses were also seen, particularly in layers III and V in response to amygdala stimulation and in layers II and III in response to subicular stimulation. Concurrent studies showed that amygdala neurons could be antidromically activated by entorhinal stimulation.

These results show an excitatory projection from the subicular complex and from the amygdala to the entorhinal cortex; and prominent inhibition, which could be either feed-back or feed-forward. The data from the layer II entorhinal neurons are particularly interesting in that these are the cells of origin of the perforant path {Steward and Scoville, JCN 169: 347-370 (1976)}, which is excitatory to dentate granule cells {Andersen et al., Acta Physiol. Scand. 66: 448-460 (1966)}. Among other circuits, therefore, these data indicate an excitatory bisynaptic projection from the amygdaloid complex to the dentate gyrus via layer II cells of the entorhinal cortex.

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176.24 IMMUNOHISTOCHEMICAL IDENTIFICATION OF GABA-CONTAINING NEURONS IN THE RAT BASOLATERAL AMYGDALA. A.J. McDonald*. (SPON: J. Buggy). Dept. of Anatomy, University of South Carolina Sch. of Med., Columbia, SC 29208.

The distribution and morphology of gamma aminobutyric acid (GABA)-containing structures in the rat basolateral amygdala (ABL) was studied using PAP immunohistochemistry. Twenty adult Sprague-Dawley rats were perfused with a solution of phosphate buffered 4% paraformaldehyde-0.5% glutaraldehyde and brains were sectioned at 50-70 µm on a Vibratome. Most animals were injected with colchicine (90 µg) 24 hrs before perfusion. A recently developed anti-GABA antiserum (Immuno Nuclear Corp.) was used at 1:3000 to 1:5000 dilutions. Three brains were counter-stained with cresylecht violet. Immunoreactive neurons were distributed throughout all parts of ABL but constituted a small percentage of the total neuronal population. Immunoabsorption of the antiserum with GABA, but not with glutamate, somatostatin, VIP, or CCK, resulted in elimination of immunostaining in ABL. Most GABA-positive cells were small to medium-sized ovoid neurons that exhibited few dendrites. In the anterior division of the basolateral nucleus, for example, GABA-positive perikarya measured 13.5 µm ± 2.0 X 9.9 µm ± 1.6 (Mean ± S.D., n=23) whereas unlabeled neurons measured 18.8 µm ± 2.7 X 15.0 µm ± 2.5 (n=23). These findings suggest that GABA-positive neurons correspond to class II, and perhaps class III, neurons described in Golgi studies (McDonald '82). In the posterior division of the basolateral nucleus, where most class I neurons are vertically oriented (McDonald '84), the majority of GABA-positive cells have a horizontal or oblique orientation similar to that of class II neurons. Small GABA-positive puncta, resembling axonal terminals, were observed diffusely scattered in the neuropil and forming pericellular baskets around large unlabeled perikarya that were the same size and shape as Golgi-stained class I neurons. These observations suggest that GABAergic class II neurons synapse with class I cells. The location of GABA-positive terminals on perikarya of numerous class I neurons may explain the profound inhibitory action of GABA noted in electrophysiological studies of ABL.

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176.25 ELECTROPHYSIOLOGICAL CHARACTERISTICS OF AMYGDALOID CENTRAL NUCLEUS NEURONS DURING DIFFERENTIAL PAVLOVIAN CONDITIONED HEART RATE RESPONDING IN THE RABBIT. J.P. Pascoe & B.S. Kapp. Dept. Psychology, University of Vermont, Burlington VT 05405.

Considerable evidence suggests that the amygdaloid central nucleus (ACE) may contribute importantly to cardiovascular adjustments in response to the presentation of conditioned emotional stimuli, possibly via direct ACE projections to cardioregulatory nuclei in the medulla (e.g., Applegate et al., Brain Res., 238, 1982). The present experiment was undertaken to obtain additional data relevant to this suggestion.

Single unit recordings were obtained from 85 histologically verified ACE neurons in 19 awake, restrained, New Zealand rabbits during differential Pavlovian conditioned heart rate responding. Conditioning involved pairing one tone (CS+), but not another (CS-), with eyelid shock. During recording, the descending ACE pathway to the brainstem was stimulated at the level of the mesencephalon in attempts to identify, via antidromic activation, those ACE neurons which contribute to this pathway.

In 14 neurons with low spontaneous rates (0.1 to 3.1 Hz) presentations of the CS+ elicited a significant increase in activity that occurred with a latency of 61 ± 24 ms and was sustained for the duration of the CS+. Much smaller increases to the CS- frequently attained significance as well. For 11 of these 14 neurons a significant negative correlation was found between conditioned heart rate response and neuronal response magnitudes. In nine neurons with comparatively rapid spontaneous rates (19 ± 14 Hz) presentations of the CS+ elicited a significant decrease in activity with a latency of 77 ± 15 ms. Smaller decreases to the CS- rarely attained significance. For five of these nine neurons a significant positive correlation was found between conditioned heart rate response and neuronal response magnitudes. The neurons described above could not be antidromically activated.

Twenty-three additional ACE neurons did satisfy the usual criteria for antidromic activation. As a population, these brainstem projection neurons exhibited little spontaneous activity (<0.01 to 0.4 Hz), a 60% decrease in activity during CS- presentations, and a virtually complete (97%) cessation of activity during CS+ presentations.

These data indicate that during the presentation of a conditioned emotional stimulus activity occurring in some ACE neurons is altered in a manner that is closely associated with concomitant cardiovascular adjustments. Under the same conditions, influences normally exerted via direct ACE projections to the brainstem appear to be withdrawn.

176.26 EFFECTS OF AMYGDALOID SEIZURES UPON HYPOTHALAMICALLY ELICITED AFFECTIVE DEFENSE BEHAVIOR IN THE CAT. M. Brutus, M.B. Shaikh*, H. Edinger, A. Ritter*, J. Barrett* and A. Siegel. Dept. of Neuroscience, UMDNJ, Newark, N.J. 07103.

Our laboratory has recently reported preliminary data suggesting that focal seizures induced by electrical stimulation of the amygdala can modify hypothalamically elicited affective defense (AD) and quiet biting attack thresholds in the cat [Neurosci. Abs., 1983, 9: 223]. The present study replicates and extends these findings.

Electrodes for stimulation and recording were stereotaxically placed into the medial hypothalamus and corticomedial and basolateral aspects of the amygdala and adjoining pyriform cortex. Initially, baseline threshold values for AD elicited by medial hypothalamic stimulation were determined over a 1-2 week period. Then, sites in the amygdala were selected which, at subseizure intensities, significantly modulated AD. The experimental paradigm that followed included stimulation of these amygdaloid sites on alternate days for 1-4 weeks at intensities that induced seizures (5 seizure trials per day). At this time, AD thresholds were regularly monitored during the postictal period. In the final aspect of the experiment, ^{14}C-2-DG was systemically injected and stimulation was applied (30 sec. on, 30 sec. off for 45 min.) to those amygdaloid sites where seizures had been generated. Brains were removed and processed for autoradiography.

Amygdaloid seizures generated from sites which facilitated AD were followed by a reduction in response threshold, while an elevation in threshold occurred when seizures were generated from sites in the amygdala which suppressed AD. Seizure foci which facilitated AD were located within the pyriform cortex and adjacent amygdaloid nuclei which utilize the stria terminalis to supply its bed nucleus and the medial hypothalamus. Foci which inhibited AD were situated more laterally and appear to employ alternate output pathways. These findings indicate that temporal lobe seizures have profound effects upon aggressive reactions and that the directionality of the effects is a function of their anatomical foci.

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177.1 ELECTRICAL BEHAVIOR AND INTRINSIC PROPERTIES OF A DISCRETE POPULATION OF SYMPATHETIC NEURONS IN THE INFERIOR MESENTERIC GANGLION OF THE GUINEA PIG. B. F. King* and J. H. Szurszewski. Dept. of Physiology and Biophysics, Mayo Medical School, Rochester, MN 55905.

A discrete population of sympathetic neurons in the inferior mesenteric ganglion (IMG) of the guinea pig functions as a center of integration for the sensory information referred to this prevertebral ganglion by mechanoreceptor elements located in the musculature of the gastrointestinal tract. Sensory information, generated by mechanoreceptors that respond to distention of the gut, is relayed to the IMG and these neurons preferentially rather than to the CNS, as shown in an earlier extensive histological and electrical study. This population of sympathetic neurons which receives mechanoreceptor input and initiates reflex relaxation, shows a considerable diversity in their electrical behavior when depolarized under 'current-clamp' conditions. Neurons which discharged phasically were differentiated further, with a subset of these neurons showing a voltage 'overshoot' at the onset of a current-step. Neurons which discharged tonically were also differentiated into two subsets. One set of these neurons showed a slowly-developing increase in potential over the period of current-clamp during which there was a marked attentuation in amplitude of successive action potentials and marked adaptation of firing-frequency. Intrinsic properties were measured and calculated for sympathetic neurons categorized into four groups according to their responses to depolarizing current-steps. The systematic quantitation of cell properties alluded to morphological and electrical differences between phasic- and tonic-discharging neurons. Differences were observed in the time-constant, specific resistance, total cell capacitance, total surface area, cell diameter and space-constant. In addition, distinctions were made for the time-to-decay (by 50% and 90%) for the afterspike hyperpolarization and to the level of negativity in potential attained during the afterspike hyperpolarization. However, it was difficult to distinguish between subsets of phasic-responding cells on the basis of their intrinsic cell properties. On the other hand, subsets of tonic-responding neurons were differentiated by the current-threshold to sustain continuous firing, and by the slope of the F/I relationship with suprathreshold current-steps. The means by which differences in intrinsic properties were manifested was not elucidated. (Supported by NIH AM 17632.)

177.2 ULTRASTRUCTURAL FEATURES OF NORMAL AND ABNORMAL-APPEARING CELIAC GANGLION NEURONS FROM AGED WISTAR-KYOTO RATS. J.A. Mascorro* (SPON: D.E. Smith). Department of Anatomy, Tulane University School of Medicine, New Orleans, LA 70112

Investigators have studied sympathetic ganglia from aged human and animal specimens in order to elucidate the morphological alterations which occur in sympathetic neurons with advancing age. Numerous morphological features have been reported, such as lipofuscin accumulation, mitochondrial enlargement, vesiculated pigment granules, myelin disorganization, senile plaque formations, laminar bodies and neurofibrillary tangles. Investigators do not agree whether the changes result from normal aging processes or perhaps are pathological manifestation(s) of a disease state. The present study utilizes very old, apparently healthy rats to illustrate the structure of aged sympathetic neurons.

Two young Wistar-Kyoto rats obtained from a colleague's SHR colony were allowed to reach 40 months in age. The animals then were anesthetized and perfused with 3% glutaraldehyde in 0.1M phosphate buffer. The celiac ganglia were processed for light and ultrastructural study following secondary fixation in osmium tetroxide.

Histologically, the celiac ganglion was a composite of large neurons, many blood vessels, myelinated and unmyelinated axons, connective tissue components, a dense capsule, as well as satellite and Schwann cells. The structure of neurons and ganglion components appeared well within normal histological limits. However, a few neurons displayed dilated spaces within their perikarya or cytoplasmic processes. The electron microscope revealed that those areas represented dilated mitochondria with fragmented cristae. The most obviously disfigured mitochondria lacked cristae and contained dense bodies and an amorphous granular material. Nissl substance and Golgi membranes appeared normal and well preserved. Lipofuscin was prominent in many of the nerve cells. This study illustrates one predominant and recurring morphological alteration in celiac ganglion neurons from 40 month old Wistar-Kyoto rats: mitochondrial degeneration. Other neuronal changes usually associated with age were not noted in the present study. Assuming that the subjects were healthy animals, it is tempting to suggest that mitochondrial dysfunction in certain old sympathetic neurons is a "true" change related to the aging process. (Appreciation is extended to Dr. Craig A. Knox, Department of Neurology, Mayo Clinic, Rochester, MN for providing the animals used in this study.)

177.3 BINDING OF THE ADENOSINE AGONIST, [^{3}H]-CYCLOHEXYLADENOSINE (CHA), TO GUINEA PIG ILEAL MEMBRANES. Michael Williams, Heather Valentine* and Greg Mack*. Nova Pharmaceutical Corporation, P.O. Box 21204, Wade Avenue, Baltimore MD 21228.

Adenosine (ado) has well characterized presynaptic inhibitory actions at myenteric ganglia (Vizi and Knoll, Neurosci., 1, (1976), 391) and receptors for this putative neuromodulator, which are insensitive to blockade by the ado antagonist, 8-phenyltheophylline, have been identified in guinea pig ileum tissue sections by autoradiographic visualization of [^{3}H]- NECA (5'N-ethylcarboxamidoadenosine) binding (Buckley and Burnstock, Brain Res., 269, (1983),374).

Using a crude guinea pig ileal membrane preparation pretreated with adenosine deaminase to remove endogenous ado, high affinity [^{3}H]-CHA binding (Kd = 3.8 ± 0.5 nM; n = 3) has been demonstrated. Such binding shows stereoselectivity, the S-diastereomer of N^6-phenylisopropyladenosine (PIA) being 10 times less effective than the R-isomer in displacing CHA, and is destroyed by boiling. Binding of CHA is sensitive to xanthine inhibition in this broken membrane preparation, 1,3-diethyl-8-phenylxanthine (DPX) with an IC_{50} value of 936 nM being 11, 65 and 128 times more potent than theophylline, caffeine and enprofylline, respectively, in displacing specifically bound CHA.

Binding of CHA to guinea pig ileum is similar in many respects to that seen in guinea pig brain and has pharmacology consistent with it being of the A-1 subtype.

177.4 EVIDENCE THAT DOPAMINE MODULATES NOREPINEPHRINE TURNOVER IN THE RAT SUPERIOR CERVICAL GANGLION DURING HYPOXIC STRESS. J.J. Brokaw and J.T. Hansen. Dept. of Cellular and Structural Biology, Univ. of Texas Health Sci. Ctr., San Antonio, TX 78284.

The rat SCG contains substantial quantities of NE (175 pmoles/SCG) and lesser amounts of DA (18 pmoles/SCG). The principal neurons are considered to be the primary sites for NE storage, and a major portion of the DA is thought to be localized in small intensely fluorescent (SIF) cells. Evidence suggests that the NE present in the SCG may represent an intraganglionic pool of neurotransmitter which is released from principal neurons during preganglionic nerve stimulation. Several electrophysiologic studies in the rabbit indicate that SIF cell DA may act, in part, to hyperpolarize principal neurons. However, there is little evidence to suggest an inhibitory role for DA with regard to NE turnover in the SCG. Therefore, we assessed the effect of hypoxic stress on NE turnover in the rat SCG as indexed by the rate of precursor DA accumulation and NE depletion following blockade of dopamine-ß-hydroxylase with diethyldithiocarbamate (DDC; 200 mg/kg, ip). Further, the potential role of DA in regulating ganglionic NE turnover was explored using spiroperidol (1 mg/kg, ip), a selective DA-receptor antagonist. Initially, 2 groups of rats were treated with DDC and exposed to either room air (normoxia) or intermittent hypoxia (5% O_2 - 95% N_2) for 2 hrs. Following the exposure, these animals and a third group of untreated controls were sacrificed by decapitation. Quantitation of SCG catecholamine levels using HPLC-EC showed a significant rise in DA content and a significant depression in NE levels in both DDC-treated groups compared to controls. However, the hypoxic group was not significantly different from its normoxic counterpart, indicating no apparent change in NE turnover. By contrast, in animals pretreated with spiroperidol 30 min prior to replication of the above experiment, the hypoxic group demonstrated a significantly greater degree of both DA accumulation and NE depletion compared to the normoxic group, indicative of enhanced NE turnover. These data suggest that DA may play an inhibitory role in regulating intraganglionic NE turnover during stress. Although we cannot exclude the possibility that spiroperidol may be exerting a centrally-mediated potentiation of sympathetic outflow, the observed effects are consistent with current hypotheses of SIF cell function. Supported by grant 83-733 from the AHA and NIH grants HL-25508 and K04 HL-00680.

177.5 INCREASED DOPAMINE (DA) METABOLISM IN THE RAT SUPERIOR CERVICAL GANGLIA (SCG) DURING PHYSIOLOGIC STRESS. D.S. Christie* and J.T. Hansen (SPON: M.D. Guthrie). Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, Texas 78284.

Small, intensely fluorescent (SIF) cells have been identified as possible interneurons in autonomic ganglia. As a prelude to studies of the functional role of dopaminergic SIF cells in the rat SCG, we have conducted experiments to determine the physiologic conditions which stimulate these cells in vivo. Dopamine is the principal SIF cell neurotransmitter in the rat SCG, and we have used the content of the DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) as an index of SIF cell activity. Measurements of DOPAC content were accomplished using high performance liquid chromatography with electrochemical detection.

To determine if SIF cells were activated during physiologic stress, three groups of animals were subjected either to hypoxia (20 min of 5% O_2), hypoglycemia (following 5U insulin/kg ip), or a cold swim (5 min in water at 5°C). All three conditions caused a significant elevation in DOPAC content in the SCG ($P<0.01$). To further determine if a non-noxious, natural stimulus would affect SIF cell activity, animals were sacrificed every 4 hrs for 24 hrs during a 14:10 light-dark cycle. A significant elevation ($P<0.01$) in DOPAC content occurred during the dark phase of the cycle, perhaps due to the activation of the pineal gland via the SCG.

Since previous investigators have proposed that SIF cells may be chemosensitive in a manner similar to the glomus cells of the carotid body, an additional experiment was conducted to determine if the elevation in DOPAC content seen during hypoxia was a direct effect or was mediated by preganglionic nervous activity. Rats, surgically decentralized by severing the sympathetic trunk just caudal to the SCG and rendered hypoxic following a 10-day recovery period, showed significantly reduced DOPAC in the SCG ($P<0.001$) and a total abolition of the rise in DOPAC seen in intact animals during hypoxia.

We conclude that SIF cells are activated during physiologic conditions which stimulate the sympathetic nervous system, and that hypoxic stress does not act directly on SIF cells but rather is mediated by preganglionic nervous activity.

Supported by grant 83-733 from the AHA and by NIH grants HL-25508 and K04 HL-00680 to J.T.H.

177.6 NEUROPEPTIDE Y (NPY)-LIKE IMMUNOREACTIVITY IN THE FEMALE REPRODUCTIVE SYSTEM. H. Traurig, R.E. Papka and J. Cotton*. Dept. of Anatomy, Univ. of Kentucky Sch. of Med., Lexington, KY 40536.

Recently NPY, a 36 amino acid residue peptide, was isolated from porcine brain and characterized (TATEMOTO, K., Proc. Natl. Acad. Sci., USA 79:5485, 1982). This peptide is part of a family of pancreatic polypeptides, some of which are present in the peripheral nervous system. Our recent studies have demonstrated the innervation of the female rat reproductive system by neurons containing peptides such as substance P and vasoactive intestinal polypeptide. Our present study provides data on the presence and distribution of NPY-like immunoreactive (NPY-I) nerve fibers in the female reproductive tract of the adult, nonpregnant rat. For this purpose, an immunofluorescence procedure was applied to formaldehyde fixed tissues that were processed as whole mounts or as cryostat sections. The areas examined included the vagina, cervix, uterus, oviduct, ovary and mammary gland nipple. NPY-I fibers generally formed a dense plexus in all organs examined. NPY-I fibers formed a particularly prominent plexus around blood vessels in all organs. In cryostat sections it was evident that the fine perivascular fibers penetrated to the junction of the tunica adventitia and tunica media. In those organs which have a smooth muscle component, e.g. cervix and uterus, NPY-I fibers were rich among the muscle fascicles and tended to be oriented parallel to the longitudinal axis of the smooth muscle fibers. In some areas, e.g. the cervix, NPY-I fibers extended from the muscle and connective tissue layers toward the epithelium and formed a subepithelial plexus. The fibers of the uterus appeared to enter the wall as large paravascular nerve trunks; from these trunks finer single fibers branched at nearly right angles to parallel the smooth muscle bundles of the muscular part of the mesometrium. In addition, some fibers appeared to terminate in the non-muscular part of the mesometrium as free nerve endings. In the ovary NPY-I fibers were seen throughout the stroma, near and in the interstitial tissue and adjacent to follicles.

The exact function of NPY-containing nerves is unclear, however it has been suggested that they may modulate vasomotor activity and influence smooth muscle activity. Thus NPY-I nerves may play an important role in the function of the female reproductive system. (Supported by BRSG #RR05374,NIH).

177.7 URINARY BLADDER AFFERENTS MAY MAKE DIRECT CONTACTS WITH LUMBOSACRAL INTERMEDIOLATERAL NEURONS. A WHEAT GERM AGGLUTININ (WGA) IMMUNOHISTOCHEMICAL STUDY IN RAT. P.J. Jannetta, I. Nadelhaft, and K.E. McKenna, VA Medical Center and Depts. of Neurosurgery and Pharmacology, University of Pittsburgh, School of Medicine, Pittsburgh,PA.

Previous studies, where the rat pelvic nerve was exposed to horseradish peroxidase, revealed extensive overlap between labelled visceral afferent collateral fibers and their terminals and labelled intermediolateral preganglionic neurons (PGN) in segments L6 and S1. To examine the possibility of direct contacts between these afferents and the PGN, we used the lectin WGA as a neuroanatomical tracer. This material was chosen because recent studies (Ruda and Coulter, '82) have shown that it could cross afferent synaptic junctions to label the postsynaptic neuron. Bladder afferents were labelled by a bilateral injection of WGA (0.2% aqueous with 0.1% bromphenyl blue for color) directly into the bladder muscle layer. The left L6 and S1 ventral roots had been transected prior to the WGA injection, to prevent retrograde labelling of PGN via leakage of WGA to the pelvic ganglion followed by transport along the pelvic nerve. After 2 or 3 days the animal was perfused with 4% buffered paraformaldehyde and 30 micron tissue sections processed immunohistochemically (nickel-enhanced PAP) for WGA. Retrograde-labelled ganglion cells were found in the L6 and S1 dorsal root ganglia and also in lumbosacral ganglia of the sympathetic chain. In the cord, labelled fibers were located in the superficial dorsal horn (LI and II) and along the lateral marginal zone extending into the area of the PGN. Labelled spinal cord neurons were identified by small black particles scattered over their profiles and proximal dendrites. We did not establish whether these granules were intracellular or on the cell's surface. Labelled cells were found in the dorsal horn marginal zone, the substantia gelatinosa, the lateral spinal nucleus, the sacral parasympathetic nucleus (PGN), and the dorsal gray commissure. Labelled afferent fibers, from the lateral marginal zone extended over the neurons of the sacral preganglionic nucleus. Some of these fibers were observed to lead directly to a neuron which also was labelled. We conclude that some preganglionic neurons may receive direct contacts from bladder primary afferent fibers.

177.8 CONTRIBUTIONS TO THE INNERVATION OF NEURONS IN THE MAJOR PELVIC GANGLION OF THE RAT. R.A. Dziurzynski*, W.G. Dail and D. Bell* (SPON: E. Reyes). Department of Anatomy, University of New Mexico School of Medicine, Albuquerque, NM 87131.

In addition to innervation by preganglionic fibers of the pelvic nerve and the hypogastric nerve, neurons in the major pelvic ganglion (MPG) of the rat are contacted by small intensely fluorescent (SIF) cells. A minority of principal neurons in the MPG are enclosed by a substance P (SP) immunoreactive nerve plexus. SP immunoreactivity is present in SIF cells and, after colchicine pretreatment, also appears in a small number of large neurons in the MPG. The apparent multiple and varied innervation of principal neurons in the MPG led to the present investigation of the relative contribution of extrinsic sources of innervation to MPG neurons, i.e., from the hypogastric and pelvic nerves, and the proportion of innervation which may be provided by intrinsic sources. Zinc iodide osmium (ZIO) staining of nerve terminals was performed in the following paradigms: (1) intact MPG, (2) MPG in which the hypogastric nerve was sectioned, (3) MPG in which the hypogastric and the origins of fibers to the pelvic nerve were sectioned. In the intact MPG, ZIO blackened terminals closely invest the cell body of most of the principal neurons when studied by a serial sampling technique. Examination of serial sections indicated that all MPG neurons received ZIO labeled terminals. Four days after interruption of the hypogastric nerve, an average of 40% of the neurons lost ZIO related terminals. This effect was most noticeable on the large diameter neurons in the MPG. When both the hypogastric nerve and the pelvic nerve were sectioned, only about 5% of the neurons remained innervated. Total decentralization also reduced but did not eliminate the SP positive fiber plexus in the MPG. The results of this study indicate that (1) the pelvic nerve provides the major input to the MPG, (2) ZIO staining indicates that intrinsic sources provide a relatively small proportion of the innervation of principal neurons, and (3) substance P positive fibers in the MPG may arise from extrinsic and intrinsic sources. Supported by NIH grant RO1NS19839-01.

177.9 EVIDENCE THAT THE HYPOGASTRIC NERVE INNERVATES SOME PENILE NEURONS IN THE PELVIC PLEXUS. W.G. Dail, K. Manzanares* and W.C. Broderick. Department of Anatomy, University of New Mexico School of Medicine, Albuquerque, NM 87131.

The vascular and intrinsic smooth muscle of the corpora cavernosa penis are innervated by parasympathetic and sympathetic fibers. It is generally agreed that preganglionic parasympathetic fibers in the pelvic nerve relay in pelvic ganglia while the sacrococcygeal chain is the ganglionic site for penile sympathetic neurons. Another sympathetic pathway to the pelvic viscera, the hypogastric nerve, is known to provide pre- and postganglionic fibers to pelvic viscera, but not to penile erectile tissue. Electrical stimulation of the hypogastric nerve has been reported to have no effect on the penile vascular bed or to cause either slight vasodilation or vasoconstriction. The singular ganglion in the pelvic plexus of the rat, with its distinct input from the pelvic nerve and the hypogastric nerve, make it an appropriate model to study this problem. Penile neurons in the major pelvic ganglion (MPG) were first retrogradely labeled with True blue. 1.5 to 2.0 microliters of dye injected into each penile crus labels approximately 100 to 150 neurons in each MPG. Met-enkephalin (m-ENK) immunohistochemistry was used as a marker for preganglionic nerve terminals in the MPG. Virtually all of the neurons in the MPG, including True blue labeled penile neurons, are enclosed by a m-ENK positive nerve plexus. In some animals, on the day of the dye injection, the roots of the pelvic nerve (from spinal nerves L_6 and S_1) were severed on the right side with the left side serving as a control. Five to seven days following this procedure, the MPG was examined for m-ENK innervated penile neurons. The majority of the blue labeled penile neurons were without a m-ENK plexus, however, some 10% to 35% of the penile neurons were enclosed by m-ENK positive fibers. In animals in which the pelvic nerve root and the hypogastric nerve contribution to the MPG were cut, m-ENK positive fibers were virtually absent from the MPG. The remaining m-ENK fibers could arise from the resident population of enkephalin positive small neurons (SIF cells), from contralateral projections of the pelvic plexus, or other unknown sources. This study has provided anatomical evidence that the hypogastric nerve conveys fibers to some penile neurons in the major pelvic ganglion of the rat. The functional significance of this pathway remains to be determined. Supported by NIH grant RO1NS19839-01.

177.10 **SEPARATE SUBDIAPHRAGMATIC VAGAL BRANCHES ORIGINATE FROM DIFFERENT LONGITUDINAL COLUMNS OF THE DORSAL MOTOR NUCLEUS OF THE VAGUS.** E.A. Fox*, F.A. Tumeo* and T.L. Powley. Lab. of Regulatory Psychobiology, Purdue Univ., West Lafayette, IN 47907.

Injections of retrograde tracers into visceral organs have typically resulted in label appearing throughout much or all of the dorsal motor nucleus of the vagus (DMV) (e.g., Kalia and Mesulam, JCN, 193,467, 1980). In contrast, some (see Norgren and Smith, Neurosci. Abs., 9,611, 1983), if not all (e.g., Rogers and Hermann, J. Auton. N.S., 7,165, 1983), experiments soaking individual vagal branches have suggested more localized maps of motorneurons.

In 24 male Sprague Dawley rats, the fluorescent tracer true blue (15µl of 10% per animal) was either injected into the pancreas, stomach, or colon. An additional 35 animals received injections (30µl of 10%) into the pancreas or the intraperitoneal cavity after a total vagotomy or a selective vagotomy that spared only one of the five subdiaphragmatic branches. Controls for leakage included examination for true blue in other viscera, the spinal cord, and the medulla (after IP applications).

After visceral injections, organ-specific patterns of label were not entirely diffuse, but they typically occupied large portions of the DMV and were also generally characterized by evidence of tracer in other tissues. In contrast, the experiments with selective vagotomies demonstrated that individual branches of the subdiaphragmatic vagus received the axons of discrete longitudinal DMV columns. The posterior gastric branch contained axons from a cell column in the medial three-quarters of the right DMV, while the coeliac branch neurons were found in the lateral quarter of the right DMV. The anterior gastric branch corresponded to a column occupying the medial three-quarters of the left DMV, while the accessory coeliac originated from the lateral quarter of the left DMV. The hepatic branch carried a small, diffuse innervation from the left DMV.

These results indicate that the subdiaphragmatic branches of the vagus are topographically organized within the DMV. Further, the fact that our and others' maps based on organ injections typically span more than one of the separate DMV columns suggests either or both of the following: (A) Viscera including the pancreas, stomach, and intestines are innervated by several vagal branches, or (B) organ innervation maps have been blurred by the tracer diffusion and leakage. (USPHS grant AM27627.)

177.11 SYMPATHETIC NERVE FIBERS IN THE RAT OROFACIAL REGION AS LABELED BY THE ANTEROGRADE TRANSPORT OF HORSERADISH PEROXIDASE-WHEAT GERM AGGLUTININ (HRP-WGA). D.F. Turner* and C.F. Marfurt. (Sponsor, W. Severs) Dept. Oral Biology, Univ. Michigan,Ann Arbor,MI 48108 and Dept. Anat.,Ind. Univ. Schl. of Med.,Northwest Center for Med. Educ.,Gary,IN 46408.

The purpose of the current report is to describe a new method for labeling sympathetic nerve fibers and their terminals in orofacial tissues. HRP-WGA solutions(2% in saline) were injected unilaterally into the superior cervical ganglion (SCG) of adult rats. One to 5 days postinjection, the animals were perfusion-fixed and the brain, spinal cord, trigeminal ganglia, and a variety of orofacial tissues processed for HRP histochemistry according to the TMB technique. Tissues were then examined critically at both light and electron microscopic levels.

The results showed that HRP-WGA injected into the SCG was taken up by the terminals of preganglionic sympathetic fibers and transported retrogradely to their cell bodies in the spinal cord intermediolateral cell columm and central gray. Additionally, the conjugate was taken up avidly by the somas of the SCG neurons and transported anterogradely into their peripheral fibers and axonal terminals.

Labeled sympathetic fibers that distributed to tissues of the eye entered the cranial vault with the internal carotid artery and coursed towards the orbit as a stout bundle of fibers attached by some loose connective tissue to the medial edge of the trigeminal ganglion. The fibers pierced the ventromedial border of the ophthalmic nerve just distal to the trigeminal ganglion and then split into numerous smaller fascicles or individual fibers that followed the terminal branches of the ophthalmic nerve. The densest network of HRP-labeled sympathetic fibers seen in the present study was found in the iris. Fibers entered the iris in radial fashion and distributed to both dilator and constrictor portions. Other labeled sympathetic fibers in the orbit formed fine plexuses around limbal blood vessels, while some entered the deep corneal stroma. The latter fibers coursed sometimes for distance of one half the corneal diameter or more before ending.

Dense plexuses of labeled sympathetic fibers were seen also around cerebral arteries, in the pineal gland, and in relation to blood vessels and sweat glands of the skin.

At the electron microscopic level,HRP-labeled sympathetic fibers were identified readily by the presence within their axoplasm of jet-black,rod-like crystals of HRP-TMB reaction product. (Supported by USPHS DE06093 and EY04923).

177.12 DIABETES INDUCED CARDIAC MUSCARINIC SUPERSENSITIVITY. G.O. Carrier, A.D. Edwards,* R.E. White* and R.S. Aronstam. Dept. of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912.

Dysfunction of the autonomic nervous system is a major complication of diabetes mellitus. Early cardiac disburbances have been attributed to abnormal parasympathetic control of the heart. The purpose of this investigation was to examine cholinergic mechanisms in diabetes.

Diabetes was chemically induced by streptozotocin in 42-43 day old male Sprague-Dawley rats. After 8-10 weeks, isolated spontaneously beating atrial preparations were prepared for in vitro heart rate measurements. Resting heart rate in the conscious diabetic rats was 303±8 beats/min compared to 401±21 beats/min for age-matched control rats. As illustrated in the table, atria isolated from diabetic rats were supersensitive to the chronotropic effect of acetylcholine, carbamylcholine and bethanechol.

Agonist	Control EC_{50}	Diabetic EC_{50}
Acetylcholine	7.4×10^{-5}	3.8×10^{-6}
Carbamylcholine	6.0×10^{-7}	1.5×10^{-7}
Bethanechol	9.0×10^{-6}	3.8×10^{-6}

There were, however, no differences in the negative inotropic responses of electrically-stimulated left atrium from diabetic and control rats to cholinergic agonists.

Muscarinic receptor populations were measured directly using [^{3}H]3-quinuclidinyl benzilate as a probe. The density of muscarinic receptors in atria from diabetic rats was 30% less than in age-matched control atria (166 vs 237 fmoles of muscarinic binding sites/mg protein.

Since the ionic basis for the negative chronotropic effect of cholinergic agonists involves enhancement of potassium (K^+) efflux and a decrease in the inward calcium current, the effects of K and Ca on atrial rate of diabetic rats were determined. There was no difference in the sensitivity of the tissue from diabetic and control rats to changes in K, while atria from diabetic rats were made sensitive to Ca. The present findings suggest that cardiac muscarinic supersensitivity in diabetes are associated with changes in postjunctional receptor-effector mechanisms. (Supported by HL31518, NS17429 and a grant from the Georgia Heart Association).

178.1 CATECHOLAMINE, GABA AND ASPARTATE/GLUTAMATE NEURONS IN THE BRAIN STEM OF HYPERTENSIVE RATS. Bang H. Hwang, C.-T. Lin* and J.-Y. Wu. Depts. of Anatomy and Physiology, Pennsylvania State University, Sch. of Med., Hershey, PA 17033.

The rat nucleus tractus solitarius (NTS) has been shown to play important roles in the cardiovascular function. The NTS contains primary afferent synapses of baroreceptors. The NTS is particularly rich in catecholamine (CA) terminals. Similarly, the parventricular hypothalamic nucleus (PVN) is heavily innervated by CA terminals and contains different peptidergic neurons including vasopressin (VP) neurons. Electrical stimulation of the PVN induces hypertension. It seems clear that both NTS and PVN are related to cardiovascular function. However, the exact mechanisms by which CA and other substances in the NTS/PVN associate with the development of hypertension remain undefined. In this study, CA, gamma-aminobutyric acid (GABA), and aspartate/glutamate neurons in the NTS and PVN of hypertensive rats were studied in order to better understand the roles that these neurons may play in the cardiovascular regulation. Male Wistar rats were unilaterally nephrectomized and treated with deoxycorti costerone acetate (DOCA) and salt to induce hypertension. Four weeks after the treatment, blood pressure of DOCA-salt rats was significantly increased. CA terminals, labeled with 5-hydroxydopamine, contained small granular vesicles. CA synapses versus total CA bouton profiles per 7220 μ^2 area of the NTS and PVN were expressed as CA synaptic frequency. The CA synaptic frequency in the PVN of DOCA-salt hypertensive rats was significantly increased. Such differences were not seen in the NTS 4 weeks after the treatment. Furthermore, by HPLC determination, the CA contents in the PVN, but not in the NTS of DOCA-salt hypertensive rats were significantly reduced. Since synapses are primary sites for the neurotransmitter release, more CA synapses in the PVN may release more CA to modulate VP neurons and result in a reduction of CA contents in the PVN. It is known that VP neurons project to the NTS. We have identified the GABA and aspartate/glutamate cell bodies and their processes by immunocytochemical method using antibodies against L-glutamate decarboxylase (GAD) and aspartate aminotransferase (AAT) respectively. Numerous GAD-positive and AAT-positive neurons were found in the NTS. These preliminary results suggest that there may be a CA-VP-GABA axis and a CA-VP-aspartate/glutamate axis in the PVN/NTS pathway to regulate blood pressure. (Supported in part by PA-AHA and NIH grant NS20978.)

178.2 NEUROANATOMICAL ALTERATIONS IN THE SPONTANEOUSLY HYPERTENSIVE RAT: GROSS MORPHOLOGICAL AND HISTOLOGICAL EVALUATIONS. D. K. Nelson*, R. L. Coulson*, R. A. Browning and J. H. Myers* (Sponsor, W. M. Yau). Medical Physiology and Pharmacology, Southern Illinois University School of Medicine, Carbondale, IL 62901

Previous studies from this laboratory (Lehr et al., Clin. Exp. Hyper., 2:123, 1980) have shown that the spontaneously hypertensive rat (SHR) exhibits morphological differences in surface brain structure when compared to Wistar-Kyoto (WKY) normotensive controls. Subsequent studies by other investigators (Nelson and Boulant, Brain Res. 226:119, 1981 and Brain Res. 261:145, 1983) have demonstrated structural differences in hypothalamic and brainstem nuclei with regard to soma cross-sectional areas and cell densities. We now report that the brain of the SHR is smaller than that of age-matched WKY rats in 5 of 7 measures of external landmarks in 94 day-old males, and in 10 of 12 gross measures in 170 day-old males. Furthermore, the SHR brain was smaller in terms of brain weight and brain weight:body weight ratio. Inasmuch as evidence describing overall sizes and locations of individual nuclei and/or fiber tracts has not been reported, it was also of interest to examine selected internal structures in terms of volumetric and stereotaxic parameters. Using coronal sections from 94 day-old rats, it was found that the volume and surface area of the nucleus of the solitary tract (NTS) are reduced in the SHR by 30% and 19%, respectively. Similarly, the volume and surface area of the dorsal tegmental nucleus of the SHR are decreased by 24% and 20%, respectively. In addition to being diminished in size, the NTS is positioned differently within the brainstem of the SHR. Specifically, the center of the NTS in the SHR is located medial with respect to the center of this nucleus in the WKY, while there is little or no variation in the dorsoventral dimension. These findings are of practical interest to stereotaxic or neurochemical investigations requiring the isolation of specific neural structures. Moreover, the involvement of these nuclei in central cardiovascular regulation (particularly that of the NTS as the primary relay of afferent baroreceptor fibers) suggests that the physiological aberrations apparent in the SHR may be related to morphological alterations in the central nervous system.

178.3 TELENCEPHALIC INVOLVEMENT IN HABITUATION OF CARDIAC AROUSAL RESPONSES TO ILLUMINATION IN THE GOLDFISH, CARASSIUS AURATUS. P.R. Laming* and S.O.E. Ebbesson. Queen's Univ., Belfast, N.I. and Dept. of Anatomy, LSU Sch. of Med., Shreveport, LA 71130.

Unilateral DC lesions were made with a carbon fiber electrode in a double-barrelled glass micropipette inserted into the posterior telencephalon of goldfish. The other barrel contained HRP for iontophoretic injection into the lesion site. Habituation deficites were greatest when lesions were made in Dc, Vi, Dp and Ep, posterior to the anterior commissure. Lesions in Dm had a lesser, though significant, effect. Larger lesions of the telencephalon had no effect on the hibituation unless they included these areas. The data therefore implicate small, restricted cell groups in habituation. The HRP preparations served to identify electrode placement and the connections of affected brain structures. Continued work with the combined lesioning and tracer infusion electrode pair should enable identification of the circuitry involved in regulating arousal during its habituation in teleosts.

178.4 NEURAL CONNECTIONS OF AN INFRALIMBIC CORTICAL PRESSOR AREA. D.C. Tucker and C.B. Saper. Dept. of Neurology, Washington Univ. Sch. Medicine, St. Louis, MO 63110.

Electrical stimulation of medial prefrontal cortex in the cat has been demonstrated to produce both pressor and depressor responses. While electrical stimulation of anterior cingulate cortex in the rat has been reported to produce depressor responses, (Morrison et al., Neurosci. Abstr. 6:817, 1980), no cortical pressor area has been demonstrated in the rat. We have mapped pressor and depressor regions in medial prefrontal cortex in a series of four adult male rats, identified a specific pressor region in infralimbic cortex, and examined its neural connections. Rats were anesthetized with 3.5 mg/kg chloral hydrate. Stimulation (200 uA, 0.5 msec pulses at 60 Hz in a 15 sec train with 45 sec between trials) was delivered via a constant current stimulator through a glass pipette (tip diameter = 60 um) filled with 2.75 M KCl. The area from 3.0 to 4.5 mm anterior and 0.7 mm lateral to bregma was mapped by stimulating at 0.2 mm intervals between 1.0 mm and 7.0 mm below the dural surface. BP responses were measured via an indwelling femoral artery catheter. Depressor responses (-5 to -25 mm Hg) were observed after stimulation of most areas of anterior cingulate and prelimbic cortex. A discrete pressor region (+5 to +22 mm Hg) was localized to the posteroventral portion of the infralimbic cortex, beneath the genu of the corpus callosum. Additional pressor responses were elicited by stimulation of nucleus accumbens.

Neural connections of the infralimbic pressor area were traced following injections of wheat germ agglutinin-HRP conjugate (3-8 nl of a 1% solution) into this region. Labeled efferent fibers coursed from the injection site through nucleus accumbens; these axons may have been responsible for the pressor response to nucleus accumbens stimulation. Reciprocal connections were found between infralimbic cortex and the lateral hypothalamic area, the parabrachial nucleus, the nucleus of the solitary tract and the ventral lateral medulla. Additional efferent projections were seen to the central nucleus of the amygdala and the bed nucleus of the stria terminalis. The profuse connections of the posteroventral infralimbic cortex with areas of the central nervous system implicated in autonomic control may underlie a role for this area in central regulation of blood pressure.

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178.5 NEUROPEPTIDE CONNECTIONS OF THE LATERAL PARABRACHIAL NUCLEUS (LPBN) WITH THE ANTEROVENTRAL THIRD VENTRICULAR AREA (AV3V). C.H. Block, R.E. Watson, Jr., and G. Hoffman-Small. Dept. of Anatomy, University of Rochester, Rochester, N.Y. 14642.

The LPBN has recently been demonstrated to serve as a critical site in the central autonomic-cardiovascular regulating network. This pontine region has projections with several major cardiovascular control centers, including AV3V of the median preoptic nucleus. Since both LPBN and AV3V contain many of the neuropeptides that have been implicated in cardiovascular modulation, we sought to examine the neuropeptidergic substrates of the potential cardiovascular projection system between LPBN and AV3V.

Microinjections of a 5% suspension of true blue were placed unilaterally, under stereotaxic guidance, into the AV3V of young adult rats. After a two week survival period, a group of the animals was treated with a 1% colchicine solution, administered intracerebroventricularly, to enhance perikaryal immunoreactivity. After an additional 24 hour survival time, the animals were anesthetized and perfused transcardially with normal saline followed by Zamboni's fixative. The brains were removed, cut on a vibrating microtome at 30 µm and the tissue was processed for immunocytochemistry (ICC) using antiserum generated against neurotensin (NT), substance P (SP), somatostatin (SS) or methionine enkephalin (ENK). Indirect immunofluorescence techniques were employed to demonstrate immunoreactive fibers and cells.

Injections that were placed in the median preoptic nucleus-AV3V region resulted in retrograde labeling of a neuronal population in the mid to dorsal aspect of LPBN. In animals that were pretreated with colchicine, many of the retrogradely filled neurons also contained neuropeptide after ICC. Specifically, at rostral LPBN levels, many SP containing neurons were filled with true blue. In addition at mid-AP levels of LPBN, a few ENK containing cells were also retrogradely labeled. In cases in which the animals did not receive colchicine, many of the retrogradely labeled cells were in apparent contact with varicosities of SP, ENK or NT immunoreactive fibers. In contrast, neither SS containing fibers nor cells were associated with the retrogradely labeled cell population.

These data demonstrate that the projections between cardiovascular centers in LPBN and AV3V are, at least in part, neuropeptidergic and underscore the potential significance of these substances in autonomic function.

Supported by NIH grant NS 16107.

178.6 THE SEGMENTAL DISTRIBUTION OF SUBSTANCE P AND NEUROPHYSIN IN THE INTERMEDIATE ZONE OF THE THORACOLUMBAR SPINAL CORD. B.J. Oldfield*, (SPON: B. Livett). Baker Medical Research Inst., Melbourne, Australia 3181.

The innervation of the intermediate zone of the thoracolumbar spinal cord has received considerable attention primarily because it contains the various subpopulations of preganglionic neurons which comprise the sympathetic outflow. The outflow from specific spinal segments, or groups of segments, has long been known to be directed to particular end organs, but more recently evidence has accumulated for an organotopic arrangement amongst autonomic subgroups of individual segments. It is possible that this functional organization, both between and within segments, is reflected in the distribution of descending pathways. The aim of this study is to examine to what extent fibers containing, substance P (SP) or the carrier molecule for oxytocin and vasopressin, neurophysin (NP) are associated with spinal sympathetic neurons (SPNs) and to determine whether these inputs are differentially distributed within the intermediate zone. Antisera to SP and NP were applied to serial horizontal sections of the spinal cords of rabbits, cats and monkeys and were localized using standard immunocytochemical procedures. In some cases, in order to co-localize SPNs and immunoreactive fibers, HRP was applied to parts of the sympathetic trunk - this tissue was examined at the light and EM levels. In general, both NP and SP positive fibers were found in close association with regions known to contain SPNs. The greatest densities of NP and SP fibers were in the intermediolateral nucleus (ILN) of the upper thoracic and mid lumbar segments. When SPNs were localized by application of HRP to the cervical sympathetic trunk there was an excellent correlation of NP fibers and clusters of cells in the ILN of the first two thoracic segments (T_1 and T_2) whereas in T_3 and T_4 some labeled cells appeared to be unrelated to NP fibers. This trend continued in mid thoracic segments where transversely orientated bands of SP and NP fibers were more predominant than those in the ILN and corresponded closely to the mediolaterally-directed groups of SPNs in the intercalated nucleus. Other regions in which immunoreactive fibers were concentrated included the area adjacent to the central canal and the lateral funiculus both of which contain SPNs. These relationships between immunoreactive fibers and SPNs which are being studied further at the EM level provide information as to the relative magnitude of the supraspinal input to different populations of SPNs and may give insights into the nature of their activation.

178.7 VAGAL INNERVATION OF THE POSTEROLATERAL WALL OF THE LEFT VENTRICLE OF THE CAT. R.B. Felder and B.N. Gupta*, Dept. of Internal Medicine and Cardiovascular Research Center, Univ. of Iowa, Iowa City, IA 52242

The cardiac branch of the vagus nerve contains both afferent and efferent fibers which subserve important cardiovascular reflex functions. Cardiac vagal afferents convey information from mechanosensitive and chemosensitive receptors to the central nervous system and thereby reflexly influence autonomic discharge to the heart and circulation. Vagal efferent fibers originating in nucleus ambiguus (NA) and dorsal motor nucleus (DMN) influence heart rate and contractility. Previous anatomical studies have examined the vagal innervation of the whole heart or of regions specifically involved in heart rate control (e.g., right atrium and AV node). We used horseradish peroxidase (HRP) methodology to investigate the afferent and efferent vagal innervation of a localized region of the left ventricular wall. In 5 cats, the posterolateral surface of the left ventricle was exposed and multiple injections (0.5-1.0 µl)of HRP (4-50%) or of the HRP-wheatgerm agglutinin conjugate (4-10%) were made (total volume approximately 20 µl). The animals were sacrificed after 36-72 hours. Both nodose ganglia and the medulla were removed, sliced into 50 µm sections and processed using standard tetramethylbenzidine histochemical methods. HRP reaction product was found in afferent cell bodies in both nodose ganglia and in vagal motor neurons in the medulla in 5 cats and in afferent fibers projecting to the nucleus of the tractus solitarius (NTS) in 3 of these. The cell bodies of cardiac vagal afferents were scattered diffusely throughout both nodose ganglia with no apparent right/left preference and with no specific focus of increased cell density within either ganglion. Vagal afferent fibers projected to medial NTS bilaterally with the greatest density of afferent endings found in commissural nucleus. Vagal motor neurons were found bilaterally in NA and DMN from 1 mm caudal to 2.5 mm rostral to obex, with greater numbers of cells visualized in NA than in DMN, and were distributed ventrolaterally in both nuclei. The data indicate that left ventricular receptors with vagal afferent fibers preferentially innervate a discrete subnuclear region of NTS bilaterally and that the preganglionic innervation of the left ventricle originates from viscerotopically organized cells in both vagal motor nuclei.

178.8 LOCATION AND DISTIBUTION OF PARASYMPATHETIC GANGLION CELLS IN THE RAT HEART. B.J. Pardini, K.P. Patel, P.G. Schmid, and D.D. Lund. Veterans Administration Medical Center and Cardiovascular Center, Department of Internal Medicine, University of Iowa, Iowa City, Iowa 52240.

A detailed description of the locus of termination of cardiac parasympathetic preganglionic neurons complements recent studies in the rat that described the compensatory change in distribution of the cholinergic marker, choline acetyltransferase, before and after unilateral vagotomy (Am. J. Physiol. 236:H620, 1979). Thus, the purpose of the present investigation was to histologically identify and describe the location and distribution of intracardiac ganglion cells in the rat. Rats (300-350 gm) were anesthetized intraperitoneally with sodium pentobarbital and were perfused with an inital rinse of 0.9% sodium chloride followed by 10% buffered formalin. Hearts were excised, post-fixed in buffered formalin for 3-5 days, dehydrated, and embedded in paraffin. Sections were cut transversely through the heart at either 20 or 40 microns. Sections were stained with either Phosphotungstic Acid Hematoxylin, Hematoxylin and Eosin or Cresyl Violet. Ganglion cells could be visualized with all three stains. Ganglion cells were typically round or ovoid (approximately 20 microns in diameter) with large clear nuclei and darkly stained nucleoli. The majority of the neurons were located in aggregates that were interspersed with a network of nerve fibers and supportive cells. The number of neurons per cluster ranged from a few to as many as 80 cells per section. The largest aggregates and majority of all ganglion cells were located on the posterior aspect of the left and right atria and on the interatrial septum at the level of the aortic valves. This bundle of cells was easily located and extended rostro-caudally for approximately 2.5 mm. Smaller clusters of neurons were located either more rostrally around the superior vena cava and ascending and descending aorta or slightly more caudally around the pulmonary vein. Few neurons were located subepicardially. These studies: 1) demonstrate the location and distribution of cardiac ganglion cells and 2) provide landmarks for further electrophysiological and immunohistochemical studies of the postganglionic parasympathetic innervation of the heart. (Supported by Veterans Administration, NIH Grant HL-24246 and NRSA Fellowship GM-09568)

178.9 DISTRIBUTION OF NEUROPEPTIDE Y (NPY)-LIKE IMMUNOREACTIVE NERVES IN THE HEART. R.E. Papka, Dept. of Anatomy, Univ. Kentucky Sch. of Med., Lexington, KY 40536

Peptides belonging to the family of pancreatic polypeptides have been localized in neurons in both the central and peripheral nervous systems. Previous work from this laboratory has concentrated on studies of the localization and distribution of nerves in the cardiovascular system which contain peptides such as substance P and vasoactive intestinal polypeptide, as well as noradrenergic and "cholinergic" fibers. To make the study of the innervation of the heart more complete, an examination of the presence and distribution of NPY-like immunoreactive (NPY-I) nerves was undertaken using guinea pigs. An immunofluorescence procedure was employed using an antiserum generated against purified NPY. The antiserum was applied to formaldehyde fixed whole mounts of tissue and cryostat sections. Tissues examined included the atrial epicardium and myocardium, ventricular epicardium and myocardium, pericardium, atrioventricular valves, ascending aorta, pulmonary artery, and inferior vena cava. A rich supply of NPY-I fibers was present in most areas of the heart. A random plexus was noted in the pericardium and epicardium, however the NPY-I fibers tended to follow the long axis of the cardiac muscle fibers in the atria and ventricles. In the valves NPY-I fibers were dense throughout the cusp and were prominent as well in the chorda tendinae. Immunoreactive fibers formed a prominent plexus of trunks and smaller branches in the adventitia of the large arteries and finer fibers were noted at the adventitia-media boundary. In general the distribution of NPY-I fibers appeared quite similar to that of noradrenergic fibers. Because of this, and the fact that there have been reports of NPY-I in peripheral noradrenergic neurons, we decided to test the effect of the adrenergic neurotoxin 6-hydroxydopamine (6OHDA) on the NPY-I nerves. Treatment with 6OHDA markedly reduced the NPY-I in all areas of the heart. Only an occasional large trunk or single fiber was evident. The function of NPY in the peripheral nervous system has not yet been established. However, one reported effect of NPY is vasomotor activity, and it has been shown to have influences on non-vascular smooth muscle and thus may function in concert with the sympathetic nervous system. These effects could be important in regulation of cardiovascular system function. (Supported by BRSG #RR05374,NIH.)

178.10 MORPHOLOGICAL ALTERATIONS OF THE AUTONOMIC NERVOUS SYSTEM IN STREPTOZOTOCIN-INDUCED DIABETIC RATS. D.D. Lund, K.S.K. Chang*, W.W. Kaelber, and P.G. Schmid. Veterans Administration Medical Center, Cardiovascular Center, and Departments of Internal Medicine, Anesthesiology, and Anatomy, University of Iowa, Iowa City, IA 52240

The deOlmos-Ingram cupric-silver staining method was used to study morphological changes in neural tissues of the autonomic innervation of hearts in streptozotocin diabetic and control rats at various time points up to one year after induction of diabetes. The control autonomic neural tissue was similar in all age groups. Diabetic animals showed progressive, time dependent degeneration in the vagus nerve, nodose ganglion, stellate ganglion, and adjacent sympathetic chain starting one week after induction of diabetes. These early changes included segmentation and fragmentation of the myelin sheaths and some cell destruction. At eight weeks, there was splitting and fiber degeneration of the vagal trunk. The ganglia from this age group contained numerous ghost cells, and an increase in the number of satellite cells. At 24 and 48 weeks, there was more pronounced neuronal and myelin disintergration. Most of the nodose and stellate ganglion cells were replace by Schwann and satellite cells. No changes were seen in the neurons of the dorsal motor, solitary, and ambiguus nuclei of the brainstem. Rats without diabetes due to pretreatment with 3-O-methylglucose did not demonstrate any toxic neuronal changes. Diabetic rats treated with insulin demonstrated preservation of both the large and small sized ganglion cells and myelinated fibers and exhibited a lesser degree of degenerative changes compared to the untreated diabetic animals. These studies demonstrate that streptozotocin produces severe time-dependent structural changes in the peripheral autonomic innervation while the parasympathetic neurons of the CNS remain intact. This toxic effect of streptozotocin can be prevented by pretreating animals with 3-O-methylglucose which prevents the development of diabetes. Insulin treatment appears to protect the animals from the degenerative changes produced by diabetes. (Supported in part by Veterans Administration and HL-24246.)

178.11 EVIDENCE FOR FOUR TYPES OF GLOMUS CELL PARANEURONS IN THE MONKEY CAROTID BODY. J.T. Hansen. Department of Cellular and Structural Biology, The University of Texas Health Science Center, San Antonio, TX 78284.

The cellular organization of the carotid body has been systematically studied in an effort to understand how peripheral chemoreceptors function. Structurally, the mammalian carotid body is composed of glomus cells, supporting cells, nerve endings and a few ganglion cells. However, little is known about the ultrastructural features of the primate carotid body and how its cellular organization compares to commonly used laboratory species such as the rat, rabbit and cat.

In this study, the monkey (M. fascicularis) carotid body was characterized morphometrically at the light and electron microscopic level. Four types of glomus cell paraneurons were identified based upon the size, density and shape of their dense-core vesicles. Glomus cell vesicles ranged from a corrected mean diameter of 131nm to 232nm and were found in cellular densities ranging from 5 to 8 vesicles per μm^2 of cytoplasm. Vesicle densities increased in cellular processes, reaching values of 2 to 3 times that observed in glomus cell bodies. Glomus cells exhibited catecholamine histofluorescence and were seen in both pre- and postsynaptic contact with terminals of the carotid sinus nerve. Glomus cells were seldom in synaptic contact with one another. Nerve endings adjacent to glomus cells averaged $1.3\mu m^2$ in area and contained an average of 34 clear-core synaptic vesicles per μm^2 of nerve ending.

Monkey carotid body glomus cell paraneurons were similar to those of other commonly studied laboratory species but their, presumably catecholamine-containing, dense-core vesicles were somewhat larger and more abundant. Cytoarchitecturally, glomus cells of M. fascicularis most closely resemble those found in the cat carotid body, where norepinephrine is the major glomus cell catecholamine.

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178.12 AUTORADIOGRAPHIC STUDIES OF MUSCARINE RECEPTORS IN HUMAN CEREBRAL ARTERIES. G. Ferrari-Dileo*, D.C. Mash and L.T. Potter (SPON: D.D. Flynn) University of Miami School of Medicine, P.O. Box 016189, Miami, Florida 33101.

Physiological studies of arteries in the pia-arachnoid membrane of laboratory mammals have suggested that adrenergic and cholinergic nerves may help regulate cerebral blood pressure. Cholinergic agonists may induce dilatation of vessels via muscarine receptors in the endothelium, or contraction by interaction with receptors in the muscle coats. Histochemical measurements of acetylcholinesterase indicate diffuse innervation, but electron micrographs suggest that innervation is limited to muscle. Assays of the binding of ^{3}H-quinuclidinyl benzilate (QNB) have suggested very low receptor levels.

We have studied muscarine receptors in human pial vessels for further information about their types and locations, and because of the need for better ways to control cerebral blood flow in such circumstances as stroke and intracranial bleeding. Vessels were obtained 2-8 hours postmortem, and were prepared by dissection, homogenization, sieving and centrifugation. Contamination with brain tissue was well below 1% by microscopic examination, staining and measurements of cerebroside. Assays by a centrifugation method showed about 0.9 pmol receptors for QNB per gram fresh vessels. Low power views of autoradiographs showed receptors in co-register with esterase-stained nerves, in keeping with knowledge of M2 receptors in cholinergic nerves. Higher power views showed muscarine receptors in all but the smallest vessels. The most striking finding was the presence of large islands of receptors in the endothelium. To judge from autoradiography most of the receptors in these vessels are endothelial; studies of whether they are M1 or M2 are in progress. The implication is that conduit vessels in humans are primarily dilated by cholinergic agonists, a point which may prove useful for therapy.

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179.1 PROJECTIONS OF STRUCTURES OF THE LAMINA TERMINALIS TO THE SUPRAOPTIC NUCLEUS IN SHEEP. R.R. Miselis, M.J. McKinley,* J.B. Simpson, M. Leventer,* and D.A. Denton.* Howard Florey Institute, University of Melbourne, Melbourne, Victoria, 3052, Australia.

The central nervous system plays the major role in integrating the various physiological parameters impinging on water balance regulation and in initiating responses to defend body water status. The neural network underlying this function has begun to be described in the rat (Miselis, et al. '79; Miselis, '81). Sheep have also been used in the study of water balance and offer the advantage of a larger brain providing greater anatomical resolution for neuroanatomical tracing studies. Free horseradish peroxidase(Sigma, VI, 30-35%) or conjugates of HRP (wheat germ agglutinin or Cholera toxin) were injected (10-100 nl) unilaterally or bilaterally via glass micropipettes into the supraoptic nuclei or into the structures along the anterior wall of the third ventricle of 17 sheep. They were sacrificed 2 or 3 days later. Their brains were sectioned parasagitally or transversely and reacted according to the protocol for TMB. In two sheep the injection sites involved primarily the SON (confirmed by heavy labeling of the neurohypophysial tract). In these cases there was a high density of retrogradely filled cells in the median preoptic nucleus(MnPo), also called the nucleus medianus. The greatest density occurred below the anterior commissure just beneath the ependyma and particularly at the ventral boundary with the organum vasculosum of the lamina terminalis (OVLT). Similarly labeled cells occurred in the dorsal MnPo, in the subfornical organ (SFO) and in the OVLT proper but in a much lower density. Results from lesion experiments in sheep indicate that the most serious deficits in secretion of vasopressin to osmotic challenges occur with damage confined to the midline structures of the anterior wall of the third ventricle below the anterior commissure. Some cells labeled above probably function in the receptor cell projection circuit controlling the secretion of vasopressin in response to physiological alterations of osmolality. Supported by a Fogarty Fellowship TW00814 and the Howard Florey Institute.

179.2 SUBFORNICAL ORGAN (SFO) CONNECTIVITY EXAMINED USING CHOLERA TOXIN-HORSERADISH PEROXIDASE CONJUGATE. M.L. Weiss, R.E. Shapiro, R.R. Miselis. Dept. of Biology, Animal Biology, Anatomy, and the Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

The SFO, described as part of a neural network underlying fluid homeostasis (Miselis, '81), is known to mediate water drinking, vasopressin release, and centrally mediated pressor response to peripherally circulating angiotensin II. The neural connectivity of the SFO has been examined in the past using a number of techniques including tritiated amino acids, free horseradish peroxidase (HRP), and fluorescent dyes. We re-examined the projections both to and from the SFO area using HRP conjugated to cholera toxin (CT-HRP, made by us) which is more sensitive than free HRP or other conjugates. Rats received pressure injections of 20-100nl of CT-HRP into the SFO and neighboring areas. The TMB protocol of Mesulam ('78) was used (with modifications) for visualization of transported label. We find heavy projections (in descending order of apparent terminal density) to: median preoptic nucleus (MnPo), organ vasculosum of the lamina terminalis (OVLT), supraoptic nucleus (SON), magno- and parvo-cellular portions of the paraventricular nucleus (PVN) of the hypothalamus, anterior periventricular area, PVN of the thalamus, dorsal perifornical area, lateral hypothalamus, lightly to the medial and lateral preoptic areas, and perhaps to a part of the bed nucleus of the stria terminalis. The number of retrogradely filled labelled neurons providing afferent input to the SFO was very small compared to the density of the efferent projections from the SFO. The SFO has predominantly efferent projections and a light afferent input. Situated outside the blood brain barrier, the SFO is in position to function as sensor for humoral signals which is probably its major source of afferent information. Supported by GM 277739, GM 07527, and MH 15092.

179.3 FINE STRUCTURAL ANALYSIS OF THE SYNAPTIC ORGANIZATION OF THE EXTERNAL ZONE OF THE RAT MEDIAN EMINENCE: AXO-AXONIC AND AXO-GLIAL SYNAPTIC CONTACTS. J.P. Card. Department of Neurology, SUNY at Stony Brook, Stony Brook, New York 11794.

Several recent investigations have provided evidence that neuroendocrine regulation of anterior pituitary function may be mediated at the level of the median eminence (ME) via axo-axonic synaptic contacts (see Negro-Vilar, Peptides 3: 305, '82 for review). It has also been suggested that endfeet of tanycytic ependyma may control release of release and inhibiting factors into the primary hypophyseal portal plexus by restricting access of axon terminals to fenestrated capillaries in the ME during various phases of the rodent estrus cycle. In the present investigation the external zone of the ME of adult, female estrus rats was subjected to systematic ultrastructural analysis in order to gain further insight into the mechanisms which control release of neuroactive substances involved in neuroendocrine regulation of anterior pituitary secretion. Reproductive cyclicity of adult female rats maintained in a standardized photoperiod (12 hrs light; light on at 0600) was monitored by daily examination of vaginal smears. Following at least three consecutive reproductive cycles, rats were perfused on the afternoon of estrus with buffered aldehyde solutions and the portion of the hypothalamus containing the ME was processed for transmission electron microscopy. Ultrastructural analysis was restricted to the external zone of the ME, primarily at intermediate levels of the rostrocaudal axis. Two major findings were observed with regard to the synaptic organization of the external zone of the ME. First, axo-axonic synaptic contacts were routinely observed throughout the external zone of the ME. In several instances, an axon terminal was observed synapsing upon another terminal in which vesicles were releasing their contents into the pericapillary space of fenestrated capillaries. Second, numerous examples of synaptic contacts between axons and tanycytic endfeet were observed. These axoglial synapses were present throughout the mediolateral extent of the ME, and it was not uncommon for a single tanycyte process to receive synaptic input from several terminals as it coursed through the external zone. These findings provide direct evidence for presynaptic modulation of the release of neuroactive substances from the ME and also indicate that invasion and retraction of tanycyte endfeet from the vacinity of portal capillaries may be subject to neural control. (Supported by NS-19714)

179.4 PHASIC NEURONS OF RAT HYPOTHALAMIC PARAVENTRICULAR NUCLEUS ARE IMMUNOREACTIVE TO VASOPRESSIN- BUT NOT OXYTOCIN-ASSOCIATED NEUROPHYSIN ANTISERUM. P. Cobbett, K.G. Smithson* & G.I. Hatton, Neuroscience Program, Michigan State University, E. Lansing, MI 48824-1117

Some neurons of the rat hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei fire in phasic bursts in vivo and in vitro, and such activity has, through indirect evidence, been related to vasopressin secretion. In this study we have attempted to determine whether phasically firing PVN neurons are indeed vasopressinergic or whether some may be oxytocinergic. A previous report on SON phasic neurons, while suggestive, did not unequivocally establish the presence of vasopressin **and** the absence of oxytocin in any of the cells studied (Neurosci. Lett. 37, 87-94, 1983). We have intracellularly injected Lucifer Yellow CH (LY) into PVN neurons in hypothalamic slices and used an immunocytochemical technique (J. Neurosci. Meth. 10, 59-69, 1984) that permits visualisation of vasopressin- and oxytocin-associated neurophysin (VP-NP and OT-NP respectively) within a **single** LY filled neuron.

11 cells injected with dye clearly displayed a phasic bursting pattern of action potentials. All phasic activity was spontaneous in that constant current injection was not required to uncover such activity. Burst and interburst intervals ranged from 1.5s to 40s in duration; the overshooting action potentials were 50-80mV base to peak and 56-88mV peak to peak. As previously observed by others in the SON, the duration of action potentials of these neurons tended to increase during the first several spikes of a burst. Except in two cases, action potentials during a burst were superimposed on a plateau potential. Subsequent examination of injected slices using epifluorescence microscopy revealed 8 single neurons and 3 dye coupled pairs of neurons. 6 single and all dye coupled pairs of phasic neurons were immunoreactive to VP-NP antiserum and were not reactive to OT-NP antiserum. Two of the VP-NP immunoreactive cells were in the predominantly oxytocinergic medial PVN while the others were in the predominantly vasopressinergic lateral subnucleus. The other two phasic neurons were not immunoreactive to either antiserum even though there was dense reaction product in neighboring cells. Thus, in the PVN, phasically firing cells are, as expected, predominantly vasopressinergic. There is no evidence for phasic cells containing oxytocin but some may contain some other peptide (CRF?). This research supported by NIH Grants NS16942 & NS01940.

179.5 INTRACELLULAR RECORDINGS AND DYE INJECTIONS OF NEURONS IN THE PERIFORNICAL/LATERAL HYPOTHALAMIC AREA OF THE RAT. G.I. Hatton & P. Cobbett, Neuroscience Program, Michigan State University, E. Lansing, MI 48824-1117.

Axons of vasopressinergic and oxytocinergic neurons of the paraventricular nucleus (PVN) of the rat hypothalamus have been shown anatomically to branch in the area between the PVN and the fornix, and neurons recorded extracellularly in this perifornical/lateral hypothalamic area (PLHA) can be synaptically activated by electrical stimulation of the PVN (J. Physiol. 338, 43P, 1983). The extremely short latency of some evoked PLHA responses, and observations of tissue stained immunocytochemically for enkephalin (Salm & Hatton, unpublished) suggest that some cells of the PLHA project to the PVN. In this study we have made intracellular recordings from and injected Lucifer Yellow CH (LY) into these PLHA cells in vitro to determine some of their electrophysiological and anatomical properties.

Coronally cut slices of male and female rat hypothalamus were incubated in an oxygenated medium. Recordings were made from 27 PLHA neurons in 23 slices. Overshooting action potentials (mean amplitude 52.7 mV, range 40-60 mV, base to peak) were recorded from all neurons of which 19 fired spontaneously. Neurons were injected with LY for 3-28 min with pulsed or constant current (<1.0nA), with appropriate precautions to prevent artefactual dye filling of neurons. Slices were fixed, dehydrated, cleared, and examined using epifluorescence microscopy. Subsequently most slices were sectioned on a freezing microtome, and the sections were examined, after counterstaining with bisbenzimide, to determine whether processes of dye filled PLHA neurons entered the PVN. Of the 27 injections, 17 produced a single dye filled neuron, 8 resulted in a pair of dye filled neurons, and 2 produced a dye filled triplet. In all cases, the somata of coupled neurons were clearly separate. Somatic diameters were 7-21 μm and 2-4 primary dendrites were observed to project from each cell body. In whole slices, in addition to processes projecting dorsally, dorsolaterally, ventrally or ventrolaterally, processes from dye filled neurons were seen to project into the somatic region of the PVN or to pass close to the ventrolateral or dorsal border of this nucleus. In sectioned material, processes from two neurons were confirmed as penetrating the somatic region of the PVN. The chemical identity of the recorded neurons is yet to be determined, as is their previously suggested role in a local neuronal circuit with PVN neurons. (Supported by NIH Grant NS 16942 and an AURIG grant from M.S.U.)

179.6 CHANGES IN THE VENTRAL GLIAL LIMITANS (VGL) SUBJACENT TO THE SUPRAOPTIC NUCLEUS (SON) DURING LACTATION. A. K. Salm, C. D. Tweedle, and G. I. Hatton, Depts. of Psychology & Anatomy & the Neuroscience Program, Michigan State University, E. Lansing, MI 48824-1117

Electron microscopic studies of the SON during dehydration and lactation suggest that, at these times, a retraction of normally interposed astrocytic processes from between the magnocellular neurons there occurs. Light microscopic immunocytochemistry (ICC) for the glial fibrillary acidic protein (GFAP), the subunit of glial filaments (Neurochem. Res. 5:1199, 1980), has shown a dramatic reduction in immunostaining for this cytoskeletal protein within the SON of lactating as compared to estrous animals (Soc. Neurosci. Abst. 9:451, 1983) suggesting that changes in this protein may mediate these apparent morphological changes. Since the SON contains a relatively small number of astrocytic somata (JCN 211:427, 1982) we used two approaches to determine if the astrocytes comprising the VGL subjacent to SON and which contribute processes to that nucleus might undergo changes concurrent with lactation. PAP ICC for GFAP was applied to 15 μm thick sections which were obtained from 3 estrous animals and 3 10-day lactating animals. Photographic negatives of the VGL (18 sections/animal), were taken with a Zeiss microscope. Images from these negatives (final magnification= 460X) were used to measure the depth of the VGL. Analysis of this variable (t-test) showed a reduction in the depth of the VGL as visualized by ICC for GFAP in lactating animals (Control±S.E.= 7.51± .28; Lactating ±S.E.=5.81± .63; $p< .05$). Control measures indicated no differences in staining density elsewhere on the sections. To ascertain what ultrastructural changes might underlie these observations, electron micrographs (12,375X)of the VGL of 3 control and 5 lactating subjects were scored on the incidence of filament bundles/area of VGL. A small, but significant reduction (t-test; Control± S.E.=6.85± .25; Lactating±S.E.=6.37± .13; $p<.05$) was found in the number of filament bundles in the glial processes of the VGL. Taken together, these two lines of evidence suggest that a reduction in the immunostainable and polymerized GFAP occurs in astrocytes of the VGL subjacent to SON during lactation. Since these cells project into the SON, such changes may mediate the glial retraction observed in the SON at this time. (Supported by NIH NS09140)

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179.7 THE AFFERENT CONNECTIONS OF THE PERIVENTRICULAR MEDIAL PREOPTIC NUCLEUS (MPN); A SEXUALLY DIMORPHIC SUBSTRATE FOR OVULATION AND PHASIC GONADOTROPIN RELEASE IN THE RAT. S.J. Wiegand. Dept. of Anatomy, Univ. of Rochester Med. Ctr., Rochester, NY 14642

The MPN, a small periventricular nucleus located at the rostral pole of the third ventricle, is larger and more densely cellular in female brains and shows a clear sex difference in vasopressin and tyrosine hydroxylase immunostaining (Watson et. al., Neurosci. Abstr. 9:454, 1983; Simerly et. al., Neurosci. Abstr. 9:1096, 1983). Lesions of the MPN, but not adjacent preoptic structures, irreversibly block ovulation and the positive feedback action of ovarian steroids on gonadotropin secretion (Wiegand et. al., Neuroendo. 31:147, 1980). In conjunction with studies on mechanisms by which lesions of the MPN might eliminate phasic gonadotropin release, we are studying the neural connections of this area.

Small pressure injections of a wheatgerm agglutinin-HRP conjugate (WGA*HRP; 5-10 nl, 0.5-1.0% sol.) were made into the MPN or adjacent brain areas. Frozen sections were reacted using a tetramethylbenzidine procedure. Injections of the MPN retrogradely label limbic brain areas, including lateral septal nuclei, bed nucleus of the stria terminalis, the amygdalohippocampal area and ventral subiculum. Fewer cells are found in the anterior and medial amygdala and in medial prefrontal and infralimbic cortex. Subcortical nuclei involved in endocrine and autonomic functions are very heavily labeled: within hypothalamus these include the median preoptic nucleus, the subfornical organ, the periventricular area, parvicellular portions of the paraventricular nucleus, the arcuate nucleus, the dorsomedial nucleus and the lateral tuberal area. However, the ventromedial nucleus is nearly devoid of labeled cells. The dorsal part of the suprachiasmatic nucleus is moderately labeled. In the brain stem labeled cells are present in the central gray, lateral dorsal tegmentum, lateral parabrachial nucleus, ventrolateral medulla and medial nucleus of the solitary tract. Scattered cells are present in midbrain and pontine raphe nuclei but the locus coeruleus is unlabeled. Markedly different patterns of labeling are produced, particularly in hypothalamus and brainstem, when WGA*HRP is injected into nearby areas such as the anterior hypothalamus or diagonal band nuclei.

These results indicate that the MPN and adjacent rostral preoptic area are in a unique position to receive and integrate limbic, neuroendocrine and visceral afferent inputs which may participate in the regulation of gonadotropin secretion.

179.8 MAMMILLARY - TELENCEPHALIC INTERRELATIONSHIPS AS DEMONSTRATED BY ANTEROGRADE AND RETROGRADE TRACERS. Mitchell L. Berk. Dept. of Anatomy, Marshall University School of Medicine, Huntington, WV 25704.

The mammalian supramammillary nucleus sends efferent projections to the hippocampal formation. The existence of a more widespread projection to other limbic or non-limbic telencephalic regions (excluding septum) is not known. As part of an on-going comparative study of hypothalamic connectivity in mammalian and avian species, the connections of the mammillary region (MR) to the telencephalon were explored in the pigeon (Columba livia) in the present study.

Efferent pathways were demonstrated by the iontophoretic injection of either WGA-HRP or ^{3}H-leucine into MR-posterolateral hypothalamus. No differences were found in the labeled projections demonstrated by the autoradiographic method and the tetramethlybenzidine reaction for HRP localization. Labeled fibers from MR course rostrally through lateral hypothalamic-preoptic areas and project to the parahippocampal area (APH) via two routes: 1)fibers pass dorsally through the septum, and 2)fibers travel laterally into the telencephalon (at preoptic levels) and course along its lateral and dorsal borders (area corticoidea dorsolateralis), partially circumscribing the telencephalon. Some of the dorsally coursing fibers may terminate on the two cell layers of the hippocampus. Other fibers course rostrally into lobus parolfactorius and stream into hyperstriatum dorsale (HD) of the Wulst, which is a region believed to participate in somatomotor/sensory functions. Fibers leave HD and enter a rostral, dorsolateral telencephalic region (RDLT) lateral to the vallecula.

Injection of WGA-HRP into APH revealed retrogradely labeled cell bodies in the lateral mammillary "nucleus", posterolateral hypothalamus and RDLT. In addition, anterogradely labeled APH fibers project to HD and RDLT.

The projection of the avian MR to a number of interrelated telencephalic sites suggests the possibility of a more widespread projection of this region in mammalian forms. Mammalian homologies of the avian telencephalic recipients of MR projections will not be made until more information is available.

179.9 ESTROGEN-SENSITIVE NEURONS IN THE RAT HYPOTHALAMUS PROJECT TO THE MIDBRAIN THROUGH THE SUPRAOPTIC COMMISSURE.
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Efferent path of 158 neurons in the ventromedial hypothalamus (VMH) was analyzed in urethane-anesthetized ovariectomized female rats. The neurons were identified by antidromic activation as having axonal projection to the mesencephalic central gray at the midcollicular level (CG). The animals had acute knife cut in either of the two major routes taken by descending VMH axons to the midbrain: the periventricular system (PVS) or the supraoptic commissure (SOC). Antidromic spike latency (range: 2.8-48.5 msec) and activation threshold (40-1000 μA) were determined for each response in fourteen PVS-cut (n=80) and sixteen SOC-cut animals (n=78). Data obtained previously from thirty females without the knife cut (n=167)(J. Physiol., Lond. 349, 273) served as control.

The frequency distribution of antidromic spike latency after CG stimulation differed between the groups of animals (Kolmogorov-Smirnov test, $p<.05$). The responses in the PVS-cut animals were fewer at 14-16 msec than in the females without the knife cut; those in the SOC-cut animals responded less frequentry at 17-19 msec (both at $p<.05$). Mean threshold values for the antidromic activation were 398 ± 16 (S.E.M.)μA for the PVS-cut group (n=39) and 367 ± 33 μA (n=31) for the SOC-cut group. Estrogen-supplement to the ovariectomized animals caused no change in the threshold in the animals with the SOC-cut (n=47; 423 ± 23 μA) but significantly decreased the value to 210 ± 26 μA (n=41) in the PVS-cut group ($p<.01$).

CG-evoked potential collided with those induced from the PVS (n=11) or the SOC (n=8), in a manner indicating that the activated axons of VMH cells pass through either of these areas on their way to the CG.

Histological analysis showed that cells with SOC projection were most numerous in the rostral VMH, while those with axons in the PVS scattered through the recorded area.

It is concluded that estrogen specifically decreases antidromic activation threshold in VMH neurons with axons descending in the SOC. Although the mechanism by which estrogen modulates the axonal excitability is unknown, it is probable that this group of VMH cells may somehow participate in the control of estrogen-dependent autonomic or behavioral functions. Supported in part by the Kudo Foundation.

179.10 DORSAL RAPHE NEURONS PROJECTING TO THE HYPOTHALAMUS IN THE HAMSTER. D.B. Michael, J.C. Hazlett & J.A. Mitchell, Department of Anatomy, Wayne State University School of Medicine, Detroit, MI 48201

The raphe nuclei are the major source of serotonergic (5-HT) projections in the nervous system. These extensive projections have been demonstrated by autoradiographic and lesion studies and include a dorsal raphe (DR) projection to the hypothalamus (HY) (Moore '78, Azmetia '78). Recent studies have demonstrated 5-HT terminals in all nuclei of HY (Steinbusch '81) and have implicated 5-HT in neuroendocrine regulation. The purpose of this study is to map DR projections to the HY by retrograde transport of horseradish peroxidase (HRP).

Sigma VI HRP, wheat germ aggulutin bound HRP (WGA-HRP; Sigma) or B subunit cholera toxin bound HRP (BHRP; List Biol) was stereotaxically injected into the HY of 33 anesthetized adult male & female golden hamsters via the transcortical approach. The animals were sacrificed, brains removed, post-fixed & sectioned at 40 um on a freezing microtome. Retrogradely labeled neurons were visualized using the benzidine dihydrochloride technique (BDHC). Controls consisted of uninjected animals & caudate putamen (CP) or hippocampus (HI) injected animals.

The injection site was confined to the HY in 28/33 animals. Retrogradely labeled neurons were observed in the DR in 14/33 animals. Although BHRP and WGA showed smaller injection sites than HRP, all 3 yielded neuronal labeling. Mammillary HY injections with some dorsal and lateral spread revealed labeling in the DR, dorsal tegmental cells in relation to the medial longitudinal fasciculus (DT-MLF), paraventricular n, and reticular thalamic n. Injections confined to the medial tuberoinfundibular HY and those confined to ventromedial n (VMH) yielded sparse but definite labeling in DR. Lateral injections in this region also showed labeling in DT-MLF. Injections in the optic HY, including the suprachiasmatic n yielded neuronal labeling in the lateral central grey rostral to DR; VMH; and the dorsomedial HY where 5-HT neurons have recently been demonstrated. CP & HI injections showed greater numbers of labeled DR neurons than HY injections. This study provides further evidence that 5-HT innervation of the HY is of DR origin although relatively few DR cells may give rise to this projection when compared with other known DR projections.

179.11 EVIDENCE FOR A MASSIVE PROJECTION FROM THE SUPRACHIASMATIC NUCLEUS TO A SUBPARAVENTRICULAR ZONE IN THE RAT. A.G. Watts* and L.W. Swanson. The Salk Institute, La Jolla, CA 92138.

The efferent projections of the hypothalamic suprachiasmatic nucleus (SCh) were investigated using iontophoretic injections of PHA-L into the nucleus. This method (Gerfen and Sawchenko, Brain Res., 290, 219, 1984) allows the visualization of terminal specializations, varicosities, and terminal boutons in anterogradely labeled fibers. The lectin is specifically taken up by neurons surrounding the injection pipette and is not taken up by fibers-of-passage.

Sprague-Dawley rats (250-400 g body weight) were injected in the region of the SCh using a glass micropipette filled with PHA-L. After survival times of 5-10 days, animals were perfused intracardially under ether anesthesia using a pH change fixation procedure. Serial 30 μm thick frozen sections were cut and processed for PHA-L immunoreactivity using rabbit anti-PHA-L serum and an avidin-biotin peroxidase reaction (Vector Labs, Inc.). The peroxidase reaction product was enhanced using osmium tetroxide and thiocarbohydrazide. In animals where the PHA-L was taken up exclusively by cells in the SCh, the major projection appeared to end in a region surrounded by the periventricular zone medially, the anterior hypothalamic area (AHA) laterally, the hypothalamic paraventricular nucleus (PVH) dorsally, and the SCh ventrally. This region extended along the anterior-posterior length of the PVH. Relatively few fibers were seen entering the PVH and these fibers showed few terminal boutons within the nucleus. Fibers throughout the subparaventricular zone were varicose and had many terminal boutons. A few fibers were seen to enter the contralateral SCh, and most of them continued on to innervate the contralateral AHA and subparaventricular zone. However, a few fibers in the contralateral SCh did show restricted terminal arborizations. More sparse projections were seen coursing dorsal to the SCh to innervate the paratenial and paraventricular nuclei of the thalamus. In some animals a sparse projection of fine fibers was also found to project laterally along the ipsilateral optic chiasm.

This study suggests that the major projection from the SCh is to a distinct subparaventricular zone, which appears to correspond at least in part, to vasoactive intestinal polypeptide- and vasopressin-stained fiber systems reported by other workers (Card et al., J. Neurosci., 11, 1289, 1981; Sofroniew and Weindl, Am. J. Anat., 153, 391, 1978)

179.12 EFFERENT CONNECTIONS OF THE BED NUCLEUS OF THE STRIA TERMINALIS (BNST). AN HRP AND AUTORADIOGRAPHIC TRACING STUDY.
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In the framework of a study on afferents to the caudal raphe nuclei, HRP injections were made in the nucleus raphe magnus and adjoining reticular formation. This resulted in labeled neurons in the lateral part of the BNST. Therefore ^{3}H-leucine injections (0,5 ul containing ± 50 uCi) were made in the BNST. The resulting pattern of labeled fibers revealed that the BNST projects to the nucleus accumbens, the diagonal band of Broca and the nucleus of the anterior commissure. Very heavy projections were to the hypothalamus, except its supraoptic, suprachiasmatic and mamillary subnuclei, and to the amygdala (mainly to its central and medial subnuclei but to a lesser extent also to the basomedial amygdaloid subnucleus and the amygdalo-hippocampal transition zone). BNST projections were also present in the paraventricular, rhomboid, parataenial and dorsomedial thalamic nuclei and more caudally in the lateral habenular, parafascicular and subparafascicular nuclei. In the mesencephalon the BNST projected to the pars compacta of the substantia nigra, the ventral tegmental area, the PAG and the cuneiform nucleus. In the caudal brain stem dense BNST projections could be traced in the locus coeruleus, the nucleus subcoeruleus, the parabrachial nuclei and throughout the lateral tegmentum of pons and medulla. In addition they were present in the nucleus raphe magnus and adjoining medial reticular formation, the dorsal vagal nucleus, the solitary nucleus and the marginal layer of the caudal spinal trigeminal nucleus. Labeled fibers could not be observed in the somatic motoneuronal nuclei III,IV,V,VI,VII,X and XII and also not beyond the level of the first cervical spinal segment.

The results indicate that the BNST projections to the brain stem are virtually identical to those derived from the central nucleus of the amygdala (Hopkins and Holstege, Exp. Brain Res. 1978, 32: 529-547).

179.13 ELECTROPHYSIOLOGICAL PROPERTIES OF VENTROMEDICAL HYPOTHALAMIC NEURONS IN GUINEA PIG AND THEIR RESPONSES TO GLUCOSE - A VITRO STUDY. T. Minami*, Y. Oomura, M. Sugimori and R.R. Llinas. Dept of Physiol., Faculty of Med., Kyushu Univ. 60, Fukuoka 812, Japan; Dept of Physiol. & Biophys. New York Univ. Medical Ctr. New York N.Y. 10016, U.S.A.

The Ventromedial Hypothalamus (VMH) is known to play a significant role in feeding, sexual and mating behavior, and is also closely involved in many forms of emotional expressions. Several anatomical and physiological studies have revealed the existance of different types of cells within this nucleus, which might correspond to their diverse functions. Therefore, in order to classify these cell types electrophysiologically, intracellular recordings were made from 45 VMH neurons in slice preparations. Some of them were also tested for their glucose responsiveness.

The value of the resting potential (range 51.4-75mV), the input resistance (range 84.4-355MΩ), and the time constant (range 6-21.4msec) varied from cell to cell. Three types of activities were recognized. The first type (45%) was characterized by a small Ca component when treated with TTX solution, small after hyperpolarization (AHP), high firing rate on intracellular current injection, and easy excitation when stimulated locally at the lateral edge of the nucleus. The second type (22%) showed a larger Ca component and AHP, low frequency firing on current injection and an excitation-inhibition response on local stimulation. The third type (33%) exhibited large hump trajectries, occasional extraspikes, big Ca components, large AHP and high frequency firing on current injection. These observations suggest that the differences in the electrophysiological properties between the first and second type of neurons can be attributed to the Ca component in the spike and to the Ca induced K current. The third type of activity can be thought to arise either from different cells with specific membrane properties or from the dendritic portions of the first and second type of cells.

Fourteen VMH neurons were also analysed for their glucose responsiveness. Three neurons (21%, the second type) depolarized (8-13mV) with a significant increase in their input resistance at high glucose concentration (30mM) and with a mean reversal potential of -89.5mV, as seen in the I-V plot. This potential can be attributed to the decrease in K conductance. Recently our group found similarly responded neurons in the nucleus tractus solitarii and dorsomotor vagal neurons, also.

179.14 DORSOMEDIAL DIENCEPHALIC CONTROL OF JUVENILE PLAY IN THE RAT. S.M. Siviy and J. Panksepp. Dept. Psychology, Bowling Green State University, Bowling Green, OH 43403.

We have reported previously that lesions of the dorsomedial thalamus (DMT) or parafascicular region of thalamus (PFA) reduces play in juvenile rats (Neurosci. Abst. 9:535, 1983). In the following work, we evaluated the behavioral specificity of these effects. Electrolytic lesions of either the DMT or PFA were performed in juvenile Long-Evans rats (21 days old). Pups were housed individually and tested for play, indexed by frequency of pinning, during 5 min observation periods every other day until 46 days of age. Play solicitation was assessed at 32 days of age by recording number or solicitations elicited towards an unresponsive partner (Thor & Holloway, Anim. Learn. Behav. 11:173, 1983). As previously found, DMT lesioned pups pinned 30% less than controls and PFA pups pinned 73% less. DMT pups were also 191% more active than controls. Play solicitations were reduced by 40% in PFA animals, but increased by 23% in DMT lesioned animals. PFA lesions eliminated opioid (naloxone and morphine) modulation of play.

Following play testing, a series of additional control experiments were conducted to assess the behavioral specificity of effects observed. Food intake following deprivation was not different among groups over 3 days of testing. However, following water deprivation, DMT and PFA animals drank 16% and 26% less, respectively, than controls. Intake of a 0.1% saccharin solution did not differ among the groups over a 1 hr period, but 24 hr intake was reduced by 23% in PFA animals. To assess foraging ability, latencies to locate 4 food pellets in a test arena were recorded. All animals displayed systematically faster search strategies over 4 days of testing, although PFA animals were somewhat slower than the other animals.

Taken together, these data argue against a generalized motivational deficit wholly explaining play deficits observed, and suggest that dorsomedial diencephalic areas may form a critical link in the elaboration of juvenile play inrats.

179.15 PLAY IN DECORTICATE RATS. L.A. Normansell* and J. Panksepp (SPON: F.G. DeEskinazi) Dept. of Psych., Bowling Green State University, Bowling Green, OH 43403

Social play is a robust instinctual process. To determine whether neural circuits which control play are subcortically organized, 11 pairs of infant Long-Evans rats were subjected to total aspirative neodecortication at 4 days of age; 12 pairs of littermates served as sham-operated controls. Dams nursed pups till 24 days of age, whereupon all animals were isolated with free access to food and water. Activity measured during this period indicated normal motor development till day 14; subsequently decorticates exhibited more than three times the activity levels of controls, indicating failure of cortical inhibition to emerge. Play was monitored every other day using the paired-encounter procedure (Panksepp, Develop. Psychobiol., 1981, 14, 327) until animals were 33 day of age (play being measured by frequency and duration of pinning). Although play of decorticates exhibited all behavioral components of normal rats, rate and duration of pinning was reduced reliably by half. Play solicitation frequency, however, was normal (albeit not as vigorously and accurately directed). After this, the responsivity of animals to pharmacological manipulations known to modify play was assessed. Decorticates exhibited normal reductions of play in response to 1 mg/kg naloxone (17/22 % reductions: control/decorticates), 0.1 mg/kg scopolamine (39/42 % reductions), and 0.5 mg/kg amphetamine (26/32% reductions). Fenfluramine (2.5 mg/kg) yielded a larger reduction in decorticates (62%) than controls (36%). Morphine (1 mg/kg) elevated play comparably in both. Decorticates paired against controls (matched for weight), were as competitive as normals (by measures of who pinned whom). In general, these results affirm that social play is a primitive instinctual function of subcortical areas of the brain.

179.16 AN ANALYSIS OF THE ORIGINS OF THE CHOLINERGIC AND NON-CHOLINERGIC SEPTAL PROJECTIONS TO THE HIPPOCAMPAL FORMATION OF THE RAT: A DOUBLE LABELING STUDY USING WGA-HRP AND AN ANTIBODY TO CHOLINE ACETYLTRANSFERASE. D.G. Amaral[1], J.Kurz[1] and F. Eckenstein[2]. [1]The Salk Institute, San Diego, CA 92138 and [2]Dept. of Neurobiology, Harvard Med. Sch. Boston, MA 02115.

These experiments were directed at determining the proportion and distribution of cholinergic septal cells which project to the hippocampal formation (HF). Twenty-four rats received injections (40-100nl) of a 1% solution of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) into different levels of the HF. A 1-in-3 series of sections through the septal complex was processed with diaminobenzidine (with $COCl_2$) for the demonstration of retrogradely labeled cells and then processed immunohistochemically by the peroxidase-antiperoxidase method for the demonstration of choline acetyltransferase (ChAT) activity with a monoclonal antibody previously demonstrated to be specific for ChAT (Eckenstein and Thoenen, 1982). The distribution of single (WGA-HRP) and double labeled (WGA-HRP + ChAT) cells was plotted using a computer-based X-Y plotter. We have adopted the nomenclature (Ch1-Ch4) of Mesulam and colleagues for the cholinergic cell groups of the basal forebrain. The medial septal nucleus can be divided into a medial region, which contains few cholinergic cells, and a lateral region in which the Ch1 cell group resides. The cholinergic projection to the HF arises from Ch1 and from the vertical and horizontal limbs of the nucleus of the diagonal band (Ch2, Ch3).

The proportion and distribution of single and double labeled cells is dependent on the site of the WGA-HRP injection. The percentage of double labeled cells throughout the septal complex ranged from 25-70.5% (mean=41.7%) in different experiments. The proportion of double labeled cells in the nucleus of the diagonal band was substantially higher (38-85%, mean=60.7%) whereas the percentage was somewhat lower (26-62%, mean=39.8%) in the lateral half of the medial septal nucleus (Ch1). The septal projection has a mediolateral component to its topography such that only the medial (noncholinergic) portion of the medial septal nucleus projects to the dorsal HF. The Ch1 cell group projects primarily to the mid and caudal levels of the HF. Similarly, the dorsal HF receives a projection from the dorsomedial portion of the nucleus of the diagonal band and more caudal levels of the HF receive projections from progressively more lateral portions of the nucleus.

This work was supported by NIH Grant NS-20004-01 and by grants from the McKnight Foundation and The Sloan Foundation.

179.17 PATTERNS OF 2DG LABELING FOLLOWING UNILATERAL STIMULATION OF THE LATERAL SEPTUM IN RATS. E. Yadin. Department of Psychology, Bryn Mawr College, Bryn Mawr, PA 19010.

Previous research in this laboratory has used unit recordings in freely-moving rats to study the possible role of the septal nuclei in emotion. In an attempt to identify other limbic structures that might be part of the same functional system the 2-deoxyglucose technique was employed. Specifically, this involved mapping of the functional connections activated by unilateral stimulation of the lateral septal nucleus.

Animals were injected intraperitoneally with [^{14}C]2DG and were stimulated via monopolar electrodes at the rate of 22 trains per minute. For 2 of the animals stimulation parameters were 0.5 sec train length at 100 pps, 0.1msec pulse duration and for the remaining 3 rats pulse duration was extended to 1msec. The current intensity used in all 5 animals was 50-60 μamp. Stimulation continued for 45 minutes at the end of which animals were given an overdose of anesthetic and perfused for 30 sec with 3.3% buffered formalin. Brains were removed and frozen in liquid freon and sections taken at 20 μm thickness, dried on a hot plate, affixed to cardboard and exposed for 10 days to an x-ray sheet.

Analysis of the radiographic images was performed at the center for Image Processing and Pattern Recognition at Drexel University. Comparisons were made between uptake of labeled 2DG on the stimulated side of the brain versus uptake on the unstimulated side. Differences between the sides were measured both in relative optical density scores and in absolute concentration of labeled material in tissue. In all animals there was increased differential labeling in the following structures: anterior olfactory nuclei, medial prefrontal cortex, nucleus accumbens, diagonal band of Broca, lateral septal nucleus, medial septal nucleus, basolateral nucleus of the amygdala, subiculum, ventral hippocampus and entorhinal cortex. In some of the animals differences between stimulated and unstimulated sides of the brain were also seen in the pyriform cortex, the medial forebrain bundle and the fornix. Presently, unit activity is being recorded from some of these target structures (e.g., basolateral nucleus of the amygdala) in search of their involvement in emotional behavior and their relation to the septal area.

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179.18 THE INFLUENCE OF CLEAN AND VARIOUS SOILED SHAVING CONDITIONS ON TWO BEHAVIORS OF MICE WITH SEPTAL LESIONS. M. Widmayer-York*, R. G. Burright* and P. J. Donovick (SPON: L.P. Spear). Dept. of Psychology and Center for Neurobehavioral Sciences, SUNY, Binghamton, NY 13901.

Brain lesions of septal nuclei are associated with a number of behavioral alterations including spontaneous alternation deficits and increased social cohesion. Environmental manipulations have been shown to alter the behavior of lesioned animals. Specifically, testing over home cage bedding, for group-housed animals, reduces the septal deficit in spontaneous alternation.

Experiment 1 was designed to clarify whether the shavings containing cues from only the individual test animal or shavings with cues from other mice alter spontaneous alternation behavior of mice with septal damage. Individually housed, genetically heterogeneous male mice, 90-120 days of age were given either large bilateral septal lesions or control surgery. One of 4 possible bedding conditions was placed under the T-maze during testing: clean, home, shavings from another mouse, or combined home + other's shavings. Consistent with previous research employing group-housed animals, isolated mice with septal lesions tested over clean shavings showed a spontaneous alternation deficit when compared to controls and this deficit was alleviated when testing occurred over home shavings. In addition, mice with lesions tested over other and home + other shavings alternated at a level midway between lesioned mice tested over clean and home shavings. The only shaving effect seen in control mice was a slight increase in alternation when tested over other's shavings.

Experiment 2 examined whether the influence of the shavings would generalize to social cohesion. Mice were individually placed in an open field with an anesthetized, unoperated, mouse. The open field was placed over one of the 4 possible shaving conditions. Measures taken included sniffing of, crawling upon and miscellaneous contact with the anesthetized mouse. The most sensitive measure was % of social cohesion (S+) due to sniffing. The expected lesion effect on this measure of social cohesion was greater in mice tested over clean bedding than for mice tested over home shavings. Testing over other or home + other shavings further enhanced this lesion effect when compared to the clean bedding condition. Control mice showed somewhat lower % of S+ due to sniffing when tested over all three soiled shaving conditions than when tested over clean shavings.

179.19 ELECTROPHYSIOLOGICAL STUDY OF CONNECTIONS BETWEEN PUTATIVE VASOPRESSIN CELLS AND THERMORECEPTIVE NEURONS OF THE DIAGONAL BAND OF BROCA/LATERAL SEPTAL AREA. J. Disturnal, W.L. Veale and Q.J. Pittman. Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 4N1.

Anatomical studies indicate that the diagonal band of Broca/lateral septal area (DBB/LS) receives afferents from arginine vasopressin (AVP) containing cells of the paraventricular nucleus (PVN), bed nucleus of the stria terminalis (BST) and suprachiasmatic nucleus (SCN). AVP has been shown to act as an antipyretic agent within this region of the brain. Extracellular recordings were made in the DBB/LS region to determine if cells in this area receive thermoreceptive information as well as afferents from putative AVP-containing cells.

Rats were anaesthetized and implanted with bipolar electrodes in the PVN, BST, SCN, fornix and amygdala. The ventral surface of the brain was exposed and recordings made throughout the DBB/LS. Cells responsive to peripheral thermal input were identified by changes in spontaneous activity in response to thermode-induced changes in the temperature of the scrotum (Ts).

Evoked responses were obtained from 191 cells and classified as antidromic or orthodromic. PVN stimulation elicited 21 antidromic and 53 orthodromic responses (23 excitatory, 30 inhibitory). Stimulation of the BST resulted in 1 antidromic and 29 orthodromic responses (10 excitatory, 19 inhibitory); SCN stimulation was ineffective on DBB/LS cells. Projections were also identified from the fornix (63 orthodromic, 3 antidromic) and amygdala (80 orthodromic, 9 antidromic).

Of 27 neurons tested for their responses to alterations of Ts (10°C-45°C), 3 increased spontaneous firing rate as Ts was raised (warm sensitive), 3 others increased spontaneous firing with decreased Ts (cold sensitive) and 21 cells were not affected by changes in Ts (non-thermoreceptive). One cell displayed a phasic activity pattern characteristic of the putative AVP neurohypophyseal neurons of the PVN. Three neurons receiving afferent thermal information were shown to be orthodromically excited (n=2) or inhibited by BST or PVN stimulation. The observation that DBB/LS cells which participate in thermoregulatory pathways also receive innervation from putative AVP-containing brain nuclei raises the possibility that such cells could play a role in mediating the antipyretic action of AVP.

Supported by MRC. JD is an AHFMR student.

179.20 AXON BRANCHING IN HYPOTHALAMO-HYPOPHYSEAL NEURONS: EVIDENCE AGAINST LONG COLLATERALS IN THE PITUITARY STALK. H.Klemfuss, S.J.Young* and P.M.Groves. Dept. Psychiatry, Univ. Calif., San Diego, La Jolla, CA 92093.

Latency jumping, an abrupt decrease in antidromic latency following a gradual increase in stimulus current, has been cited as evidence for axon branching in several brain regions, including neurosecretory axons originating in the paraventricular nucleus (PVN). However, it is also possible that latency jumping is due to activation of a more proximal site on a single, unbranched axon. The present study used electrophysiological methods to determine whether latency jumping constitutes evidence for axonal branching in the pituitary stalk.

Stimulating and recording electrodes were placed in the pituitary stalk and PVN, respectively, of male rats under urethane anesthesia. The stalk was stimulated with pulses of < 1.0 ma of 0.2 msec duration at 1 Hz to locate PVN cells fulfilling the standard criteria for antidromic activation. In some cells, a latency jump of at least 1.0 msec could be produced with increased current. Thresholds for low threshold, long latency spikes and higher threshold, shorter latency spikes were determined for these cells. Low and high current thresholds, x 1.2, were used to evaluate branching as follows.

The difference in latencies between the spikes initiated by low and high currents was defined as the latency jump time (LJ). Relative refractory period (RRP) was taken as the shortest separation interval between two high current pulses for which two antidromic spikes could be recorded in the PVN. The collision interval (CI) was defined as the minimum interval between a low current pulse and a high current pulse which resulted in both long and short latency spikes in the PVN. In an unbranched axon the CI should be equal to LJ plus RRP. In a branched axon, the CI should equal the time for the first action potential to travel antidromically to the branch point and orthodromically to the spike initiation site on the second branch, plus the RRP. Letting T represent the time required for a spike to travel from the branch point to the initiation site in the second branch, CI = 2T + LJ + RRP. In 12 PVN neurons tested, T = 0.03 ± 0.04 msec. We conclude that latency jumping in the hypothalamo-hypophyseal system occurs in unbranched axons. The existence of latency jumping alone is not sufficient evidence for axon branching without appropriate measure of CI and RRP.

180.1 THE EFFECTS OF MEDIAL PREFRONTAL CORTEX STIMULATION ON HEART RATE IN THE AWAKE RAT. R.R. Terreberry and E.J. Neafsey. Dept. of Anat., Loyola Univ. Med. Ctr., Maywood, IL 60513.

We have recently reported a substantial projection from the rat medial prefrontal cortex to the solitary nucleus (Terreberry and Neafsey, BR 278:245-250, 1983). The present study investigated the functional significance of this projection by monitoring the effects of intracortical microstimulation of the medial prefrontal region on heart rate (HR) in unanesthetized, awake, behaving rats (n=11). Under Nembutal anesthesia each animal had EKG leads embedded into the chest and back musculature, and the leads were led subcutaneously to the back of the neck where they were fixed to the skull with dental acrylic cement. Glass-insulated tungsten microelectrodes were stereotaxically implanted into the cortex (3.0-3.7mm rostral to bregma, 1.0mm lateral to midline, depth of 3.5-4.0mm), and cemented in place. Following a two day recovery period, each animal was placed into a Plexiglas testing chamber and allowed to move freely while the HR was monitored and stimulation delivered via a flexible cable attached to the rat's head. The stimulation parameters were 5 sec trains of negative .5 msec pulses at 25-50 Hz with currents between 50 and 300 μamps. After 2-4 sessions, the animals were perfused and the brains sectioned and stained to reconstruct the electrode tracks.

Of the 11 animals studied thus far, four responded to stimulation with a marked decrease in HR (10-30% below baseline rate), while one animal responded to stimulation with an increase in HR (16% above baseline rate). In 4 animals these responses were obtained with currents of 150 μamps or less. The HR decreases were most obvious when the baseline HR was high (>500 bpm). These HR decreases have also been observed in rats (n=3) anesthetized with ketamine HCl with threshold currents less than 50 μamps; in one animal the threshold current was 10 μamps. The administration of atropine totally abolished the HR decreases. The effective cortical area was localized to the infralimbic cortex (area 25); stimulation of the more dorsal prelimbic region had no effect on HR. The ability of atropine to successfully block the HR decrease suggests that the response may be vagally mediated, with the vagal activation perhaps occurring by way of the direct prefrontal cortex-solitary nucleus projection.

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180.2 CARDIOVASCULAR ADJUSTMENTS ELICITED BY ELECTRICAL STIMULATION OF FRONTAL CORTEX IN CONSCIOUS RABBITS. Shirley L. Buchanan, D. A. Powell, and James Valentine*, WJB Dorn VA Hospital, and University of S.C., Columbia, SC 29201

Stimulating electrodes were implanted in different areas of the frontal cortex in New Zealand albino rabbits. After a two week recovery period the medial ear artery was cannulated and cardiovascular as well as respiratory and electromyographic changes were recorded subsequent to electrical stimulation through these electrodes.

Stimulation at all sites resulted in bradycardia. However, a midline strip beginning at the mid callosal level and extending to the anterior frontal pole and a strip of lateral cortex extending throughout the perirhinal sulcus of the frontal cortex (insular region) resulted in bradycardia which was more long lasting and occurred at lower thresholds than stimulation of other areas. Bradycardia elicited by stimulation of dorsolateral isocortex was accompanied by pressor responses, but midline and perirhinal stimulation always resulted in depressor responses. Responses elicited from more posterior perirhinal regions appeared to be longerlasting and of greater magnitude than that elicited by anterior insular or midline stimulation. Stimulation at all sites was accompanied by increases in respiration rate and decreases in depth. Stimulation of midline cortex and isocortex also resulted in increases in electromyographic activity (i.e., movement), but stimulation of the perirhinal region often did not.

The administration of beta (propranolol) and alpha (phentolamine) adrenergic blockades revealed that the bradycardia elicited by more dorsal isocortical stimulation was due to baroreceptor activation, since alpha blockade greatly attenuated and, in many cases, abolished it. In addition, muscarinic cholinergic receptor blockade with methyl atropine always abolished the bradycardia elicited by stimulation of isocortex. The administration of atropine, in combination with beta adrenergic blockade, revealed that the bradycardia associated with midline and perirhinal stimulation was not entirely due to vagal release, but was partially due to sympathoinhibitory mechanisms. These data thus show that although cardiovascular changes can be elicited from all areas of the frontal cortex by electrical stimulation, the agranular midline and insular regions produce inhibitory changes that are qualitatively different from those elicited from other frontal regions.

180.3 CRYOGENIC BLOCKADE OF THE CENTRAL NUCLEUS OF THE AMYGDALA ATTENUATES AVERSIVELY CONDITIONED CARDIORESPIRATORY RESPONSES. J.X. Zhang and R.M. Harper. Neuroscience Program, Brain Research Institute, and the Dept. of Anatomy, UCLA, Los Angeles, CA 90024.

The central nucleus of the amygdala (ACE) has strong projections to respiratory and cardiovascular control areas in the parabrachial pons and brain stem. Neurons in the ACE exhibit phasic discharge with the cardiac and respiratory cycle, and electrolytic lesions of the ACE abolish the bradycardic response to conditioned aversive stimuli. This study examined the effects of a reversible blockade of the ACE during extinction of an aversively conditioned cardiorespiratory response. Female cats were anesthetized with ethrane, and two cryoprobes were stereotaxically placed into the ACE bilaterally. Electrodes were inserted into the neck musculature, crural diaphragm, orbital plate, and frontal bone to record nuchal EMG, respiratory activity, eye movement, and cortical EEG, respectively. Femoral cannulae were inserted for recording arterial pressure. Cats were subjected to 60 trials of paired tone (1000 Hz, 5 sec) with shock (100Hz, 1 sec, concurrent with tone offset) administered to the nuchal region, which resulted in unconditioned effects of blood pressure rise, bradycardia, and respiratory rate increase. Immediately following conditioning, forty extinction trials were presented. During extinction trials, the ACE was alternately cooled bilaterally (cryoprobe tip temp=0°C, 30 sec) for a single tone presentation, and then permitted to warm for 2 min before the next tone presentation. Cooling of the ACE during extinction trials resulted in a marked diminution of both the cardiovascular and respiratory conditioned effects. The ACE was also cooled during both normal waking and quiet sleep states. Under these conditions, there were no dramatic changes in respiratory or cardiac patterning. These results support the hypothesis that the ACE plays a role in both respiratory and cardiovascular regulation during conditioned aversive responses.

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180.4 LEARNED CARDIOVASCULAR MODIFICATION DURING PHYSICAL EXERCISE IN MACACA MULATTA. M.I. Talan* and B.T. Engel* (SPON: M. Selmanoff). Gerontology Research Center, NIA, NIH, Baltimore City Hospital, Baltimore, MD 21224

In the course of evaluation of extent to which cardiovascular system could be modified by operant conditioning, the possible constraints on the heart rate (HR) response to physical exercise have been studied. Three, chronically chaired monkeys weighing about 8 kg each were trained operantly to slow heart rate using visual feedback and shock avoidance. Once stable performance was achieved, they were trained to lift 8 kg at a rate not less than 18 per min using auditory feedback and the same shock avoidance as in HR training. When the monkeys demonstrated stable rates of weight lifting, then exercise and HR slowing trails were combined so that animals were required to lift the weight and to slow HR at the same time. The combined (C) and regular exercise sessions (E) were run alternately while HR, intraarterial systolic and diastolic blood pressure (BP), O_2 consumption, CO_2 production, and number of weight pulls were recorded continuously. The results showed that during C sessions HR increases were significantly less (sometimes below baseline level) than during E sessions, in spite of sufficient work produced in both sessions (average work performed during each session was 130 - 200 kg/m). Analyses of BP responses, rate pressure products, O_2 consumption and CO_2 production indicated that the cardiovascular performance during C sessions was consistently more efficient--i.e., associated with lower rates of change per unit O_2 consumed--than was cardiovascular performance during E sessions.

180.5 CIRCADIAN PATTERNS OF HEART RATE AND BLOOD PRESSURE IN THE CHAIRED MONKEY. G. Bardos,* M. Talan * and B. T. Engel* (SPON: D.R. Jasinski). Gerontology Research Center, NIA, NIH, Baltimore City Hospitals, Baltimore MD 21224.

Heart rate (HR) and blood pressure (BP) are thought to be stable and highly correlated in various conditions. However, recent data show that in chronically chaired monkeys, there were diurnal changes and consistent differences in the variability of HR and BP, and in the correlations between HR and BP across the day. (Engel, B.T., unpublished observations). The purpose of the present work was to study these parameters in chronically chaired monkeys (Macaca mulatta) during cardiovascular training.

The monkeys were operantly conditioned to control heart rate or systolic blood pressure (SBP). Data were collected also from monkeys that had to control HR during exercise. A shock avoidance paradigm was used for the training (Gottlieb, S.H. and Engel, B.T., Psychophysiology, 16: 528, 1979). HR, SBP and diastolic pressure (DBP) were continuously monitored through an implanted arterial cannula. Records were averaged and recorded in every 5th or 10th min. The booth doors were closed during the training period (between 10:30 and 16:30). The animals were fed about 17:00. A normal light dark cycle was maintained with a 19:00-7:00 dark period. Data were analyzed in groups according the type (workdays or weekends) and period (i.e., morning, handling, training and evening periods, respectively) of the day.

Despite considerable interindividual variability in all of the parameters recorded, our data show the mean values as well as correlations between HR, and SBP and DBP, respectively, were changed by the training. During periods corresponding to handling and training, HR and BP were usually poorly correlated on the weekends. After training progressed, both the correlations and the absolute values of HR and BP became higher. Otherwise, the circadian pattern was not essentially altered. The means and the correlations were especially vulnerable to changes during the training sessions, depending on the type of the training and on the behavior of the animal in the given session. The absolute values of HR, SBP and DBP were changed (generally raised) as a result of the training.

180.6 STATE-DEPENDENT DISCHARGE INTERACTIONS OF NEURONS IN THE INFRALIMBIC CORTEX AND THE CENTRAL NUCLEUS OF THE AMYGDALA WITH THE CARDIAC CYCLE. R. C. Frysinger, R. M. Harper and R. D. Frostig, Dept. of Anatomy and Brain Research Institute, UCLA, Los Angeles, CA 90024

Neurons in the medial frontal (infralimbic) cortex project strongly to areas surrounding the central nucleus of the amygdala (ACE), and the ACE in turn projects heavily to cardioactive areas in the parabrachial pons and brain stem. Neurons in the ACE discharge phasically with the cardiac cycle, and this discharge is altered by sleep state. The objective of this study was to examine the nature of state-related neuronal discharge impinging on the ACE. Adult female cats were anesthetized with ethrane and electrodes inserted for recording lateral geniculate nucleus and frontal cortex EEG, costal diaphragmatic and nuchal EMG and eye movement. Microdrives were implanted to permit bundles of nine microwires to be lowered through the right and left ACE and the left infralimbic cortex. Arterial pressure was monitored continuously through a chronic femoral arterial cannula advanced into the descending aorta. Recording began one week following surgery, and up to four cells were simultanously recorded along with the relevant cardiorespiratory and state assessment parameters. A high percentage of infralimbic cells show a discharge timing relationship with the cardiac cycle across all states, with a higher correlation with the cardiac cycle in quiet and REM sleep than during waking. Unlike cells in most brain areas, infralimbic cortical neurons discharged more rapidly in both quiet and REM sleep than during waking. This higher discharge rate was accompanied by clustered burst-pause discharges. Examination of cross correlations between cells within the infralimbic cortex, and between the infralimbic cortex and the ACE revealed a high degree of common input to entire networks of cells within the infralimbic cortex during sleep, and inhibitory influences of cortical neurons on ACE neurons. We suggest that neuronal discharge in the infralimbic cortex may modulate ACE neuronal activity, that these cortical neurons play a role in cardiovascular control, and that neurons in this cortical area are subject to a common input from another as yet unidentified source during sleep.

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180.7 AMYGDALOID INFLUENCES ON BRAINSTEM NEURONES IN THE RABBIT. J.S. Schwaber, S.A. Turner*, P. Moruzzi*, K.M. Spyer*. E.I. du Pont de Nemours & Co., Glenolden Lab, Glenolden, PA 19036; D-1000 Berlin 65, FRG; Centro Cardiologico, Via Parea 4, Milano 20138; Department of Physiology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF.

The central nucleus of the amygdala (CeN) in the rabbit is known to exert powerful influences on behavior and on the cardiovascular system (Kapp, et al., 1982). In particular, electrical stimulation therein evokes a marked bradycardia and hypotension. Recent neuroanatomical studies have indicated reciprocal connections between this nucleus and the dorsomedial medulla, specifically involving regions of the nucleus of the tractus solitarius (NTS) and dorsal vagal nucleus (DVN) (Schwaber, et al., 1982). The present study has been designed to examine whether these anatomical connections may be involved in mediating the cardiovascular responses observed by stimulating the CeN.

Experiments were performed on rabbits anesthetized with urethane (1.5 g/kg, i.v.) or chloralose (90 mg/kg, i.v.), paralyzed with flaxedil and artificially ventilated. Unit activity in the dorsmedial medulla was recorded extracellularly using glass microelectrodes during electrical stimulation of the aortic (AN) and vagus (VN) nerves. Stimulation within the CeN was effected using bipolar concentric electrodes, firstly to identify points eliciting cardiovascular responses (0.1-0.5 ms pulses, 50-60 μA, at 100 Hz for 5 secs) and, subsequently, to investigate the influence of such stimulation at 1.0 Hz on the activity of medullary neurones.

In total, 97 neurones have been studied in detail; 84 located in the NTS, 13 in the DVN. Twenty-four neurones localized within the NTS were excited by electrical stimulation of the CeN (onset latency 3-24 ms, 19 units; 40-70 msec, 5 units). Ten of these were also excited orthodromically on AN stimulation; 2 on stimulation of the VN; one neurone was excited by AN, VN and CeN. Thirteen vagal efferent neurones in the DVN were identified by their antidromic responses to electrical stimulation of the VN. Two of these were excited also by stimulation of the AN but none were influenced by CeN stimulation.

These results suggest that the cardiovascular responses elicited from the CeN in the rabbit may involve the activation of the central pathway of the baroreceptor reflex through direct action on NTS neurones.

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180.8 **MODULATORY ACTIONS OF THE CAUDAL INFERIOR OLIVARY NUCLEUS ON THE BARORECEPTOR REFLEXES IN THE CAT.** **J.S.Kuo*, R.H. Liu* and C.-T. Yen. Dept of Medical Research, Veterans General Hosipital, VACRS*, and Dept of Zoology, Natl. Taiwan Univ., Taipei, Taiwan, Rep. of China.**

Electrical stimulation of the caudal part of the inferior olivary nucleus (ION) produces cardioinhibition in chloralose-urethane anesthetized cats. This vagally mediated cardioinhibition potentiates reflex bradycardia (RB) induced by systemic phenylephrine and diminishes reflex tachycardia (RT) induced by nitroglycerin (Kuo et.al. Brain Res 177: 373 (1979)). To further elucidate the role of the ION in the baroreflex, glutamate stimulation and electrolytic lesion of the ION were performed in this experiment. 0.2 to 0.3 μl 1M sodium glutamate solution was injected into the ION via a Hamilton syringe. It produced a reduction of heart rate of 30 to 80 beats/min for 5 to 10 minutes. This glutamate-induced bradycardia also potentiated RB and diminished RT. However, 2 mA DC current electrolytic lesion of the ION resulted in no apparent alteration of the basal arterial blood pressure and heart rate. Neither was any significant change in the magnitude and duration of the drug-induced RB or RT observed. These results suggest that the ION may not be a primary component of the neural loop subserving the baroreceptor reflex. The ION can, nevertheless, assume a modulatory role on the baroreflex by way of the vagal nerves.

180.9 RAPHE-SPINAL NEURONS INHIBIT PREGANGLIONIC SYMPATHETIC NEURONS DURING HEMORRHAGIC HYPOTENSION. P.S. Blum and J.A. Spath, Jr.*, Department of Physiology, Jefferson Medical College, Philadelphia, PA 19107

Previous studies in this laboratory suggest that a descending serotonergic pathway inhibits preganglionic sympathetic neurons during hemorrhagic hypotension in the anesthetized cat (Spath and Blum, Adv. Shock Res. 10:111, 1983). Descending serotonergic neurons are located in the medullary raphe nuclei and the link is well established between raphe-spinal neurons and effects upon sympathetic activity (Adair et al, Brain Res., 128:141, 1971). To date, however, little information is available regarding the response of these neurons to hypotension. These studies, therefore, were done to investigate the response of raphe neurons to hypotension in adult cats anesthetized with sodium pentobarbital. In three experiments, multiunit recordings were made from the medullary raphe region during a four hour hemorrhagic hypotension protocol. Two of the three animals showed a direct relationship and the third animal showed an indirect relationship between blood pressure and integrated multiunit activity. In another series of experiments, single unit recordings were made from neurons in the medullary raphe region and these neurons were tested for a response to changes in arterial blood pressure. Recordings from three neuons showed a slowly adapting increase in activity following a nitroprusside-induced depressor effect or a decrease in activity following a phenylephrine-induced pressor effect. Four neurons showed the opposite response to blood pressure changes. These data do not show directly that raphe-spinal nerons inhibit preganglionic sympathetc neuons during hemorrhgic hypotension. They do show that raphe neurons are affected by hypotenion and maintain a response during a prolonged stimulus (up to 90 min). These observations suggest that raphe neuons could produce both inhibition or excitation of sympathetic neurons. Additional experiments are necessary, however to detail the action of these neurons on sympathetic neurns during hypotension. (Supported in part by NIH grant GM 30473)

180.10 CNS-MEDIATED PRESSOR EFFECTS OF CLONIDINIVE IN THE UNANAESTHETIZED RAT F.M.A. Corrêa and J.A.S.Magro* Dept. Pharmacology School of Medicine of Ribeirao Preto, 14100 Ribeirão Preto-SP-Brazil.

The intravenous (iv) injection of clonidine has well known effects on the blood pressure (BP) in anaesthetized rats. The BP response is characterized by a transient rise followed by a long - lasting hypotensive response. The first component is related to a direct action on receptors in the blood vessels whereas the hypotensive response is mediated through central inhibition of the sympathetic outflow. Similar hypotensive responses were observed after intracerebroventricular (icv) administration of clonidine to anaesthetized rats. However opposite effects were observed in unanaesthetized rats. Clonidine caused only long-lasting pressor responses when administered iv or icv to unanaesthetized rats. The pressor responses to icv clonidine were reduced by 50% after hypophysectomy, suggesting the involvement of vasopressin in the mediation. The possible participation of the sympathetic system can be ruled-out since ganglionic blockade with hexamethonium did not affect the pressor response to icv clonidine neither increased the inhibition of the response observed in hypophysectomized rats. Because clonidine has direct effects on the blood vessels and cross the blood-brain barrier, the remaining pressor effect observed in hypophysectomized rats can be due to the leakage of clonidine into the peripheral circulation. The present results indicate the existence of a central pressor pathway involving vasopressin release, activated by clonidine in unanaesthetized rats and silent in anaesthetized ones, in addition to the depressor system evident in the anaesthetized animal.

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180.11 ACUTE CARDIOVASCULAR AND HORMONAL RESPONSES FOLLOWING PREOPTIC PERIVENTRICULAR ABLATIONS. S.L. Bealer. Univ. Tenn. Center for Health Sci., Memphis, TN 38163.

The periventricular tissue surrounding the anteroventral portion of the third ventricle (AV3V) is critical for fluid and electrolyte regulation, and cardiovascular homeostasis. Immediately following electrolytic ablation of this brain region, rats develop an acute natriuresis and increase in blood pressure. Lesions in this brain area subsequently lead to a chronic decrease in plasma and blood volume, and an increase in extracellular fluid volume. The purposes of the present experiments were to characterize the acute effects of AV3V lesions on heart rate, and plasma levels of corticosterone (P_{cort}) and aldosterone (P_{aldo}) during the acute post-lesion natriuresis.

Prior to surgery, all animals were implanted with catheters in the femoral artery. On the following day, rats were anesthetized with ether, and underwent either ablation of the AV3V region, or control surgery. Blood pressure, heart rate, and sodium excretion were monitored for 4 h following CNS surgery. Plasma volume was then determined in some animals by calculating dilution of 125-I labeled serum albumin. Separate groups of rats were decapitated, and trunk blood was collected for determination of P_{aldo} and P_{cort} by radioimmunoassay.

Characteristically, rats with AV3V lesions excreted a greater amount of sodium during the 4 h post-surgery observation period than control-operated rats. Blood pressure and heart rate returned to presurgery levels (108 ± 5 mmHg; 380 ± 10 beats/min) in control operated animals within 1 h following surgery, and remained stable. However, rats which received a lesion of the AV3V region showed a significant hypertension and sustained bradycardia (130 ± 8 mmHg; 290 ± 12 beats/min) following the immediate post-surgery depression in these cardiovascular parameters. Furthermore, rats with AV3V lesions had significantly smaller plasma volume (3.75 ± 0.1 ml/100g BW) and significantly greater P_{aldo} (72 ± 15 ng/dl) and P_{cort} (52 ± 10 ng/ml) than control-operated animals (4.02 ± .1 ml/100g BW;16 ± 4 ng/dl; 23 ± 8 ng/ml, respectively).

These data demonstrate that AV3V periventricular ablation results in acute natriuresis and decrease in plasma volume, accompanied by an increase in P_{aldo} and P_{cort}. Furthermore, the acute pressor response following these lesions is associated with a decrease in heart rate. (Supported by USPHS HL-25877.)

180.12 STIMULATION IN THE DORSAL MEDULLA DECREASES CARDIAC CONTRACTILITY IN RATS.
S.L. Stuesse, E.D. Koval Jr.*, L. Cauller*, and S.E. Fish, Neurobiology Program, Northeastern Ohio College of Medicine, Rootstown, OH 44272.

Cardiac preganglionic parasympathetic fibers originate in two brainstem areas, the nucleus ambiguus and dorsal motor nucleus (DMN). It has been postulated that in cats there is a separation of function such that the nucleus ambiguus influences cardiac automaticity and the DMN controls cardiac contractility (Geis and Wurster, Circ. Res., 46:606, '80). We are studying whether such a separation of function exists in another mammal, a rat.

Anesthetized and beta-blocked Long Evans rats (500-700 gm) were placed in a stereotaxic apparatus. A bipolar pacing electrode was placed in the right atrium through the superior vena cava. A pressure transducer was positioned in the left ventricle through a small intercostal incision. The chest was reclosed, and the animals allowed to breathe spontaneously. The surface of the dorsal medulla was stimulated with monopolar stainless steel microelectrodes (0.2ms square wave pulses, 20/sec, 0.6-5.0 v). Sites of stimulation were later located in frozen transverse brainstem sections with the Prussian blue reaction for iron. Heart rate, left ventricular pressure (VP), and times of stimulation were recorded under paced and unpaced conditions. The VP was digitized and processed on a microcomputer. The maximum dP/dt of the rising phase of the VP curve was used as an index of contractility. Areas were located in the dorsal medulla (from 1200 μm caudal to 200 μm rostral to the obex) which slowed heart rate when stimulated. Under paced conditions dP/dt was markedly decreased when the tip of the electrode was in the DMN nucleus or the medial portion of the solitary complex. This decrease in contractile force was found from 300 μm caudal to 200 μm rostral to the obex and not at more caudal regions. These data indicate that stimulation in the dorsal medulla in rat decreases both cardiac automaticity and force of contraction.

180.13 DISTRIBUTION OF BLOOD FLOW, ARTERIAL PRESSURE AND HEART RATE RESPONSES TO HINDLIMB MUSCULAR CONTRACTION: EFFECTS OF BARORECEPTOR DENERVATION. T.G. Waldrop* and J.H. Mitchell* (SPON: S. Speciale). The University of Texas Health Science Center at Dallas, Dallas, TX 75235

Tetanic contraction of hindlimb muscles of anesthetized cats causes increases in arterial pressure and heart rate; however, distribution of cardiac output has not been determined. Therefore, the purposes of the present study were to measure blood flow to various tissues during muscular contraction and to evaluate if the baroreceptor reflex modulates the cardiovascular responses to muscular contraction. Adult cats anesthetized with a mixture of chloralose and urethane were studied. Radioactive-labeled microspheres were injected through a catheter into the left atrium in order to determine blood flows (ml/g/min) to various tissues during rest and during muscular contraction. A laminectomy was performed to expose the L_7 and S_1 ventral roots which were cut and peripheral ends placed on stimulating electrodes. The ventral roots were stimulated at 40-50 Hz (3 x motor threshold, 0.1 msec. pulse duration) to produce tetanic contraction of the triceps surae muscle group. Developed tension was measured by attaching the calcaneal tendon to a force displacement transducer. Contraction of the hindlimb muscles of cats with intact baroreceptors caused increases in mean arterial pressure (Δ 43 mmHg) and heart rate (Δ 4 b/min). In addition, blood flow during muscular contraction increased to the left ventricle (Δ 0.546 ml/g/min), to the right ventricle (Δ 0.386 ml/g/min) and to the contracting gastrocnemius muscle (Δ 0.065 ml/g/min). Blood flow to visceral organs did not change during contraction of the hindlimb muscles. Mean arterial pressure (Δ 52 mmHg) and heart rate (Δ 18 b/min) responses were slightly greater in baroreceptor-denervated (bilateral transection of the vagus and carotid sinus nerves) animals. However, blood flow responses were similar in both groups. In conclusion, tetanic contraction of hindlimb muscles causes increases in arterial pressure and heart rate which are accompanied by increased blood flow to heart tissue and to the contracting hindlimb muscle. The baroreceptor reflex appears to attenuate the heart rate and pressor responses. However, baroreceptor denervation does not alter the distribution of blood flow occurring during muscular contraction.

180.14 NUCLEUS TRACTUS SOLITARIUS (NTS): DOES IT MASK THE INTERACTION OF BLOOD PRESSURE AND THERMAL ENERGETICS? Doreen M. Fyda and J. Roger Wilson. Department of Psychology, University of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2

Blood pressure (BP) is viewed as generally unresponsive to all but extreme thermal challenges despite evidence that BP and thermoregulation (TR) are co-regulated. Baroreceptor reflexes presumably control their balance. Since baroreflex deafferentation exaggerates vascular responsiveness it may aid studying the dynamics of the underlying BP-TR interactions. BP and metabolic adjustments to mild thermal challenges were assessed following lesions of the mid-NTS the termination site for afferent fibers from the carotid sinus. Accordingly, 16 restraint-adapted rats were shaved and exposed to mild thermal challenges in a ventilated metabolic chamber (Pre-Surgery). Two sessions, each approximately 10 hs in duration were distributed across a 48 h period. In the first session the rats were exposed to successive 90-min bouts of incremental warm challenges (27°, 31°, & 35°C) with interpolated periods of baseline (23°C) temperatures. The second session was identical except that incremental cool challenges (19°, 15°, & 11°C) were used. Half of the animals (N=8) were then given bilateral anodal electrolytic DC lesions of the mid-NTS, while the remaining rats served as Sham-operates. Post-operatively the rats were ventilated acutely on 50-100% atmospheric oxygen. Two weeks later they were chronically implanted with a descending aortic catheter and after 48 hrs recovery re-exposed to the warm and cool challenge sessions (Post-Surgery). Measures obtained during presurgical thermal challenges included Respiratory Quotients (CO_2 production/O_2 consumption), rectal, tail and thoracic skin temperatures. Direct arterial BP and blood pH/PCO_2/PO_2 were also monitored during post-surgical thermal challenges. Results showed that NTS lesions (a) eliminated BP-cardiac reflexes to bolus i.v. injections of acetylcholine and phenylephrine, (b) potentiated *both* phasic-pressor and tonic-depressor adjustments that occurred in response to cool and warm challenges, respectively, (c) inhibited thermal-evoked alterations in expired metabolic gases without altering RQs or body temperatures, and (d) increased sensitivity to heat-induced metabolic acidosis. It appears that the centrally debuffered preparation leads to bidirectional hemodynamic, but suppressed metabolic, responsiveness to thermal challenges. This reifies the notion that in mammals endothermy is a principle factor in the evolution of aerobic capabilities. (Supported by NSERC Grant A6404 to JRW)

180.15 PERIPHERAL VASOPRESSIN: EFFECTS ON FOOD INTAKE, WATER BALANCE, AND BLOOD PRESSURE OF CONSCIOUS, UNRESTRAINED ANIMALS. J.A. Czaja, T.A. McCaffrey*, & E.A. Baronowsky*, Dept. Psych. Sci., Purdue Univ., W. Lafayette, IN 47907.

We have previously found that estradiol stimulation reduces blood pressure, cardiovascular responsiveness, and ingestive behaviors of ovariectomized guinea pigs (McCaffrey & Czaja, Neurosci. Abst. 9, 954, 1983). Since estradiol has been reported to elevate circulating vasopressin, two experiments were conducted to determine the consequences of increased peripheral vasopressin and to evaluate the potential role of vasopressin in mediating the cardiovascular and behavioral effects of estradiol. The first experiment involved tests of conscious, unrestrained guinea pigs (GPs) which had been ovariectomized and implanted with carotid and jugular catheters. Systolic and diastolic pressures and heart rate were measured for 10 sec prior to, and 60 sec following, infusion of 1.6 µg norepinephrine (NE). These measurements were made in triplicate immediately before and at 1, 6, 12 and 24 hours after subcutaneous injections of either 5 units vasopressin tannate in oil (Pitressin, N=7) or the oil vehicle alone (N=7). Surprisingly, vasopressin had no significant influence on resting blood pressures nor on the pressor responses induced by NE. However, vasopressin produced a significant decline in resting heart rate and blunted the NE induced bradycardia. These effects were maximum at 1 hour following vasopressin treatment ($t(12)=4.73$, $p<.001$ for resting heart rate, $t(12)=4.90$, $p<.001$ for bradycardia). To substantiate the ability of the above vasopressin treatment to influence water regulation, an additional 10 GPs received single injections of 0.5 and 5.0 units of vasopressin tannate in oil and oil alone in counterbalanced order. Compared to oil injections, treatment with 5 units of vasopressin significantly reduced 24 hour water intake ($t(9)=2.55$, $p<.05$) and urine output ($t(9)=2.34$, $p<.05$) while producing a moderate, nonsignificant, depression in food intake ($t(9)=2.12$, $p>.05$). In GPs, the finding that estradiol can have independent effects on food intake and water intake has led to the suggestion that these effects are mediated by different mechanisms (Czaja, Butera & McCaffrey, Behav. Neurosci., 97, 210-220, 1983). The present results are consistent with the possibility that vasopressin may mediate the observed effects of estradiol on water regulation, but not the changes in blood pressure and food intake.

180.16 CARDIOVASCULAR RESPONSES FOLLOWING ACTIVATION OF THE SYMPATHETIC NERVOUS SYSTEM AND RENIN ANGIOTENSIN SYSTEM BY INTRAVENOUS HYDRALAZINE IN THE CONSCIOUS CAT. J.W. Hubbard, M.A. Nathan, T.K. Keeton*, and R.A. Buchholz. Dept. of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284.

Hydralazine is a commonly used antihypertensive vasodilator which elicits a reflexively mediated increase in plasma renin activity (PRA), plasma norepinephrine (NE) and epinephrine (E) concentrations, and heart rate (HR). However, few studies have examined the changes in total peripheral resistance (TPR), cardiac output (CO), stroke volume (SV) and HR which accompany these sympathetic and endocrine responses to hydralazine. In addition, there is little information about the effects of hydralazine on mean arterial pressure (MAP) in the conscious, normotensive cat. Thus, this study examined the cardiovascular and neuroendocrine responses to intravenous hydralazine in the conscious cat.

Six cats of either sex, weighing between 3.2-5.9 kg, were instrumented with arterial and venous catheters and an electromagnetic flow probe placed around the ascending aorta. All cats were given a minimum of 7 days of postoperative recovery prior to obtaining any physiological measurements. On the day of testing, the cats were placed in a sound attenuating cubicle and allowed to adapt to this environment for 30 minutes prior to obtaining baseline data. A 5 minute sample of MAP, HR, and CO was obtained and then a 1 ml blood sample was taken for assay of plasma NE, E, and PRA. Hydralazine was then administered (1 mg/kg, i.v., in a 1 ml volume, over a 2 min period) and a 5 minute sample of cardiovascular variables were taken at 15 and 30 minutes post-infusion. Blood samples were also taken at the end of these time periods. This table summarizes the results:

*p < .05; **p < .01	Control	15 min	30 min
MAP (mmHg)	102±5	94±4	97±2
TPR (mmHg·min/ml)	.22±.04	.13±.02*	.12±.01*
CO (ml/min)	474±87	722±95**	810±69**
HR (bpm)	171±10	205±9**	216±11**
SV (ml)	2.8±.6	3.5±.4*	3.8±.4*
PRA (ng AI/ml/hr)	.8±.1	36.5±9.4*	55.4±9.3*
NE (pg/ml)	252±44	1425±388*	1156±143*
E (pg/ml)	88±22	351±134*	406±103*

These results show that in the conscious cat, hydralazine elicits a significant reduction in TPR, which appears to be antagonized by an increase in HR, SV, PRA, and the sympathoadrenal system. Supported by HL 27046, HL 24529, and NIH RR07187

180.17 AREA POSTREMA STIMULATION IN DOGS RELEASES ADRENOMEDULLARY CATECHOLAMINES. K.L. Barnes, K.B. Brosnihan, and C.M. Ferrario. Cleveland Clinic, Cleveland, OH

Previous studies have shown that in dogs electrical stimulation of the area postrema (AP) produces a sympathetically mediated increase in arterial pressure. A direct assessment of the contribution of catecholamine release to this pressor response has not been investigated. The present study examined the effects of stimulation of the area postrema on adrenal medullary catecholamine secretion and hindlimb vascular resistance.

In eight dogs anesthesized with chloralose (66 mg/kg, iv) and pre-medicated with morphine (2 mg/kg im) arterial pressure was monitored via a brachial artery catheter and adrenal secretion rate was assessed in situ. Femoral blood flow was recorded using an electromagnetic flow probe; a nonocecluding catheter was inserted into a femoral vein for sampling of blood. The AP was stimulated continuously for one minute using a teflon-insulated electrode (0.2 msec pulse, 50Hz, and 20, 50, or 80 μA). Plasma samples were analyzed for catecholamines by radioenzymatic assay. AP stimulation at the three current levels resulted in graded pressor responses, associated with graded increases in adrenal secretion of both epinephrine (ESR) and norepinephrine (NSR). Hindlimb vascular resistance (HVR) was not changed, but there was a tendency for the norepinephrine gradient (HNV-A) across that bed to be increased.

	Control	20 μA	50 μA	80 μA
MAP, mmHg *	115 ± 6	127 ± 7	144 ± 7	142 ± 8
ESR, ng/min *	5.7 ± 2.1	7.6 ± 2.2	11.5 ± 5.1	97.6 ± 52.4
NSR, ng/min *	1.4 ± 0.3	1.8 ± 0.3	2.7 ± 1.0	12.9 ± 6.2
HN(V-A), pg/ml	41 ± 26	52 ± 28	176 ± 95	128 ± 80
HVR, units	6.4 ± 2.9	5.7 ± 2.1	4.1 ± 1.0	4.8 ± 0.9

Mean ± SE; * $p < 0.01$ by analysis of variance.

The data showed that activation of preganglionic spinal sympathetic pathways during electrical stimulation of the area postrema causes increased secretion of norepinephrine and epinephrine from the adrenal medulla. The tendency for femoral vein norepinephrine to increase above base line values suggests that electrical stimulation of the AP facilitates neuronal release of the transmitter in this bed. A counteracting effect of adrenal epinephrine might explain the failure of AP stimulation to increase HVR. (Supported by NHLBI grant, HL-6835).

180.18 VENOUS AFFERENT ELICITED POSTSYNAPTIC POTENTIALS IN TRICEPS SURAE MOTONEURONS. F.J. Thompson and B.J. Yates. Dept. Neurosci., Univ. of FL Coll. of Med. and Vet. Med., Gainesville, FL 32610.

Recently, some details of the electrophysiological properties (Thompson and Barnes, 1979), CNS projection patterns (Thompson et al., 1980), and sensory properties (Thompson et al., 1983) of limb venous afferents were presented. These studies have indicated that limb venous afferents include Group II afferents which are activated by low amplitude distentions of the femoral-saphenous vein wall. Reflex connections of limb venous afferents to lumbar skeletal muscle motoneurons were revealed by ventral root recordings and recordings of electrical activity of hindlimb skeletal muscles (Thompson et al., 1982).

The purpose of the studies reported here was to investigate postsynaptic potentials in identified triceps surae motoneurons elicited by limb venous afferent stimulation. These studies were performed on adult decerebrate, spinal cats. Intracellular potentials were recorded using 6-10 M glass pipette microelectrodes filled with 3 M KCl.

These studies revealed that brief, low intensity stimulation of femoral venous afferents elicited postsynaptic potentials in the triceps surae motoneurons. The minimum latency of these potentials was 7.5 msec. The potentials were complex waveforms composed initially of serial EPSPs. The peak of the initial EPSP occurred within 4 to 5 msec from onset. The appearance of additional serial EPSPs began on the falling slope of the initial EPSPs and extended the entire excitatory period for 25 to 30 msec. The amplitude of the EPSPs, elicited by stimulation twice threshold for the most excitable fibers, was 1 to 2 mV. The excitatory potentials were terminated by the onset of an IPSP which reached a peak amplitude of 1 to 3 mV. Although the precise onset latency of the IPSP could not be clearly distinguished, the peak occurred 50 to 60 msec following stimulation.

The authors propose that the venous afferent modulation of triceps surae motoneuron excitability indicated in this and previous studies is consistent with a venous afferent contribution to skeletal muscle tone. This veno-somatic reflex is suggested to provide a neural substrate for reflex increases in skeletal muscle tone to resist orthostatic blood pooling in leg skeletal muscles. (Supported by NIH ROI HL 25619, and Air Force College of Aerospace Medicine F33615-82-D-0627).

180.19 EFFECTS OF VARIOUS CALCIUM CHANNEL ANTAGONISTS ON ALPHA$_1$-ADRENERGIC RECEPTOR MEDIATED PHOSPHOLIPID SYNTHESIS AND CONTRACTILE FORCE IN THE RABBIT EAR ARTERY. Thomas L. Smith and Sue Piper Duckles, Vet. Adm. Med. Ctr., Tucson, AZ 85723 and Dept. of Pharmacol., Univ. of Arizona, Tucson, AZ 85724.

Receptor-mediated phosphatidylinositol (PhI) turnover is closely associated with Ca^{2+} influx and intracellular Ca^{2+} mobilization in a large number of tissues. The precise nature of the linkage of PhI turnover to Ca^{2+} signalling however, remains elusive. Recently, we characterized the PhI response to a variety of hormones in the rabbit ear artery (Smith and Piper-Duckles, Fed. Proc. 42:650, 1983) and demonstrated a close correlation between the ability of a given drug to stimulate PhI turnover and its ability to develop contractile force. Since receptor-mediated vascular smooth muscle contraction is Ca^{2+} dependent, it was of interest to determine the effects of Ca^{2+} channel antagonists such as the dihydropyridines (lidoflazine, nicardipine, nitrendipine) and diltiazem on receptor-stimulated ear artery contraction and PhI turnover. Aliquots of minced arteries (3-5 mg) were incubated with [^{32}P] and indicated drugs in a buffered physiological cell solution for 1 hr. Normal [Ca^{2+}] was 2.5 mM. Phospholipids from each sample were extracted, isolated by TLC and [^{32}P] incorporation quantitated. Under normal Ca^{2+} conditions, maximally effective norepinephrine (NE) ($1x10^{-5}$M) elicited a 4-5 fold increase in [^{32}P] incorporation into PhI. Omission of Ca^{2+} from the medium had no effect on the magnitude of the PhI response to NE. However, under the same conditions the NE stimulated contractile force was greatly reduced. Basal incorporation of [^{32}P] into PhI was unaffected in the presence of either lidoflazine, nicardipine, diltiazem, or nitrendipine ($1x10^{-6}$M). At this concentration, nicardipine and nitrendipine significantly reduced the magnitude of the PhI response to NE ($1x10^{-5}$M), whereas diltiazem and lidoflazine had no effect. Similarly, the contractile response to NE was significantly inhibited by nitrendipine ($1x10^{-6}$M) but not by diltiazem. It is concluded that the PhI response to NE is independent of Ca^{2+} influx and that nitrendipine and nicardipine may be acting at other membrane sites in addition to the Ca^{2+} channel. (Supported by the American Heart Association, Arizona Affiliate and by the Veterans Administration).

180.20 THE NATURE AND MECHANISMS OF NEURALLY-MEDIATED RELAXATION RESPONSE OF RAT MESENTERIC ARTERY. M.S.Kannan* & A.E.Seip* (Spon. G. Rajakumar*). Neurosciences Department, McMaster University Health Sciences Centre, Hamilton, Canada.

Field stimulation (FS) of segments of rat superior mesenteric arteries results in two types of mechanical events: an excitatory (contractile) and an inhibitory (relaxation) response. Previous studies demonstrated that the excitatory phenomenon was predominantly adrenergic in nature. In the present study, we investigated the nature as well as the mechanisms of the inhibitory response to FS. Arterial segments were maintained at their optimal calibre in vitro at 37°C in a Physiological Salt Solution between a pair of platinum electrodes. FS at optimum voltage, 0.5 ms pulse duration, 10s pulse train, and at frequencies ranging from 1 to 15 pps resulted in graded relaxations that were sensitive to tetrodotoxin (0.1ug/ml) and guanethidine (10uM) (n=6). Norepinephrine (NE, 10uM) maximally relaxed these arteries and the response to FS at 15 pps was 62±3% of the NE maximum (n=4). Propranolol (3.3uM) and atropine (3.3uM) had no significant effects on the size of the inhibition (n=4). Prior exposure to indomethacin (3uM), however, resulted in partial inhibition of the relaxation to FS (44±5% inhibition, n=5). Naloxone (3uM), a non-selective opiate antagonist, also inhibited this relaxation (59±8% inhibition, n=3). Substance P (up to 1uM) did not alter the tone of the vessels, but partially inhibited the relaxation to FS at all frequencies studied (44±9% inhibition, n=3). Exposure to neuronal (cocaine, 1 to 10uM) and extraneuronal (estradiol-17β, 3uM) uptake blockers did not alter the amplitude as well as the time course of the relaxation to FS. The results of these preliminary studies are interpreted as follows: (i)the relaxation to FS is neural in origin and dependent on the activity of sympathetic nerve-endings, but not on uptake mechanisms for termination of effects; (ii)it is non-adrenergic and non-cholinergic in nature and may be mediated by endogenous opiates; (iii) prostanoids appear to mediate and/or modulate a component of the inhibition; and (iv)substance P may modulate release through a presynaptic mechanism, although a direct smooth muscle effect cannot be ruled out.

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180.21 THE RADIOMETRIC EXAMINATION OF VASCULAR EVENTS.
A. J. Tahmoush, M. Zuzu*, Dept. of Neurology, Hahnemann University, Philadelphia, Pa. 19102.

Radiometric techniques provide a simple, inexpensive and indirect measure of arterial and microvascular events. However, the physiological basis for these signals has not been established. Therefore, an in vitro system was designed which included a Harvard Model 931 Infusion Pump, Statham P23AA Pressure Transducer, Biotronex Electromagnetic Blood Flowometer, and either a rigid glass cell or compliant artery. The rigid glass cell served as a simple model of transparent microvessels. Pulsatile Ringer's lactate or human blood flows between 12 - 84 cc/min were employed. In the artery studies, the radiometric signal increased as the pressure and flow increased. The signals were similar for Ringer's lactate and human blood. In the glass cell studies when blood was employed, the radiometric signal decreased as the flow and pressure increased. Linear regression analysis between the radiometric signal and both flow and pressure signals showed a high correlation ($r>0.85$) No change in the radiometric signal occurred when Ringer's lactate was employed as the solution.

These studies indicate that the radiometric signal is a useful index of arterial or microvascular pressure and flow changes. They also suggest that backscatter from the opaque artery wall is the predominant source of the arterial signal whereas changes in the optical properties of flowing blood is the predominant source of the microvascular signal.

SENSORY TRANSDUCTION

181.1 MOLECULAR ANALYSIS OF A VISUAL MUTATION IN DROSOPHILA.
F. Wong,* K.M. Hokanson* and L.T. Chang*. (SPON: K.W. Yau). Marine Biomedical Institute & Dept. Physiol. Biophys., Univ. Tex. Med. Br., Galveston, TX 77550-2772.

Of the many visual mutations in Drosophila melanogaster thus far isolated, a small number of them are thought to affect intermediate steps of phototransduction. To understand the link between these mutations and their physiological effects, it is necessary to study the genes and their products. Therefore, we have attempted to isolate and to characterize the transient receptor potential (trp) gene. Mutations in this gene are known to cause a reduction in the rate of occurrence of quantum bumps which are the unitary responses to single photons.

The trp mutation is fully recessive and has been mapped to bands 99C5-6 on the third chromosome. This limits the location of the trp gene to a region consisting of about 60 kb of DNA. A previously cloned DNA segment has been found to hybridize to the trp region as demonstrated on chromosome squashes. Using fragments of this cloned DNA as probes to select overlapping DNA segments from a genomic library, we have cloned a stretch of DNA which includes the trp gene.

The stretch of DNA is found to contain genes that encode for three major mRNA species. One of these mRNA species is missing in the mutant. On the other hand, no obvious specific change in DNA has been identified in the mutant based on analyses of restriction patterns generated by endonucleases.

These results would suggest that the trp mutation alters the DNA sequence in a small region containing signals necessary for the normal expression of the gene or for the processing of the mRNA. Accordingly, the defective receptor potential observed in the mutant may be due to the lack of a protein that is important to the occurrence of quantum bumps.

181.2 INVERTEBRATE PHOTORECEPTORS THAT MAY SIGNAL THE WAVELENGTH OF LIGHT.
Robert S. Schehr* and Eduardo R. Macagno. (SPON: E. E. Holtzman). Dept. Biol. Sciences, Columbia University, New York, N.Y. 10027

Compound eye photoreceptors of Daphnia magna fall into at least three spectral classes (maximum sensitivities approximately at 450, 510 and 590nm--Schehr and Macagno, Neurosci. Abst., 9, 98.9, 1983). The responses of these cells are not, however, univariant. The most striking phenomenon is a hyperpolarization that, for example in the 510nm class, can be seen at wavelengths longer than 510nm. At low intensities, at these wavelengths, the response is purely hyperpolarizing. As intensity is increased, a positive-going component arises and ultimately the only remnant of the hyperpolarization is a notch at the leading edge of a depolarizing response. The saturating depolarization, at these wavelengths, is smaller than at wavelengths where there is no hyperpolarization.

A possible source for the hyperpolarization within the recorded photoreceptor is the photointerconvertible rhodopsin-metarhodopsin system responsible for both the late receptor potential and the prolonged depolarizing afterpotential (PDA). The action spectrum of the back reaction (metarhodopsin to rhodopsin) and, hence, the shutting off of the PDA is often different from that for the forward reaction (a species dependent property). The shutting off of the PDA could be seen as a wavelentgh-dependent hyperpolarization. However, this seems to be ruled out since, after the stimulus is turned off, the cell returns to its original resting potential instead of relaxing to a new level as would be the case if a PDA had been suppressed.

We think that other photoreceptors belonging to a spectral class different from that of the recorded cell are the ultimate sources of the hyperpolarization. At least two mechanisms, both providing the necessary sign inversion, seem possible: chemical synaptic and a scheme in which return photocurrent is constrained, by the existence of high resistance barriers, to flow through photoreceptor axons. This latter scheme has been proposed as the explanation for apparently similar hyperpolarizations seen in the compound eyes of a locust and a butterfly (Shaw, Nature, 255, 480, 1975; Horridge, et al., J. Comp. Physiol., 150,271,1983). But in all of these cases, including Daphnia, the physiological preparations leave synaptic connections intact and in Daphnia synaptic inputs onto photoreceptors have been seen in the electron microscope (Macagno et al., PNAS,70,57,1973). Experimental tests of these two hypotheses are under way.

181.3 LUMINANCE INCREMENT SENSITIVITY OF THE LOCUST PHOTORECEPTOR. L.R. Owens*, T.E. Cohn. Univ. of Cal. School of Optometry, Berkeley, CA 94720.

This study investigates the role of the steady state membrane noise during light adaptation for locust photoreceptors. Intracellular recordings of the mean and variance of the receptor potential are measured during stepped increases in background intensity. As the background level increases, the standard deviation (S.D.) of the potential increases, reaching a maximum of 2.75mV near the linear range of the cell's V/log I curve. At higher intensities, the noise has more power at higher frequencies, but the amplitude of the noise decreases.

The photoreceptor's V/log I curve was found at each level of increased background intensity using increment and decrement stimuli. For the dark-adapted cell, the dynamic range is on the order of 5 log units of stimulus intensity. The results from the V/log I curves are used to generate an increment-threshold curve, a plot of log threshold vs. log background intensity. When a fixed criterion equal to 2.75mV is used at each background level, there is no effect on the increment threshold for dim background levels. When the background is increased further, the log increment threshold rises linearly with log light intensity, obeying Weber's law: $\log \Delta I = k \log I$, where $k=.9$. When the measured S.D. of the membrane potential is used to assess the threshold, threshold rises linearly but with a slope closer to .6 than to .9.

To conclude, when a fixed criterion is used to determine threshold, the receptor's sensitivity is under-estimated at both low and high background intensity levels. An assumed fixed criterion has the effect of making the slope appear to lie closer to 1.0 than it actually does, obscuring effects that could otherwise be attributed to quantum fluctuations. The measured S.D. values should be used in adopting threshold criterion to ensure an accurate representation of the underlying processes which limit the sensitivity in the system.

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181.4 RECTIFYING PROPERTIES OF THE LIGHT-ACTIVATED CONDUCTANCE OBSERVED AT THE MICROSCOPIC AND MACROSCOPIC CURRENT LEVEL IN LIMULUS VENTRAL PHOTORECEPTORS. K. Chinn*, J. Bacigalupo*, and J. E. Lisman. (SPON: E. Silinsky). Dept. of Biology, Brandeis University, Waltham, MA 02254.

It has previously been shown in Limulus ventral photoreceptors that light causes the opening of ionic channels whose single-channel conductance (~40 pS) is constant over the voltage range -80 - +50 mV (Bacigalupo and Lisman, Nature 304: 268, 1983). We found that the probability of a channel being in the open state is nearly constant at potentials more negative than reversal potential ($V_{rev} \sim +10$ mV) but becomes highly voltage-dependent at potentials positive of V_{rev}, increasing 7-fold at potentials within 30 mV positive of V_{rev}. We examined whether the macroscopic currents showed a similar rectification. Cells were voltage-clamped at a holding potential of -70 mV and were depolarized to a variety of different potentials. At each potential, the cells were exposed to a 100 ms light flash which occurred 200 ms after the voltage step onset. The shape of the light response changed with voltage, becoming greatly prolonged at potentials positive of V_{rev}. Because of this, we used the total charge moved rather than the peak current amplitude as a measure of the light response size. We normalized this charge movement by the driving force and have given it the name g'. At potentials negative of V_{rev}, g' was almost constant while at potentials within 30 mV positive of V_{rev} g' increased between 3 and 20-fold for 7 different cells. This is within the same range as the increase in the probability of a single channel being open. Large increases in the light-activated conductance at potentials positive of V_{rev} have also been shown in vertebrate rods (Baylor and Nunn, Biophys. J. 41: 125a, 1983) and barnacle photoreceptors (Brown et al., J. Physiol. 208: 385, 1970). We examined whether divalent ions were important in this rectification by lowering external divalent ions (from Ca = 10mM, Mg = 48mM to Ca = 0.3mM, Mg = 3mM). Rectification still occurred but in this case at potentials positive of V_{rev} g' reached saturation, which was never observed in normal Ringer (ASW). Thus, we have found that rectification is dependent on the gating properties of the channel. While these gating properties seem to be influenced by divalent ions, they are not dependent on them.

181.5 OCTOPAMINE AND cAMP ANALOGUES PARTIALLY REPRODUCE A CIRCADIAN CLOCK'S EFFECT ON LIMULUS PHOTORECEPTORS. Leonard Kass, Janice L. Pelletier, George H. Renninger and Robert B. Barlow, Jr. Eastern Virginia Medical School, Norfolk, VA; Albert Einstein Coll. Med., Bronx, NY; University Guelph, Guelph, ONT; Syracuse Univ., Syracuse, NY.

A circadian clock in the Limulus brain transmits optic nerve activity to the lateral eyes at night. This efferent activity increases retinal sensitivity by changing the anatomy and the physiology of the photoreceptors (Barlow et al, 1980, Science 210:1037). Octopamine is a putative transmitter of the clock's action. (Kass and Barlow, 1984, J. Neurosc., in press) When injected into the lateral eye, octopamine increases cAMP levels (Battelle et al, 1982, Science 216:1250). We further investigated the possible roles of cAMP and octopamine by observing effects of various agents on intracellular photoreceptor recordings.

Photoreceptor potentials were recorded from retinal slices maintained in an organ culture medium (Bayer and Barlow, 1978, J. Gen. Physiol. 72:539-564). These potentials were similar to those recorded in situ during the day: they had large resting potentials (-60mV) and they manifested (dark) spontaneous and light-evoked potential fluctuations (quantum bumps up to 50mV). When octopamine (200uM) was added to the bathing medium, physiological changes induced were characteristic of those seen when recording in situ at night; i.e., spontaneous quantum bumps were reduced in frequency and the slope (gain) of the intensity-response function was increased (Kaplan and Barlow, 1980, Nature, 286:393). Other pharmacological agents which also increased photoreceptor sensitivity by increasing signal (gain) and decreasing noise (spontaneous quantum bumps) were: naphazoline (a potent octopamine agonist; 25uM), forskolin (a putative adenylate cyclase activator; 250uM), and dibutyrl-cAMP or 8-bromo-cAMP (cAMP analogues; 250uM).

A possible scheme: a circadian clock in the brain releases octopamine at efferent synaptic terminals on photoreceptor cells. Octopamine activates adenylate cyclase which increases intracellular cAMP and triggers changes in photoreceptor anatomy and physiology. According to this scheme, the efficacy of cAMP action is not dependent upon the specific identity of the endogenous efferent neurotransmitter(s).

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181.6 SEROTONIN REDUCES K CURRENTS AND ENHANCES A Ca^{2+} CURRENT IN HERMISSENDA TYPE B PHOTORECEPTORS. R. Wu and J. Farley. Princeton Univ., Princeton, NJ (Spon: K. Jennings).

Farley & Alkon (this volume) have documented long-term increases in a voltage-dependent Ca^{2+} current (I_{Ca}) in Type B photoreceptors produced by associative training. Unlike the associative reductions in I_A (Alkon et al., Science, 1982) or I_C (Farley & Alkon this volume) which have been linked to increases in intracellular calcium (Ca_i^{2+}) during training, I_{Ca} is relatively unaffected by physiological increases in Ca_i^{2+}. Here, we suggest that serotonin, or a similar neuromodulator, may play a role in training-produced changes in I_{Ca}, I_C, and I_A.

5-HT (.1mM) in normal ASW results in a pronounced enhancement of the peak and steady state light response of the ligated Type B. The enhancement persists for at least 45 minutes following extensive washout of 5-HT, and reflects the combined result of three separate effects of 5-HT: 1) reduction of two outward K^+ currents (I_A and I_C), 2) enhancement of I_{Ca}. In the unclamped B cell, block of the delayed rectifier (I_K) by the addition of 100 mM TEA to the bath failed to abolish the enhancement of the steady state light response by 5-HT (pre: 19.0 ± 1.46 mV; post: 22.9 ± 1.69; $t(8) = 5.03$; $p < .01$), indicating that 5-HT's effect is largely independent of I_K. In contrast, block of the fast, rapidly inactivating K^+ current (I_A) by the addition of either 1 or 10 mM 4-AP prevented the 5-HT enhancement (pre: 25.68 ± 1.45mV; post: 25.72 ± 1.85). Thus, a major portion of 5-HT's effect upon the light response is mediated by reduction of I_A.

Voltage-clamp results for the B cell confirmed these hypotheses. I_A was isolated by the addition of 100mM TEA to the bath and by substituting 10mM Ba^{2+} for Ca^{2+} (to minimize I_C). Under these conditions 5-HT reduced peak A current amplitude at 0 mV in 9 of 9 cells tested (10.85 vs. 5.14 nA; $T(8)=7.39$). Isolation of I_{Ca} was achieved by a bath comprising 10mM 4-AP; 100mM TEA, 10mM Ba, and 300mM K^+ ($E_K=0$). Steps from $V_h=-60$ to 0 mV (E_K) revealed the presence of a net inward current which is blocked by cadmium. Addition of 5-HT enhanced the magnitude of this inward current in 5 of 5 cells examined (1.85 vs. 3.50 nA; $T(4)=8.12$). I_C was isolated by blocking I_A (10 mM 4-AP), I_K (100 mM TEA), and by increasing (100 mM) external Ca^{2+}. Addition of 5-HT reduced I_C in 6 of 6 cells (13.42 vs. 8.42 nA; $T(5)=4.26$).

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181.7 CUMULATIVE DEPOLARIZATION OF HERMISSENDA TYPE B PHOTORECEPTORS: IONIC BASIS AND THE ROLE OF CALCIUM. L. Grover & J. Farley (Spon: M. Lampert) Princeton Univ., Princeton, NJ 08544

Pairing-specific long-term reductions in a fast, rapidly inactivating K^+ current (I_A) [Alkon et al., Science, 1982] and a calcium-activated K^+ current (I_Q) [Farley & Alkon, this volume] have been reported for Hermissenda Type B cells following associative training. Increased intracellular calcium levels (Ca_i^{2+}) has been suggested to cause the reduction of K^+ currents and to play a role in the cumulative depolarization and decreased conductance that B cells exhibit during acquisition of learning. We have asked: 1) To what degree does cumulative depolarization of B cells occur in conditions which minimize A and C current? 2) How strict is the requirement for a transmembrane flux of Ca^{2+} for cumulative depolarization?

Ligated B cells were exposed to an in vitro simulation of associative training which entailed five paired, or unpaired, presentations of light and depolarizing (Δ +15 mV) current stimulation. In ASW (10 mM Ca^{2+}), B cells exhibited a pairing-specific cumulative depolarization [2.0 min post training: paired = 5.83 ± 1.74 mV vs. unpaired = 3.17 ± 1.13 mV; T(5) = 2.27; $p < .05$]. When trained in a solution which blocked I_A (10 mM 4-AP) and minimized other K^+ currents (100 mM TEA; 10 mM Ba^{2+}; 0 Ca^{2+}), B cells exhibited a pairing-specific cumulative depolarization here as well [paired: 4.20 ± 1.23 mV vs. unpaired: 1.20 ± 2.83 mV] whose magnitude was ~80% of that seen in normal ASW. Similar results were obtained when B cells were current-clamped at hyperpolarized (> -70 mV) levels of membrane potential throughout the inter-trial interval, so as to preclude a contribution of cumulative voltage-dependent inactivation of residual K^+ currents to these results. Substituting Sr^{2+} for Ba^{2+} also produced the same outcome [paired: 6.67 ± 1.67 mV vs. unpaired: 3.04 ± 4.14 mV]. These results suggest that pairing-specific cumulative depolarization of B cells can still occur when A and C currents have been suppressed. Moreover, transmembrane flux of Ca^{2+} is not a strict requirement for the occurrence of cumulative depolarization.

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181.8 TEMPORAL RELATIONSHIP BETWEEN CALCIUM TRANSPORT AND PHOTOVOLTAGE IN FROG RODS. Geoffrey H. Gold* (SPON: W.H. Miller). Dept. of Physiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

High resolution extracellular free Ca measurements along the distal surface of the isolated bullfrog retina were made by a method modified from that of Gold & Korenbrot (PNAS 77, 5557 (1980). In the present configuration, the retina is mounted receptor-side up in a perfusion chamber and the planar Ca electrode is positioned above the retina. The ion-selective membrane is mounted on the front of a microscope objective, allowing the retina to be viewed through the membrane with IR illumination. The distance between the electrode and retina can be determined visually and the electrode can be raised and lowered during an experiment to change perfusate composition. The active area of the membrane (1 mm diameter) is surrounded by a coaxial reference electrode (3 mm diameter). This new electrode configuration eliminates contamination of the Ca signal by the trans-retinal potential (ERG), which was found in the previous experiments. The rod photovoltage is isolated from the ERG by addition of 25 mM Asp and 10 uM Ba to the Ringer's solution, which contained 110 mM Na, 2.5 mM K and 1 mM Ca.

The light stimulated extracellular Ca concentration changes in the outer segment layer are quantitatively similar to those reported previously. Light stimulates an efflux of Ca leading to a rise in extracellular Ca that is followed by an influx which returns extracellular Ca to its initial level (i.e., the rods take up all of the Ca which was initially extruded). However, the waveform of the Ca flux and photovoltage are dissimilar; the Ca transport reverses from efflux to influx before the photovoltage returns to the dark level.

These results imply that 1) if Ca transport and photovoltage depend solely on intracellular Ca then their dependence on Ca must change following illumination, or 2) Ca transport or photovoltage are regulated by other substances in addition to Ca.

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181.9 TAURINE ADDITION MIMICS THE EFFECTS OF LOWERING $[Ca^{++}]_o$ ON CONE AND RPE RETINOMOTOR MOVEMENTS. Allen Dearry* and Beth Burnside.* (SPON: Matthew M. LaVail). Dept. Physiology-Anatomy, Univ. of California, Berkeley, Ca 94720.

We have compared the effects of varying extracellular taurine concentration to those of varying $[Ca^{++}]_o$ on retinomotor movements in cultured retinas. Retinomotor movements are coordinated photoreceptor and RPE pigment granule movements which occur in response to light changes. In darkness, cone myoids elongate and pigment granules aggregate toward the basal end of RPE cells. In light, cone myoids contract and RPE pigment granules disperse.

We have previously shown that $[Ca^{++}]_o$ has pronounced effects on retinomotor movements in cultured green sunfish retinas (J. Gen. Physiol., 1984). When isolated dark-adapted (DA) retinas were cultured in constant darkness, cones contracted to their light-adapted (LA) positions if $[Ca^{++}]_o$ was $>10^{-3}$M. When $[Ca^{++}]_o$ was $<10^{-6}$M, cones retained their DA positions; intermediate $[Ca^{++}]_o$ produced intermediate cone myoid lengths. Cone contraction was 100x more sensitive to $[Ca^{++}]_o$ in the presence of A23187 suggesting that effects of altering $[Ca^{++}]_o$ were mediated by altering $[Ca^{++}]_i$. We now report that adding taurine to a medium containing 1.8mM Ca^{++} mimics the effect of lowering $[Ca^{++}]_o$. When taurine was added to a solution having 1.8mM Ca^{++}, cones retained their DA positions during dark culture. Taurine was maximally effective at >5mM; lower concentrations produced intermediate cone myoid lengths. Similarity of taurine and $[Ca^{++}]_o$ dose-response curves suggests that the effects of each may be mediated by a common mechanism. Taurine and $[Ca^{++}]_o$ also influenced the ability of DA cones to contract in response to light onset. When DA retinas were cultured in light, cone contraction was partially inhibited by either $<10^{-6}$M Ca_o^{++} or 5mM taurine in the presence of 1.8mM Ca_o^{++}.

Taurine and $[Ca^{++}]_o$ also affected the occurrence of dark-induced retinomotor movements. When LA retinas with attached RPE were cultured in the dark in the absence of taurine, cones elongated and RPE pigment aggregated only if $[Ca^{++}]_o$ was between 10^{-5} and 10^{-7}M. In the presence of 5mM taurine, cones elongated and RPE pigment aggregated even in 1.8mM Ca_o^{++}. Neither taurine nor $[Ca^{++}]_o$ affected cone or RPE retinomotor positions when LA RPE-retinas were cultured in constant light.

Thus, in any given dark/light condition, the effects of taurine on cone and RPE retinomotor movement closely paralleled the effects of lowering $[Ca^{++}]_o$. All results obtained by lowering $[Ca^{++}]_o$ to 10^{-6}M could be reproduced by adding 5mM taurine to a medium containing 1.8 mM Ca_o^{++}. Therefore, we suggest that either reducing $[Ca^{++}]_o$ or adding taurine favors dark-adaptive retinomotor movements by decreasing $[Ca^{++}]_i$.

181.10 BARIUM CHLORIDE REMOVES THE OUABAIN-INDUCED INCREASE IN THE AMPLITUDE OF THE ROD RECEPTOR RESPONSE. A.E. Walter and *A.J. Sillman. Dept. of Animal Physiology, Univ. of California, Davis, CA 95616.

The mass receptor potential of the excised, superfused retina of the bullfrog was isolated with sodium aspartate. Rods were selectively stimulated by using very dim flashes of light. In the presence of 0.1 mM ouabain, strophanthin, the amplitude of the rod receptor response first transiently increased and then progressively decreased. As shown previously by the intracellular work of Torre (J. Physiol. (Lond.) 333:315, 1982) the rods of the marine toad respond in the same fashion to another cardiac glycoside, strophanthidin. Interestingly, we find that the ouabain-induced transient increase in the amplitude of the rod receptor response was completely eliminated by 0.4 mM barium chloride. However, barium did not affect the rate at which the amplitude of the rod receptor response decayed in the presence of ouabain. Therefore, barium is not interfering with the ability of ouabain to block the sodium-potassium pump and to, thereby, eventually collapse the sodium and potassium gradients. It is equally unlikely that barium exerts its effects on the ouabain-induced transient by virtue of its known ability to alter the conductance of potassium channels (Armstrong et al., J. Gen. Physiol. 80:663, 1982) because a substantial ouabain-induced transient occurs even when the external potassium concentration is elevated to a level sufficient to impede the conductance of the potassium channels of the rods. We propose that barium removes the ouabain-induced transient increase in the amplitude of the rod receptor response by reducing the coupling ratio of the postulated electrogenic sodium-potassium pump of rods.

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181.11 EFFECTS OF IBMX AND $MnCl_2$ ON THE RESPONSES OF BULLFROG RODS AND CONES. Lawrence W. Haynes and Arnold J. Sillman. Dept. of Animal Physiology, University of California, Davis, CA, 95616.

The concentration dependent effects of IBMX on the aspartate isolated photoreceptor responsess of the superfused retina of the bullfrog were investigated. Low concentrations of this phosphodiesterase inhibitor produced reversible increases in the response amplitude of rods. The threshold for this increase was near 1μM. The rod response amplitude increased with increasing IBMX concentrations between 1μM and 280μM. At concentrations of IBMX of 1mM and 2.8mM, the rod response amplitude increased just as with lower concentrations, but this increase was transient rather than stable in that it was followed by a decrease in rod response amplitude. In contrast to the rods, cone receptor response amplitude was depressed by IBMX in a concentration-dependent fashion with a threshold near 10μM. This reversible depression forced the receptor response asymptotically toward zero. In no case was an increase in cone response amplitude observed. In addition, a high concentration of Mn^{2+}, which acts as a guanylate cyclase inhibitor, produced a prompt depression in rod response amplitude which spontaneously recovered somewhat with time. Removal of Mn^{2+} resulted in a large increase in rod response amplitude to levels well above those seen during the initial control period. As with IBMX, the cone response amplitude was depressed by Mn^{2+}. However, with Mn^{2+} this depression was irreversible. These data support the importance of the role of cyclic nulceotides in both rod and cone photorecptors, but indicate that there are differences between the two classes of photoreceptor with respect to the enzymes which are involved in transduction.

181.12 NUCLEOTIDE MODULATION OF CALCIUM BINDING TO ISOLATED BOVINE ROD OUTER SEGMENT MEMBRANES. E. P. Meyertholen* and R. N. Lolley. Jules Stein Eye Institute, UCLA Sch. of Med., CA 90024, and V.A. Medical Center, Sepulveda, CA 91343.

A role for calcium in light-mediated electrophysiological events has been identified in vertebrate photoreceptors, but the mechanisms which regulate the concentration of intracellular calcium or its binding/release from rod outer segment (ROS) membranes remain elusive. We have recently investigated the binding of 45-Calcium to partially purified ROS membranes from bovine retinas. Employing the technique of equlilibrium dialysis, minimal calcium binding was observed in the soluble fraction after 18 hr of incubation at 4°C. The ROS membranes bound calcium in a dose-dependent manner, and the binding appeared to saturate at high calcium concentrations. Scatchard plot analysis indicates the presence of at least two binding sites. Estimation of kinetic parameters (assuming that there are two independent sites) suggest a high affinity site (Kd = 0.08 mM) with a binding capacity of 25 nM/mg protein, and a low affinity site (Kd = 0.91 mM) with a binding capacity of 162 nM/mg protein. Calcium binding to washed ROS membranes appears to be light-insensitive.

The magnitude of the calcium binding can be modulated by the addition of certain nucleotides to the dialysis media. The addition of 0.1 ATP reduces the amount of calcium bound to the membranes by 60-90% throughout a range of calcium concentrations between 0.1 μM and 1.0 mM. This effect is reversible, since calcium binding increases to control levels when the ROS membranes are transferred to media without ATP. The ATP-modulation of calcium binding is dose-dependent, and a linear reduction of calcium binding is measured between the range of 0.005-0.1 mM ATP. The ATP-modulation is lost when the membranes are boiled in water. The addition of methylene-ATP (a non-hydrolyzable analog of ATP) has no effect on the calcium binding, suggesting that the hydrolysis of ATP is necessary for the reduction of calcium binding. In addition to ATP, other nucleotides (cGMP, GTP and cAMP) resulted in small (<20%) reductions in the measured binding. It is possible that ATP may act in ROS to regulate calcium binding and, thereby, influence the intracellular concentration of calcium. (Supported by the National Retinitis Pigmentosa Foundation and by the Medical Research Service of the Veterans Administration.)

181.13 ANATOMICAL AND FUNCTIONAL EFFECTS OF HEMICHOLINIUM ON THE RETINA OF DUTCH RABBITS. M. P. White, A. Negi*, P. A. Hock* M. Jain*, and M. F. Marmor*. Dept. of Ophthalmology, Veterans Admin. Hospital and Stanford Univ., Palo Alto, CA 94304

Photoreceptor morphology and retinal function in Dutch rabbits were studied after single intravitreal injections of hemicholinium (18 ug) or saline. As others have reported in albino rabbits, there is a progressively abnormal appearance of the basal outer segment region. At 3 - 5 days after injection the outer segments are lost entirely. The effect is reversible for rods. After 14 - 17 days short rod outer segments are observed. Histological examination shows only subtle changes in the retinal pigment epithelium with no pyknosis or loss of this cell layer.

Electroretinogram recordings show a decrease in a, b and c wave amplitudes. A and b waves are decreased by 30% after 1 - 2 days. The c wave is decreased by 50% after 1 day and by 3 - 4 days is completely abolished. Thus, the time course of effect is different for b and c wave; the effect may be greater on c wave. Even after 5 days, there is a measurable b wave, although no outer segments appear to remain in histological sections. Using iodate during the early stages of hemicholinium reaction, we find that slow PIII is also reduced. All components of the electroretinogram recover. Recovery of amplitudes of the a and b waves is slower than predicted by the 10-day turnover of rod outer segment membrane.

Ophthalmoscopic abnormalities of the fundus are severe. Pigment clumping is seen over most of the retina. We systematically varied needle placement during hemicholinium injection and were able to obtain different patterns of damage in the fundus. This finding suggests that a combination of diffusion gradients and a critical threshold produce the pattern of fundus damage.

Initial detailed histological study has been limited to posterior and equatorial retina. Because of apparent sparing of peripheral retina when viewed funduscopically, we are currently analyzing the regional extent of damage within the eye. Significant sparing of peripheral rod outer segments would explain the b wave level remaining in the injected eyes. We are not yet able to affirm or eliminate this possibility, but preliminary results indicate that outer segments are lost even at the ora serrata.

181.14 ISOLATED CONE PHOTORECEPTORS SUITABLE FOR ELECTROPHYSIOLOGICAL STUDY. A.V. Maricq* and J.I. Korenbrot. Dept. of Physiology, University of California, San Francisco, CA 94143

Preparations of large quantities of isolated functional rod photoreceptors have aided the electrical and biochemical investigations of these cells. Unfortunately, no similar preparations have been available to study cone photoreceptors. We have developed a procedure which reproducibly yields large numbers of isolated cone photoreceptors from the retina of the lizard, sceloporous orcutti. This retina is an excellent source of cone photoreceptors; single cones comprise the major component of the photoreceptor layer and rods are not observed. Large yields of isolated cones were obtained from these retinas using a variety of enzymatic dissociation techniques. Prolonged digestion with neutral protease released many cells with intact outer segments and axons. In some instances cone photoreceptors maintained their morphological connection with second order cells. Digestion with either the more vigorous protease papain, or with a mixture of collagenase/hyaluronidase produced higher yields of cones, a larger fraction of which lacked outer segments and axons. Under all conditions most cells remained morphologically stable for two to three hours. These cells, free of interphotoreceptor coupling, are well suited for electrophysiological investigations. The cells could be immobilized on collagen coated glass coverslips using concanavalin-A. Electrical recording from the cone inner segment using the "patch clamp" technique revealed several types of voltage dependent channels, including a hyperpolarization activated channel carrying inward current and a depolarization activated channel carrying outward current.

181.PO NONLINEAR DYNAMIC INTERACTIONS BETWEEN PAIRS OF PHOTONS ABSORBED IN LOCUST PHOTORECEPTORS. A.S. French and J.E. Kuster*. Department of Physiology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

In some invertebrate photoreceptors at low light levels, each absorbed photon produces a fluctuation in membrane potential, or bump. Responses to stronger stimuli consist of summed bumps, and the summation is approximately linear for dim flashes. However, recent work on fly photoreceptors indicates that peak membrane depolarization to a flash increases less than linearly when only 10 or more photons are absorbed together, while nonlinear changes in time course of the response occur when about 4 photons are present in a flash.

We have now studied the electrical response of locust compound eye photoreceptors to flashes containing only a few photons and find that when the flash contains more than 1 photon the amplitude of the response grows less than linearly and the time course becomes faster. To examine the dynamic properties of these nonlinearities we used pairs of flashes, each containing an average of 1 photon, with variable flash separation. The nonlinear components of the response were obtained by subtraction of the predicted linear summation, based on single photon responses in the same cell, from the actual response. The major nonlinear interaction was a depression of sensitivity caused by transduction of a photon. In addition, there was evidence for early facilitation of the second photon response by the first, as has been suggested for other photoreceptors.

Optimum interaction between photons occurred with a small delay (about 10 ms) suggesting that nonlinear summation of receptor current at the membrane might account for our results. We therefore examined the response to single photons in cells where the membrane was depolarized by injecting current through the recording electrode during the flash. Current waveforms were either simple steps of the same amplitude as the normal response, or were exactly the same shape as the single photon response. No evidence of nonlinear behavior was found in either case. These results have significance for models of phototransduction and spatial interactions between photons within receptors.

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INVERTEBRATE MOTOR FUNCTION AND BEHAVIOR

182.1 ASSOCIATIVE LEARNING CHANGES INTRINSIC TO HERMISSENDA TYPE A PHOTORECEPTORS. W. Richards and J. Farley (Spon: B. Campbell). Princeton Univ., Princeton, NJ 08544

Previous research has implicated Type B photoreceptors in Hermissenda's eyes as sites expressing long-term changes in excitability, following repeated light-rotation pairings. The enhanced depolarizing response to light of B cells from trained animals has been correlated with decreased light-evoked activity in Type A photoreceptors (Farley & Alkon, J. Neurophysiol., 1982), which project via polysynaptic pathways to motoneurons subserving phototaxis (Goh and Alkon, J. Neurophysiol., 1984; Lederhendler et al., 1982). Here, we report that Type A photoreceptors (both the medial and lateral) exhibit persistent, pairing-specific decreases in their light-evoked generator potentials, on retention days following associative training.

Hermissenda were trained in the standard manner (50 trials/day; 3 days) and exposed to either light-rotation pairings, or random presentations of these two events. Following re-tests of phototaxic behavior at 24 or 48 hr retention intervals, the nervous systems were removed and intracellular recordings were obtained from ligated Type A cells. A cells from paired animals exhibited significantly smaller steady-state generator potentials (n=9; 13.12 $\pm$ 2.21 mV) than those from random control (n=8 21.03 $\pm$ 2.74 mV) or untrained animals (n=14; 20.10 $\pm$ 2.52) [$t(15)=2.13$; $t(21)=1.84$; p's < .05]. No differences in peak light responses nor resting potentials were apparent among groups.

Voltage-clamp analysis of Type A and B cells from untrained animals reveal the same complement of voltage dependent outward (K^+) and inward (Ca^{2+}) currents, but differences in their relative current densities. Peak amplitude of A currents (at 0 mV; V_h=-60) in the A cell (29.93 $\pm$ 4.65 nA; n=7), were significantly smaller than those in either the lateral B (44.72 $\pm$ 5.97 nA; n=9) or the medial B (74.79 $\pm$ 10.05 nA; n=7). In contrast, the calcium-activated K^+ current (I_c) is bigger in the A cell (25.40 $\pm$ 2.00 nA) than in either the medial (16.42 $\pm$ 2.35 nA) or lateral B cell (12.45 $\pm$ 2.95 nA). This difference in C current may account for the characteristically smaller steady-state light response, the more rapid accomodation, and the light-induced after-hyperpolarization that is characteristic of A vs. B photoreceptors (Alkon & Fuortes, 1972). Whether the same conductance changes that are produced by associative training in the B cells also occur in A cells is the subject of current investigation.

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182.2 A SMALL FIBER PROJECTION SYSTEM IN THE VENTRAL NERVE CORD OF LUMBRICUS TERRESTRIS CAUSING POTENT LONGITUDINAL MUSCLE INHIBITION. J.L. Johnson. Dept. of Physiol. & Pharmacol., USD School of Medicine, Vermillion, SD 57069

Attempts to discern longitudinal muscle inhibition in Lumbricus by peripheral nerve stimulation has proved unsuccessful (J. Exp. Biol., 60:453, 1974). Thus, longitudinal muscle inhibition was analyzed in Lumbricus by stimulating the free ventral nerve cord (VNC) feeding to a section of body wall connected to a force transducer. Unless otherwise stated, the VNC was stimulated by bipolar Ag/$AgCl_2$ electrodes at a frequency of 10 Hz (pulse duration of 0.2 msec). Stimulation of the VNC at 0.5-0.6 Volts evoked only conducted dorsal giant fiber responses and weak longitudinal muscle contractions. Potent contractile responses were seen which were graded in amplitude from 2-4 Volts stimulation to VNC. Potent longitudinal muscle inhibition was seen when stimulating the VNC at 5-10 Volts. The amount of inhibition was graded in nature between this stimulus range. During the time of inhibition, the longitudinal muscle was much less responsive to tactile stimuli or direct electrical excitation. Using a constant 10 Volts stimulation strength to the VNC, stimulation at 10-20 Hz evoked a potent longitudinal muscle inhibition, while stimulation at 40-80 Hz induced graded contractile responses which were capable of overriding this inhibitory effect. Switching back to 20 Hz again after the 80 Hz stimulation resulted in a profound longitudinal muscle inhibition again. No long latency inhibition of motoneurons was seen that could account for this inhibitory effect on longitudinal muscle activity. Several interneuronal types were located in the VNC which were excited by the small fiber projections after a long conduction time latency. The small fiber projections in the VNC leading to longitudinal muscle inhibition would seem to be separate and distinct from the lower threshold population leading to potent longitudinal muscle excitation via the small excitatory motoneurons. In conclusion, there is a high threshold small fiber projection system in the VNC of Lumbricus which causes a potent longitudinal muscle inhibition. The ability of this high threshold system to cause muscle inhibition seems to be highly dependent upon the stimulus frequency, since during high frequency stimulation, the excitatory motoneurons can override this inhibitory effect.

182.3 Microsurgical lesions reveal functional significance of horizontal cells in optomotor behaviour of flies.

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The horizontal cells of flies are giant output neurons of the optic lobes that respond selectively to horizontal motion in the visual environment. The effect of microsurgical lesion of the cells on visually induced flight behaviour was investigated in blowflies (Calliphora erythrocephala). The results provide evidence in favour of the hypothesis that the horizontal cells of each optic lobe control yaw torque generation and hence turning of the fly to the ipsilateral side. Moreover the existence of an additional yaw torque control system in each optic lobe was revealed. This additional system seems to remain unaffected by the lesion. It is activated by horizontal and vertical motion in either direction in the ipsilateral visual field and induces turning of the fly towards the stimulated side irrespective of the direction of motion. There is evidence that the additional system is inhibited by contralateral horizontal and vertical motion.

182.4 DRAGONFLY FLIGHT: MATCHING NEUROMUSCULAR SYSTEMS TO UNSTEADY FLUID MECHANISMS. C. Somps*, M. W. Luttges and J. A. Beel*, Aerospace Engineering Sciences, University of Colorado, Boulder, Colorado 80309.

Quite recently, insects have been shown to depend upon unsteady separated flows in generating the lift necessary to support hovering. These observations have had a considerable impact upon fluid mechanists seeking to understand the principles of novel lift generation mechanisms. The same observations have special implications for the neuromuscular control of the wing kinematics necessary for generating and utilizing such complex flows. The present studies focus upon the flight of dragonflies. Wing kinematics are described for free flight, tethered and "automaton" specimens. The effects of these wing kinematics are corroborated by an integrated force measurement as well as flow field visualization. The precision and stereotypy of wing-flow field interactions supportive of high lift generation make rather specific demands upon the motor output systems supporting dragonfly wing kinematics. Direct electrical stimulation of thoracic neural and muscular systems show that wing kinematics are relatively immune to disruption by side variations in stimulus parameters. Nevertheless, increases in both wing beat amplitudes and frequencies lead to enhanced lift generation without altering wing-flow interactions. Common feedback controls for dragonfly flight, lighting, mean flows, horizon alterations and wing loading, produced only minor modifications of wing kinematics. Axiosymmetric variations in wing kinematics were similarly modest but appear able to support the aerodynamic characteristics documented for dragonflies. These results are discussed in terms of the importance of central pattern generators compared to peripheral feedback controls. Response sterotypy is evaluated as a major determinant in the use of particular forms of motor output control systems.

182.5 FLIGHT MOTOR PROGRAM IN BIFUNCTIONAL MUSCLES APPEAR LATE IN DEVELOPMENT OF THE COCKROACH, PERIPLANETA AMERICANA. J.G. Malamud and D.R. Stokes*. Dept. of Developmental & Cell Biology, Univ.of Calif., Irvine, Ca 92717 and Dept. of Biology, Emory Univ., Atlanta, Ga 30322.

Adult males of Periplaneta americana will fly if thrown into the air, while females flutter their wings a few times and fall to the floor (S.Kramer, Proc. Tenth Intl. Cong.Entoml, 1:569). The wing area of males is larger than that of females, although female body mass averages 1.5 times that of males (thus wing loading is much greater in females). Mean tethered flight duration of 20 day post terminal molt (p.t.m.) males is 15 minutes; females average 10 seconds. However, flight frequency is 30 Hz (at 30° C) for both. Newly emerged adult males do not fly. They show progressive improvement in tethered flight duration following molting through 20 days p.t.m.

The bifunctional muscles 177c (basalar) and 177a (tergo-trochanteral) are synergistic coxal depressors but antagonistic wing depressors (177c) and wing elevators (177a). Myograms of both male and female 20 day p.t.m. adults show an alternating 177a and 177c flight program, although it is somewhat erratic in females. Last instar nymphs of both sexes will adopt a flight posture; however, flight EMG's cannot be elicited. Flight EMG's first appear in the levator muscle (177a) where they consist of bursts of potentials of mixed amplitudes. Burst duration (and number of potentials) in 177a typically decreases with maturation of flight capability. The depressor muscle (177c) fires after 177a, as a single spike, and occurs irregularly in young adults. By 20 days p.t.m., the two muscles have attained the mature adult pattern and firing is completely reciprocal.

The absence of a flight motor program in the late nymphal stages of P. americana is in contrast to its presence in locusts and crickets where flight motor units are activated as early as the 4th and 7th instar, respectively.

182.6 THORACIC INTERENEURONS EXCITED BY GIANT INTERNEURONS OF THE COCKROACH. R.E. Ritzmann and A.J. Pollack*, Department of Biology, Case Western Reserve University, Cleveland, OH 44106.

The giant interneuron (GI) system of the cockroach has been the subject of many neurobiological and behavioral studies. Although mounting evidence suggests that the GIs are involved in the wind-mediated escape behavior, no thoracic neurons have ever been clearly shown to be directly excited by them. In this report, we describe 6 thoracic interneurons that are monosynaptically driven by GIs. Four of these are interganglionic interneurons and two are intraganglionic. All are excited by either dorsal or ventral GIs but to date none have been shown to be excited by both populations.

One interneuron, which we call Lambda cell, has a 25 micron axon in the T2-T3 connective, making it a giant interneuron in its own right. It has been recorded 1 for 1 in conjunction with ventral GIs 1,2 and 3. In addition, it follows intracellular stimulation of ventral GIs at 100Hz. In several cases Lambda cells were recorded in conjunction with dorsal GIs. In no case was there evidence for monosynaptic connections in these trials. In at least one experiment Lambda cell fired action potentials during dye filling each time a hyperpolarizing current was turned off. Each such burst of action potentials was correlated with a burst of action potentials in leg nerves. Thus Lambda is both excited by ventral GIs and excites leg motor neurons.

Two other interganglionic interneurons were also found to be monosynaptically excited by ventral GIs but not by dorsal GIs. Another interganglionic interneuron, which we have named T cell, is monosysnapticaly excited by dorsal GIs. However, it has not yet been tested with ventral GIs.

The results of these experiments suggest that the distinction between dorsal and ventral GIs that has been noted previously is maintained in the postsynaptic interneurons. This work was supported by NIH grant 1 R01 NS17411-01 to R.E.R..

182.7 RESPONSES OF THORACIC INTERNEURONS OF COCKROACH TO WIND PUFFS OF DIFFERENT DIRECTIONS. J. Westin and R.E. Ritzmann. Dept. of Biology, Case Western Reserve Univ., Cleveland, OH 44106.

Cockroaches exhibit a directional escape response to wind puffs (Camhi and Tom, J. comp. Physiol.,128:193,1978). Information on wind direction is encoded in giant interneurons which extend from the abdominal ganglion to the head (Westin et. al., J. comp. Physiol.,121:307,1977). They synapse with a population of higher order interneurons in the thoracic ganglia which have been described by Ritzmann and Pollack(Soc. of Neurosci. Abstr.,1983). These higher order interneurons are presumed to be involved in controlling escape running and/or flying. To understand how these behaviors are controlled, it is thus important to determine if directional information is preserved in the higher order interneurons.

We recorded intracellularly from individual higher order interneurons, and delivered reproducible wind puffs from various angles around the animal. We plotted the number of action potentials vs. wind direction on polar coordinates. The cells were filled with lucifer yellow. Both wholemount morphology and tract location were determined. We found that, indeed, many of the higher order interneurons encode information on wind direction. Moreover, some of the curves suggest that the higher order interneurons receive input from more than one GI.

This work was supported by NIH grant 1 RO1 NS 17411-01 to R.E.R.

182.8 INTERSEGMENTAL INTERNEURONS SENSITIVE TO LEG SENSORY STRUCTURES IN THE COCKROACH. Murrain, M.P. and R.E. Ritzmann. Department of Biology, Case Western Reserve University, Cleveland, OH 44106.

Intersegmental sensory information from the legs is important for the coordination of walking in the cockroach (Pearson and Iles J. Exp. Biol. 58:725-744 1973 and Hughes J. Exp. Biol. 34:306-333 1957). Sensory information from the legs is also important in the switching from the flight to the walking behavior in the cockroach (Kramer and Markl J. Physiol. 24:577 1978). We are interested in how sensory information is transmitted from one segment to another, specifically for the coordination of walking, as well as the switching from the flight to the walking behaviors. We did two experiments which address this question. The first was a series of ablation experiments to determine the leg sensory stuctures most invovled in intersegmental coordination of walking. In these experiments, specific leg sensory structures were ablated, and films were taken of walking animals. The films were analyzed frame-by-frame, comparing experimental and control animals, where no leg sensory structures were ablated. The trochanteral campaniform sensilla and hair plates were the sensory structures most involved (of the 5 studied) in intersegmental coordination of walking.

We have identified a group of intersegmental interneurons in the cockroach which respond to stimulation of specific leg sensory structures. The intersegmental interneurons were recorded from intracellularly from the T_2-T_3 connectives, or in the T_3 neuropil. They were filled with Lucifer Yellow. The sensory structures were stimulated with a vibration delivered via a micropipette attached to a speaker, which was driven by a function generator. Responses seen in these cells included: one or a group of spikes on onset of stimulation, activity throughout the stimulus, or complete inhibition of activity.

These cells are part of a population of cells which respond to various sensory structures on the leg of the cockroach. The identification of these cells will be very important for the understanding of how sensory information is used not only in the intersegmental coordination of walking but also in switching from the flight to the walking behavior.

This work was supported by NIH grant 1 RO1 NS17411-01 to R.E.R..

182.9 SYNAPSE DISTRIBUTION ON TWO DENDRITIC FIELDS OF A LOCAL CIRCUIT INTERNEURONE IN THE LOCUST. A.H.D. Watson* and M. Burrows, Dept. of Zoology, Univ. of Cambridge CB2 3EJ, England.

Spiking local interneurones in the locust metathoracic ganglion are the primary integrators of sensory information from the hind legs (Siegler, M.V.S. and Burrows, M., J. Neurophysiol.,50, 1281-1295, 1983). They also mediate local postural adjustments of a leg by virtue of their output connections with motor neurones (Burrows, M. and Siegler, M.V.S., Science 217, 650-652, 1982). One group of these interneurones is characterised by two distinct fields of neuropilar branches linked by a single fine process, one ventral the other more dorsal. The afferents from sensory hairs overlap only with the ventral field and the branches of the leg motor neurones only with the dorsal field. Could these two fields represent distinct sites for synaptic inputs and outputs? To test this idea, interneurones were characterised physiologically and then stained intracellularly with Horseradish Peroxidase so that the structure and distribution of their synapses could be examined with the electron microscope.

The ventral field is composed of stout secondary neurites which give rise to numerous fine processes, often less than 0.2μm across, but of uniform diameter along their length. They lie mainly within a distinctive region of neuropile composed mainly of processes less than 1μm in diameter. Input synapses predominate. They are made from processes that contain small agranular, or larger granular synaptic vesicles. Output synapses also occur, either from the larger neurites or from occasional small varicosities on finer branches of the interneurone.

The dorsal field is composed of neurites with many vesicle-filled varicosities up to 2μm across joined by neurites of less than 0.2μm in diameter. Output synapses predominate and occur typically from the varicosities, each of which may contain four or five synaptic sites. The synapses have round agranular vesicles approximately 50nm across, clustered near presynaptic bars. A small number of input synapses are made both onto the varicosities themselves and the neurites between them.

The ventral and dorsal fields could represent dendrites and axon terminals respectively, and the linking process which is sheathed in glia and makes no synapses, the axon. This is consistent with the expected ventral site of sensory input and the dorsal site of motor output.

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182.10 CHROMATOPHORE MOTONEURONS IN THE SQUID, LOLLIGUNCULA BREVIS. F. Dubas*, G.P. Ferguson*, R.T. Hanlon* and H.M. Pinsker. (SPON: J.E. Kanz). Marine Biomed. Inst., Univ. of TX Med. Br., Galveston, TX 77550-2772.

Coordinated chromatophore activity in different regions of the body of cephalopods produces various patterns that transmit intra- and inter-specific visual signals. A chromatophore organ comprises a pigment-containing cell surrounded by 15-20 radially arranged muscle fibers whose contraction and relaxation cause expansion and retraction of the pigment cell. Individual chromatophore muscle fibers are visible under the light microscope. Previous studies indicated that chromatophore muscles are innervated by axons whose somata are located in the subesophageal mass of the brain. Our purpose is to identify the chromatophore motoneurons of L. brevis, a species with a simple pattern repertoire and only yellow and brown chromatophores.

To locate the cell bodies of putative chromatophore motoneurons, HRP was injected in the vicinity of chromatophore muscles in the posterior mantle of live squids. After 7-10 days, HRP-labelled somata were visible in the ipsilateral posterior chromatophore lobe of the subesophageal mass. A single localized HRP injection labelled up to 10 isolated or clustered somata in different regions of the lobe.

A semi-intact preparation was developed for extra- and intracellular electrophysiology. The whole animal was stabilized in a lucite chamber and the aorta perfused with oxygenated, buffered artificial sea water containing a vasodilator. The subesophageal mass was exposed and a 75-100 micron glass pipette used to stimulate the posterior chromatophore lobe extracellularly. Favorable preparations lasted up to 4 hr.

Stimulation (1-20 Hz) of the chromatophore lobe showed that chromatophore expansion followed one-for-one to about 15 Hz, above which tetanic muscle contraction was observed. Focal threshold stimulation showed that chromatophore neurons have highly localized and repeatable motor fields whose size varied from 3-40 chromatophores, in general of the same color. Preliminary results suggest overlapping motor fields and multiple innervation of single chromatophores. Mapping the chromatophore lobe indicated that neurons activating chromatophores in adjacent parts of the body were not necessarily adjacent in the lobe. Intracellular analysis of the putative chromatophore motoneurons is now being conducted with lucifer-filled microelectrodes.

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182.11 THE DORSAL LIGHT REFLEX OF THE CRAYFISH: SUSTAINING FIBER TO MOTONEURON CONNECTIONS, RECIPROCAL INHIBITION AND SPATIOTEMPORAL SUMMATION. By R.M. Glantz, H.B. Nudelman and B. Waldrop, Biology Dept., Rice University, Houston, TX 77251.

Stalk eyed crustaceans exhibit compensatory eye movements in response to body rotation and changes in the distribution of ambient illumination. The light elicited eye movements are called dorsal light reflexes because they are principally sensitive to illumination of the dorsal half of the retinula. The reflexes are mediated by oculomotor neurons which arise in the anterior motor cluster of the protocerebrum and project to the periphery via the optic nerve motor bundle. The functional connectivity between identified visual interneurons (sustaining fibers - SF) and oculomotoneurons was assessed by simultaneous recording and crosscorrelation analysis. A small group of SFs (3) exhibit monosynaptic excitatory connections to an identified tonic oculomotoneuron. The excitatory interactions are found exclusively between SFs and oculomotoneurons with similar and/or overlapping excitatory receptive fields. A second group of SFs (2) exhibit inhibitory connections to the motoneuron. The excitatory receptive fields of these SFs correspond to the inhibitory receptive field of the motoneuron. The motoneuron firing rate is thus determined by the absolute firing rates of 5 SFs, each weighted in accordance with the strength and sign of its synaptic actions. Because the SFs are themselves modulated by a surround inhibitory mechanism, two layers of reciprocal inhibition control the motoneurons. The collective actions of the SFs are sufficient to produce all of the steady state visual behavior of the motoneurons including the increment in firing rate elicited by illumination, unique features of the motoneuron receptive field and differential sensitivity to blue light and polarized light. Pairs of SFs which converge on the same motoneuron sum their effects linearly. Thus the joint interaction of two SFs on a motoneuron is equal to the sum of the two postsynaptic effects taken separately. The ensemble information code, at the SF level of the optomotor pathway, is a set of differentially weighted mean firing rates. The weightings reflect differences in the strengths of the several SF-to-motoneuron interactions. One consequence of these differences is a selective weighting of the effects of illumination (in different regions of visual space) on the compensatory eye reflex. Supported by N.S.F. grant no. BNS 8312296.

182.12 TACTILE ACTIVATION OF INTERNEURONS WHICH PRODUCE ABDOMINAL MOVEMENTS IN CRAYFISH. J. Jellies and J. L. Larimer. Department of Zoology, University of Texas, Austin, TX, 78712.

Positional adjustments of the crayfish abdomen can be produced by stimulating the axons of individual interneurons in the abdominal CNS (Kennedy et al., J. Exp. Zool., 165:239, 1967). It is also known that positional changes can be evoked by "natural" stimulations and reflexes (Larimer and Eggleston, Z. vergl. Physiol., 74:388, 1971; Page, J. Comp. Physiol., 102:65, 1975; Page and Jones, J. Exp. Biol., 99:339, 1982) but it is not clear how afferent input gains access to these interneurons.

Intact Procambarus clarkii were restrained ventral side up in a saline filled dish and one ganglion was exposed and supported on a platform. Neuropilar impalements were made in this ganglion using lucifer yellow filled microelectrodes. The body surface was then stimulated with a fine brush, evoking activity in the tonic motoneurons.

Of the 134 positioning interneurons examined, one group responded to touch with short latency trains or bursts of impulses. Where known, these cells had their somata in the terminal ganglion and had consistent receptive fields which overlapped in the tail-fan. A second set of positioning interneurons were not strongly activated by touch but their discharge frequencies were influenced. This second group of interneurons had their somata in more rostral ganglia and had less well defined receptive fields.

There are two major features of this work. First one group of interneurons appears to be synaptically closer to tactile sensory input than the other. Secondly, there are extensive synaptic interactions among these positioning interneurons (Jellies and Larimer, Neurosci. Abst., 9:382, 1983). Therefore, afferent input would probably activate constellations rather than single interneurons because of both overlapping receptive fields and interneuronal interactions.

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182.13 OUTPUT PROPERTIES OF PREMOTOR INTERSEGMENTAL INTERNEURONS IN THE POSTURAL SYSTEM OF LOBSTER. K.A. Jones and C.H. Page. Dept. of Biological Sciences, Rutgers University, Piscataway, NJ 08854.

We have identified 11 interneurons (INs), which drive the tonic motoneurons (MNs), by recording intracellularly from their somata in the second abdominal ganglion. Each IN when depolarized induced either a flexion or an extension motor program. All of these INs spiked and their axons projected into either the anterior or posterior connective. Five of these intersegmental INs could be repeatedly identified in different lobster preparations; these were assigned identifying numbers.

The output properties of these INs were studied with respect to three pairs (left and right) of tonic MNs: 1) peripheral inhibitor to the extensors (e5), 2) peripheral inhibitor to the flexors (f5), 3) the largest flexor excitors (f6). Two flexion evoking INs have specific and direct excitatory synaptic connections with these MNs. One of these, IN 201, produced short latency e.p.s.p.s in the left e5 and the right f6; the other, IN 301, produced e.p.s.p.s in both left and right f6s. The short latency of these responses and the constant appearance of the response following every IN spike suggest that the connections are monosynaptic.

All five INs also activate other intersegmental INs. For example, both IN 201 and 301, which have axons that project posteriorly, activate ascending units that spread a weaker but in all respects identical motor program to the first abdominal ganglion. Thus, although INs 201 and 301 clearly have a premotor or driver IN function, they also have the capacity to turn on other sets of INs that spread the motor program to distal ganglia. To test for the possibility that some INs were contributing to the motor program generated by other INs, we impaled pairs of INs to test for synaptic coupling. Of seven different pairs tested, three pairs showed excitatory connections while a fourth showed weak inhibition. The strongest excitatory connection was monosynaptic between ascending IN 503 and IN 201. While IN 503 had an extension motor program in the most anterior two segments, it produced a flexion in the posterior segments. A careful analysis of the motor programs of both INs when depolarized separately, suggested that the 201 motor program contributed significantly to the motor program initiated by IN 503.

182.14 EVIDENCE THAT SEROTONIN MEDIATES RESTRAINT-INDUCED INHIBITION OF THE CRAYFISH'S LATERAL GIANT ESCAPE RESPONSE. J. Gunther*, D. L. Glanzman, and F. B. Krasne (SPON: M. E. Barish). Department of Psychology, UCLA, Los Angeles, CA 90024 and Marine Biological Laboratories, Woods Hole, MA 02543.

When a crayfish is restrained -- for example, by being picked up and held -- its lateral giant (LG) escape response is suppressed (Krasne and Wine, J. Exp. Biol., 63:433, 1975). Recently, Glanzman and Krasne (J. Neurosci., 3:2263, 1983) found that serotonin (5-HT) also suppresses the LG escape response; moreover, the effects of 5-HT upon the neural pathway underlying the escape response closely parallel those produced by restraint. This finding raises the possibility that 5-HT might mediate restraint-induced inhibition of escape behavior.

To test this possibility, we treated crayfish with 5,7-dihydroxytryptamine (5,7-DHT), a cytotoxic analogue of 5-HT which selectively damages serotonergic neurons (e.g., Glover and Kramer, Science, 216:317, 1982). Crayfish, weighing 4.5-9.0 g, were injected with 2.0-4.0 mg of 5,7-DHT. The injections contained 2.5×10^{-2} M 5,7-DHT in a carrier solution of HEPES-buffered crayfish saline with 0.1%-0.5% sodium L-ascorbate.

We examined the effects of 5,7-DHT on serotonergic pathways in crayfish with immunohistochemical techniques. The nervous systems of 5,7-DHT-treated crayfish were depleted of 5-HT as indicated by their reduced 5-HT immunofluorescence compared to nervous systems of control animals. Also, the toxin produced an abnormal brown pigmentation in serotonergic cell bodies.

To assess the neurotoxin's effect on escape behavior, we compared the stimulus threshold necessary for eliciting an LG response in restrained 5,7-DHT-treated and restrained normal crayfish. The sensory neurons for the escape response were stimulated by taps to the animals' abdomens and activity of the LGs, the command neurons for escape, was monitored with extracellular electrodes. Escape responses were more readily evoked in restrained 5,7-DHT-treated animals than in restrained normal animals ($p < .01$).

These results suggest that 5-HT is necessary for restraint-induced inhibition of the LG response. Possibly, 5,7-DHT disrupted a tonic inhibitory system unrelated to restraint, thereby facilitating escape in the toxin-treated animals. However, escape does not appear to be under tonic inhibition, as indicated by data from acute experiments including intracellular recordings from the LGs.

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182.15 PHENTOLAMINE BLOCKS INHIBITION OF SWIMMERET BEATING BY INHIBITORY COMMAND INTERNEURONS. B. Mulloney, A.G. Bradbury*, L. D. Acevedo* & W. Hall* Zoology, Univ. California, Davis, CA 95616.

Three pairs of command interneurons that inhibit the rhythmic beating of swimmerets in crayfish have been described by Wiersma & Ikeda (1964). Stimulation of each of these interneurons effectively inhibits the motor pattern that drives the swimmerets. Bradbury & Mulloney (1982) discovered that octopamine mimicked this inhibition; perfusion of the system with 10^{-6} to 10^{-4} M octopamine also inhibited the motor pattern. To test the hypothesis that some inhibitory command interneurons use octopamine as their normal transmitter, we tested the ability of a well-characterized octopaminergic blocker, phentolamine (Evans, 1981), to block the inhibition by command interneurons.

Bundles of axons that contained an inhibitory command interneuron were dissected from the connective between abdominal ganglia 1 and 2. The swimmeret motor pattern was recorded from first roots of ganglia 2 through 5. The entire abdominal nerve cord was continuously perfused through the ventral artery with either control or test solutions under pressure. The control solution was normal crayfish saline with 10^{-5} M proctolin; the test solutions were the normal crayfish saline with 10^{-5} M proctolin plus various concentrations of phentolamine. Each interneuron was tested by stimulation for 30 seconds at 30, 40 and 60 Hz during perfusion with control solution, the test solution and again by the control solution.

Phentolamine competitively and reversibly blocks inhibition of the swimmeret rhythm by one of the inhibitory command interneurons described by Wiersma & Ikeda. In the working range (10^{-6} to 10^{-4} M phentolamine), this block can be partially overcome by stimulating the interneuron at higher frequency.

Certain other inhibitory command interneurons are insensitive to phentolamine. These two inhibitory pathways also differ in that the phentolamine-sensitive pathway reduces burst duration but does not affect burst period, but the phentolamine-insensitive pathway has a pronounced effect on burst period.

These results are consistent with the hypothesis that certain of the inhibitory command interneurons of the crayfish swimmeret system use octopamine as a transmitter.

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182.16 **AFFERENT EXCITATION OF COMMAND-EVOKED BEHAVIOR.** by Ted W. Simon* and Donald H. Edwards, Jr. Department of Biology, Georgia State University, Atlanta, Ga. 30303.

The debate over the appropriateness of the term "command neuron" has focused on whether cells that can release organized motor outputs or behavior when stimulated electrically mediate the same responses to natural stimuli. We wish to report that a motor response and behavior of crayfish that are released by electrical stimulation of command neurons can also be released by excitation of a pair of sensory neurons, the caudal photoreceptors (CPRs). The motor response produced by CPR excitation appears to be mediated by excitation of those same command neurons.

Light directed at the abdominal ventral surface of a blinded, freely behaving crayfish will evoke backward walking and repetitive postural tail flexions. Light directed at the last abdominal ganglion of a restrained animal excites the CPRs, which fire a brief, high-frequency burst followed by a sustained lower frequency train of spikes. The CPRs are known to make no connections within the abdomen, but instead project their axons into the rostral part of the ventral nerve cord. We have found that if the initial burst of the CPRs is greater than 60 Hz, the light stimulus will evoke leg motion and a sustained bursting discharge from the abdominal tonic flexor motoneurons. These same responses occur when a single CPR is stimulated electrically at 70 Hz. Identical motor responses can be evoked by electrically stimulating axons on the lateral margin of one abdominal ganglionic connective. These axons are strongly excited by CPR stimulation and conduct spikes into the abdomen from the rostral CNS. They are active throughout the motor response to CPR excitation and are also active during spontaneous motor bursts that are accompanied by leg motion. These axons occupy the same location in the nerve cord as a set of command neurons that, when tonically stimulated, can produce backward walking and repetitive abdominal flexion. Our current conclusion is that motor responses to abdominal illumination occur because CPRs excite command neurons for these responses.

182.17 DIFFERENT NEUROPILS IN THE ABDOMINAL GANGLIA OF A CRAYFISH HAVE DISTINCTIVE INTEGRATIVE FUNCTIONS. E.M. Leise and B. Mulloney. Zoology, Univ. of California, Davis, CA 95616.

In crayfish, the core of each abdominal ganglion contains four longitudinal tracts of axons running between ganglia that alternate with commissures connecting hemiganglia. Distinctive synaptic neuropil occurs in 4 major areas: in two lateral bean-shaped neuropils (LN), in a ventral horseshoe-shaped neuropil (HN), and within the tracts and commissures as tract neuropil (TN) (Skinner 1982, Ph.D. Thesis, UC Davis). To discover the functions of these different neuropils, we exploited the functional segregation of motor and sensory axons in peripheral nerves that leave the ganglion.

Each abdominal ganglion innervates peripheral structures via 3 pairs of nerve roots. The first roots innervate that segment's ventral appendages, the swimmerets. The anterior branch of this root innervates the return stroke muscles of the swimmeret; the posterior branch innervates the power-stroke muscles. The anterior branch of each second root contains mainly sensory axons from the body wall; the posterior branch contains axons to the extensor muscles and from the muscle receptor organs. The third roots innervate only the flexor muscles. The first two pairs of roots carry both sensory and motor axons. The third roots carry only motor axons.

A series of cobalt backfills of nerve roots from the abdominal ganglia of the crayfish, Pacifastacus leniusculus, were examined in both whole mounts and in thick sections. Individual motor neurons filled with HRP were also studied. Each LN contains dendritic arborizations from both branches of its ipsilateral first root with a few dendrites from the contralateral posterior branches of the first and second roots. Different regions of the HN receive arborizations from first and second root axons. The first roots send some fibers to the ventral arms of the HN but not to the curved portion. The posterior branch of the second root arborizes extensively throughout most of the HN. By contrast, results to date show that fast flexor axons have virtually no branches in the LNs or HN but arborize extensively in the TNs of the dorsal commissure and tract. Together, these results indicate that discrete regions of neuropil within each abdominal ganglion receive dendrites from different nerve roots and hence mediate different motor activities.

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182.18 CENTRAL MODULATION OF PROPRIOCEPTIVE INPUT TO THE ISOLATED 4th THORACIC GANGLION OF THE CRAYFISH. P.Skorupski*, K.T. Sillar* and B.M.H.Bush* (SPON: S.J.W.Lisney). Dept. of Physiology, University of Bristol, Bristol BS8 1TD, U.K.

Crustacean walking is an example of a rhythmic behavior where there has not yet been a direct demonstration of a central pattern generator (cpg). To investigate interaction between any such cpg and pertinent mechanosensory inputs, we have developed a preparation of the isolated thoracic nerve cord of the crayfish. The only sensory input to the CNS is from the intact thoracic-coxal muscle receptor organ (TCMRO) of the right 4th walking leg, connected by its two large non-spiking afferent fibers to the penultimate thoracic ganglion.

Recording from the cut ends of the nerve roots to the promotor, remotor and levator muscles reveals a substantial amount of centrally patterned activity, in the absence of any phasic sensory feedback. Many preparations show spontaneous, rhythmic bursts of impulses in promotor and levator motor neurons alternating with remotor bursts. These have the appropriate phase relationships for forward walking, although the cycle period is usually 2 to 5 times longer than that observed in intact animals. In quiescent preparations a similar motor output pattern can be evoked in various ways, e.g. by stimulating a nerve root. Intracellular recordings from a variety of walking motor neurons and local nonspiking interneurons during such activity reveal large membrane potential oscillations phase-locked to the peripherally recorded rhythm, suggesting graded synaptic control. Widespread inhibitory and excitatory connections between motor neurons may also contribute to pattern generation.

This preparation is also reflexly viable since controlled stretch of the TCMRO normally evokes a promotor resistance reflex, similar to that observed in crabs (Cannone & Bush, 1980, J.Exp.Biol.86, 275-331). However this reflex is highly variable and can be centrally modulated in several ways, particularly during rhythmic motor output. Recordings from the central terminals of the TCMRO afferents during such activity reveal that these receive central input correlated with the oscillatory drive to the motor neurons. This input, which can produce 8mV shifts in membrane potential, may represent central modulation of reflex function. In one experiment, for example, we recorded from a promotor motor neuron and applied stretch stimuli to the TCMRO during rhythmic motor output. During promotor bursts the afferent input was excitatory and increased spike frequency, while during remotor bursts this input became reversed in sign. This may be important in gating sensory input during locomotion.

182.PO SENSORIMOTOR INTEGRATION IN THE CRAYFISH SWIMMERET SYSTEM W.J. Heitler. Gatty Marine Lab., St. Andrews, Fife KY16 8LB, Scotland.

There are 3 sensory systems monitoring the swimmerets of the crayfish Pacifastacus leniusculus; an elastic strand spanning the swimmeret base innervated by 2 non-spiking stretch receptors (nssrs) with central cell bodies, a similar elastic strand innervated by several spiking stretch receptors with central cell bodies, and a variety of spiking hair cells and stress detectors. Inter-ganglionic interneurones have been identified which receive input from spiking receptors, but these have not been studied in detail. Research has concentrated on the nssrs, which both depolarize with swimmeret retraction. Injecting depolarizing current into a single nssr inhibits several retractor motorneurones, while injecting hyper-polarizing current excites them. Various interneurones are also affected. Sinusoidal current injected into a nssr at a slightly different frequency to that of spontaneous rhythmic activity induces beat-frequency modulation in the motorneurones, as does applying sinu-soidal movement at the same frequency to a single swimmeret. There is no indication of oscillator entrain-ment. Holding a swimmeret retracted decreases the amplitude of membrane potential oscillation expressed by retractor motorneurones of the same ganglion, while holding it protracted has the opposite effect. Movement applied to the swimmeret of one ganglion can also modulate the amplitude of the rhythm expressed in motorneurones of adjacent ganglia. Thus the nssrs are primarily concerned with amplitude modulation of the swimmeret rhythm through resistance reflexes, and the sensory systems of a single swimmeret do not appear to have significant access to the central pattern generator.

Some motorneurones display non-linear plateau-like responses to injected current. Similar responses can be elicited by swimmeret movements, and are probably mediated by the nssrs. This suggests that an additional function of the nssrs may be control of the plateau "flip-flop" in motorneurones.

LOCOMOTION I

183.1 THE WORK OF THE CAT HINDLIMB MUSCLES DURING LOCOMOTION. W.B. Marks, G.E. Loeb, and W.S. Levine*, J.P. Chapelier*, and W.M. Roberts*. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205 and Dept. of Electrical Engineering, Univ. of Maryland, College Park, MD 20747.

When walking at a constant velocity on a level surface, the only net work done by the muscles of the cat hindlimb is against internal friction, which is relatively small. Muscle work done to cause oscillatory motions should sum to zero, although individual muscles may do mostly positive work (active force generation while shortening) or negative work (active force generation while lengthening). Also, many muscles cross more than one joint; their force may represent positive work on one skeletal segment combined with negative work at another.

To survey these patterns, we developed geometrical models of the anatomical arrangements of 33 cat hindlimb muscles to calculate muscle length trajectories during walking from digitized, videotaped stick figures. Muscle force output was measured directly by implanted strain gauges or (more usually) estimated from chronically implanted EMG records. The table divides the muscles into classical extensors and flexors (based on period of normal activity in the step cycle), and indicates the direction of the length change (+ shortening, - lengthening, 0 isometric) caused by the motion at joints across which they insert (Hip, Knee, Ankle, Toes) during active force generation. Two symbols indicate sequential motion during a single EMG burst; two lines indicate two EMG bursts/step cycle. NET shows the work based on the overall length trajectory of the muscle. Several biarticular muscles appear to convert positive work done on one limb segment into zero or negative net work through motion of an adjacent limb segment. This strategy may improve overall efficiency by taking advantage of the low energy consumption of actively lengthening muscle. However, it may complicate control and coordination considerably.

FLEXORS	H	K	A	T	NET	EXTENSORS	H	K	A	T	NET
iliopsoas	+				+	biceps anterior	+				+
sartorius medial	+	+			+	rectus femoris	-	+			-
sartorius anter.	+	+			+	sartorius anter.	-	+			-
semitendinosus	-	+			0	semitendinosus	+	-			0
tibialis anterior			+		+	vastus lateralis		0			0
flexor dig. long.			-	+	-	vastus intermed.		-0			-0
						gastrocnemius		0	0		0
						soleus			0		0
						flexor hall. long.			0	-	-

183.2 A QUANTITATIVE COMPARISON OF HINDLIMB MUSCLE ACTIVITY AND FLEXOR REFLEXES IN NORMAL AND DECEREBRATE CATS DURING WALKING. S.H. Duenas*, G.E. Loeb and W.B. Marks. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205

After high decerebration, cats can generate spontaneous locomotor movements of the hindlimbs. However, it remains unclear which of the details of normal motor coordination are well-preserved and which parts of the gait generating apparatus may be operating in a different state. We have addressed this question by chronically implanting a variety of length, force, and EMG recording devices in order to record the normal activity patterns during normal walking with and without electrical stimulation to cutaneous nerves (to generate gait-dependent flexor reflexes), and then decerebrating the animal and recording the analogous activities. During decerebrate locomotion, an elastic support at the back absorbed whatever weight was unsupported by the extensor muscles.

The kinematic pattern of knee joint motion in decerebrate walking covered the same angular range as normal but without the usual E1 phase extension prior to footfall and without E2 yielding at footfall. Knee extension force (measured at the patellar ligament) could be nearly normal in amplitude and temporal pattern (early post-decerebration), but frequently dropped to 20-30% of normal over several hours of intermittent walking. The EMGs of all three monarticular knee extensors (vastus muscles) were reduced in amplitude but normal in timing. The EMG amplitude of the hip extensor anterior biceps was greatly increased and the ankle extensors were divided, with reduced soleus and normal or increased lateral gastrocnemius activity. Pure flexors of the hip (iliopsoas) and ankle (tibialis anterior) generated increased EMG bursts at appropriate times. Biarticular muscles had complex amplitude changes which were often different for each of their two phases of recruitment per step cycle. Only posterior biceps had a significant change in the timing of its activity.

Flexor reflexes in the normal included complex patterns of short and long latency excitation and inhibition, depending on site of stimulation (saphenous or sural nerve), phase of the step cycle, and anatomical function of the muscle. Almost all reflex responses disappeared for identical stimuli delivered during decerebrate locomotion, in muscles with increased, as well as those with decreased, locomotor EMG activity. However, greatly reduced decerebrate recruitment of knee and ankle extensors might have obscured their typical inhibitory reflexes.

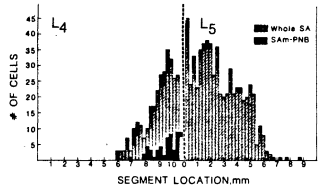
183.3 ARCHITECTURAL FEATURES OF SHORT, SERIES MUSCLE FIBERS IN CAT SARTORIUS AND TENUISSIMUS MUSCLES. A.J. Rindos, G.E. Loeb, F.J. Richmond and O. Morris. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205, and Dept. of Physiol., Queen's Univ., Kingston, Ont., Canada

The cat sartorius and tenuissimus muscles generate active tension during large physiological excursions. According to the sliding filament hypothesis, such motion should cause large changes in tension output with changes in the thick/thin filament overlap and rate of cross-bridge motion. However, this assumes that the motion of the whole muscle is conveyed proportionally to individual muscle fibers and sarcomeres in such nonpinnate muscles. To test this we optically measured sarcomere intervals in longitudinal slices from sartorius and 32 other hindlimb muscles (with pinnate and nonpinnate architectures) using 6 limbs set at various joint positions in rigor. When plotted as changes in length relative to minima observed, sarcomere length changes for most muscles were either proportional to muscle length (nonpinnate muscles) or greater (pinnate muscles), in agreement with traditional muscle architecture models. In tenuissimus, however, sarcomere length changes were proportionately much smaller than muscle length changes (slope 0.21); in sartorius, a similar tendency was masked by variability among different parts of the muscle and over time. Both results suggest significant, heretofore undescribed, elastic series elements.

To understand the possible architecture underlying such series elasticity, we dissected and measured the lengths and locations of individual muscle fibers in gold-stained, glycerine permeated, complete longitudinal fascicles from anterior and medial sartorius. Individual muscle fibers were 1/6th to 1/7th the length of their fascicle, which ran the length of the muscle without apparent connective tissue planes or inscriptions between fibers. The fibers had long tapering ends which interdigitated with other fibers. A similar organization was noted in tenuissimus by S. Cooper in 1929 (J. Physiol. Lond. 67,1), but has never been modeled mechanically. We hypothesize a connective tissue arrangement which conveys tension among the parallel and series fibers and permits an elastic shifting of degree of interdigitation of the muscle fibers, conserving sarcomere length for large physiological excursions in these two and perhaps other similar muscles.

183.4 ORGANIZATION OF THE CAT SARTORIUS MOTONEURON POOL. C.A. PRATT, W.J. YEE*, C.M. CHANAUD and G.E. LOEB. LABORATORY OF NEURAL CONTROL, NINCDS, NIH, Bethesda, MD 20205

The cat sartorius muscle (SA) is a homogeneous anatomical unit (ie., devoid of any intramuscular partitions) which is functionally heterogenous (see adjacent poster by Loeb et al.). Previous work from this laboratory showed that the SA motoneuron (MN) pool is functionally divided into three independent task groups, one associated with SA pars medialis (SAm) and two with SA pars anterior (SAa), each subserving a kinematically distinct task during locomotion (Hoffer et al., Neurosci. Abstr. 8,946, 1982). In the present study, retrograde transport of horseradish peroxidase (HRP) was used to determine the size and spatial distributions of MNs innervating SAa and SAm. The whole SA nerve in one limb and a single longitudinally-running primary nerve branch (PNB) going to either SAa or SAm in the other limb were cut and soaked in a 30% solution of HRP.

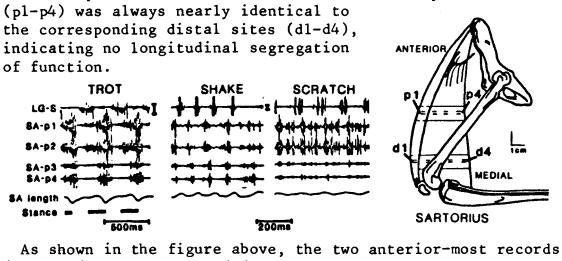


The SA MN pool, as measured in 8 cats, was usually about 1.5 spinal segments in length ($\bar{X}$=14mm) and was located between the midportions of L4 and L6. SAa MNs tended to be located in the caudal portions of the SA MN pool while SAm MNs were rostrally situated. The figure shows the spatial distribution of MNs supplying the second-most medial SAm PNB vs. that of the whole SA nerve in one cat. All but one of the SAm MNs, a gamma MN, were located in the rostral 14%-35% of the SA nucleus. The size distributions for the 511 SA MNs and the 43 SAm PNB MNs were similar. In each, gamma MNs comprised an unusually high 50% of the total. Our data indicate that there is a topographical relationship between the rostro-caudal position of SA MNs in the spinal cord and the medial-anterior termination of their axons in the muscle. There was a tendency for SAa PNB nuclei to be more elongated than SAm nuclei; this is consistent with the SAa nuclei having more than one task group, but the SA MN pool does not appear to be strictly segregated on the basis of task groups.

183.5 SEGREGATION OF NORMAL AND REFLEX ACTIVITY IN THE CAT SARTORIUS MUSCLE. G.E. Loeb, C.A. Pratt and W.B. Marks. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205.

Different patterns of EMG activity occur in the medial part of the cat sartorius muscle (SAm) than in the anterior part (SAa) during walking. SAm (a knee and hip flexor) actively shortens during early swing phase; SAa (a knee extensor and hip flexor) actively shortens during late swing phase and actively lengthens during late stance phase. There appear to be three distinct task groups of motoneurons recruited separately to generate each of the three EMG bursts (Hoffer et al., Neurosci. Abstr. 8, 946, 1982).

The present study was designed to define more accurately the spatial distribution of the various EMG patterns during walking, trotting, shaking, scratching and flexor reflex activity. Eight separate bipolar electrodes were positioned on the inner surface of the SA in two fascial surface patch arrays (proximal and distal), with four sites distributed in each array. The time course of EMG from each proximal sites (p1-p4) was always nearly identical to the corresponding distal sites (d1-d4), indicating no longitudinal segregation of function.



As shown in the figure above, the two anterior-most records (p1 & p2) were always similar to each other but were often different from the two medial records (p3 & p4). Phase differences were distinct during slow and fast walking and were subtle during shake. Scratching produced mainly amplitude differences. Similar activity was recorded from all four sites during flexor reflexes evoked by electrical shocks to the saphenous and sural nerves during walking. The variety of relative timings and amplitudes of EMG from the two parts of the SA muscle, as well as the abrupt boundary between them (note differences between adjacent electrodes p2 & p3) suggest there is a functional subdivision of the SA nucleus which is embedded in the central pattern generators for each movement and which permits task dependent recruitment of any combination of these two parts of the muscle.

183.6 SPEED AND GRADE EFFECTS ON FORCE AND EMG IN CAT SOLEUS AND MG MUSCLES. R.J.Gregor, R.G.Lovely, V.R.Edgerton, R.R.Roy and M.K.Kuehl Brain Research Institute and Dept. of Kinesiology, UCLA, LOS ANGELES, CA 90024

The response of cat hindlimb extensors to the changing kinetic demands imposed by increased treadmill speeds has been documented (J.Neurophysiol. 41:1203-1216,1978). While EMG response to increased grade has been reported for the guinea pig (J.Appl.Physiol.,52:451-457,1982) no data are available in cat muscle. This study assesses the relationship between muscle force production and associated EMG, in-vivo, in the cat medial gastrocnemius (MG) and soleus (SOL) muscles as a function of treadmill speed and grade.

Fifty-five step cycles in one cat were analyzed at speeds ranging from 1.3m/s to 2.7m/s and grades ranging from 0 to 30 per cent. Force transducers were surgically implanted on the SOL and MG tendons (J.Biomechanics, 16:691-701,1983) and bipolar electrodes (50um) implanted into the belly of each muscle (Brain Res., 117:529-533,1976). Three additional cats were implanted for EMG and studied over a range of speeds and grades. Records were digitized off-line using a Digital MINC 23 computer. Duration of force and EMG, integrated EMG (IEMG), impulse, peak force and EMG, and mean EMG (IEMG/duration) were measured.

EMG response to speed and grade was similar in all cats. As speed of locomotion increased IEMG and impulse decreased in both the SOL and MG. Peak and mean SOL EMG did not change implying a constant activation. Peak SOL force, however declined. Peak and mean EMG and peak force in the MG increased with increased treadmill speed. While duration of force and EMG declined in both SOL and MG with increased speed this was not the case at a constant speed with increased grade. EMG durations did not change. As grade increased, SOL IEMG was relatively constant while impulse and peak force declined. Integrated EMG, peak force and impulse increased in the MG. Additionally, the force impulse and IEMG were highly correlated across speeds and grades for the SOL (r=0.91) and MG (r=0.96).

The reasons for decreased SOL force at relatively constant activation might be related to mechanical changes such as an increased role of synergists and variations in the time of peak force relative to shortening or lengthening velocities. Supported by NIH Grant 16333

183.7 GATING OF FORELIMB REFLEX PATHWAYS DURING LOCOMOTION IN INTACT CATS. T. Drew*, J. Provencher* and S. Rossignol. Centre de recherche en Sciences Neurologiques, Département de physiologie, Université de Montréal, Montréal, Québec, Canada, H3C 3J7.

Mechanical perturbation of the hindlimb of the cat during the swing phase of locomotion evokes responses which cause the obstructed limb to be first moved away from, and then over, the obstruction. This response is achieved principally through reflex activation of flexor muscles at around, or during the time of their locomotor activation. The response elicited in the forelimb muscles by a similar stimulation, although serving the same function, is achieved by a reflex activation of muscles both in phase and out of phase with the period of their locomotor activity.

In chronically implanted, unrestrained cats walking on a treadmill, the forelimb was either mechanically perturbed during the swing phase (6 animals) or the superficial radial nerve was electrically stimulated by a cuff electrode (3 animals). Electromyograms (EMGs) were recorded from flexor and extensor muscles of the elbow. When the cat was at rest, threshold stimulation (T), defined as the current needed to evoke a just detectable flexion reflex, evoked a small excitatory response only in the brachialis. Stimulation during the swing phase of locomotion caused an increase in the amplitude of this response; the same stimulus during the stance phase was ineffective.

Stimuli at 2*T, which were well tolerated by the animal, not only caused a marked increase in the amplitude of the brachialis response (L=8-10ms) evoked during swing but also induced large excitatory responses in cleidobrachialis (L=8-10ms) and in the two principal elbow extensors, the long and lateral heads of triceps (L=10-12ms). Kinematically the initial result of this co-activation was a pause in the forward progression of the limb, followed by a retraction of the humerus against the body. There was also a strong ventroflexion of the wrist, and an elevation of the scapula. Following this, the elbow was hyperflexed and then extended to place the foot normally. With stimuli occurring in late swing, the foot was occasionally placed prematurely. These late mechanical effects reflected changes in the EMG bursts at latencies of 40-70 ms. Stimuli at this strength given during stance generally caused no response in either flexor or extensor muscles.

Mechanical perturbation of the limb in swing caused effects similar to that of the electrical stimulation, with almost simultaneous activation of the brachialis, cleidobrachialis and the elbow extensors.

Thus, although the probability of exciting the elbow flexors closely parallels the time when these muscles are active, the largest responses in the extensor muscles were elicited in a period where the muscles are silent. It is concluded that the period of reflex responsiveness of these muscles during locomotion may be dissociated from the period of their normal activation in the step cycle. (Funded by the Canadian MRC).

183.8 RESPONSE TO PERTURBATIONS DURING LOCOMOTION IN HUMANS M. Belanger & A.E. Patla, Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada. N2L 3G1

Compensatory responses, in six muscles of the lower limb in humans, to stimulation (a 20 ms train, 10-1ms pulses, 4-5 X threshold) at five phases of the stepcycle were quantified and the appropriateness of the response examined. Five trials for each condition were ensemble averaged and then the normal cycle was subtracted from the perturbed one, yielding the reflex response. The area under the EMG curves for both the normal and the perturbed cycles were determined for 100ms starting 20ms following the onset of the stimulus. At heelstrike (HS), in response to stimulation the foot is quickly lowered to the ground while the knee is prevented from collapsing by an increase in extensor activity. In early (ES) and late (LS) stance the foot appears to be removed from the stimulus by ankle dorsiflexion, while the knee is maintained in extension by increased extensor activity. The lift-off and the subsequent swing phase are facilitated by an increase in flexor activity at the ankle, knee and hip at toe-off (TO). During midswing (MS), the foot can be withdrawn from the stimulus by enhanced flexor activity at both the ankle and hip. The latency and area data indicate that the order and amount of the muscle response are modulated during the stepcycle. The leg muscles had a shorter latency than the thigh muscles (distal to proximal response). Temporal data revealed a tendency for the perturbed cycle to be shorter than the normal cycle when the stimulus was applied at HS, ES and MS, while it was of equal or slightly longer duration than the normal during LS and TO. The major difference between the response in cats and in humans is the enhanced dorsiflexor response during ES and LS. This may be due to the high stimulus intensity used in the study. In summary, the compensatory responses in the ipsilateral limb are clearly matched to the phase of the stepcycle to provide stability and allow for locomotion to continue.

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183.9 SOME PROPERTIES OF THE HUMAN LOCOMOTOR PROGRAM Aftab E. Patla, Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada. N2L 3G1

The human locomotor program, which is characterised by a stride-to-stride repeatable muscle activity patterns, was studied under three conditions of walking on a treadmill: a) forwards at different speeds (0.5 natural speed to 2.0 N.S.), b) at three stride lengths (short, normal, long) and c) backwards. Surface EMG, from seven subjects, were recorded from: tib ant, sol, gast, vast lat, rect fem, biceps fem, semi tend and erector spinae. Rectified, filtered (10 msec) and ensemble averaged stride data for each conditiom were rubberbanded to normalise stance and swing duration values. To analyse synergies between patterns and changes in muscle activity patterns, EMG patterns were correlated. The r value measures similarity between patterns and the slope (b) indicates the gain. The following properties along with the rationale emerged. Property #1: There is separate program for extensors and flexors at each joint. - a) r values between extensors across joints (SO & VL r = -.06) and antagonists at a joint (TA & SO r = -.35) are low compared to r values between synergists at a joint (SO & GA r = .75) suggesting a motor program for each joint; b) r values between normal and large stride length are poor for flexors (BF 5 = -.1 & ST r = -.05) compared to extensors (VL r = .4 & ST r = .2) suggesting a separate program for flexors and extensors; c) postural muscle (ES) activity show a time lock with stride events for different speeds and long stride length suggesting they are programmed, but ES activity for backward and short stride length walking is different suggesting functional specification; d) a higher r value between forward extensor activity and backward flexor activity (GA & TA r = .62) suggesting flexibility of the program; e) with changes in speed from normal value, r values for each muscle reduced suggesting a change in pattern of muscle activity. Property #2: There is a distal to proximal increase in gain with independent control over extensor and flexor activity level. - a) with increase in speed proximal extensor muscles (VL at 1.5NS b = 2.6) increase more than distal muscles (SO at 1.5NS b = 2); b) for long stride knee extensor activity is higher than for short stride (short b = 3.1, long b = 4), but the flexor activity increases are opposite.

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183.10 ANKLE FLEXOR ELECTROMYOGRAM ALTERED BY LEARNING DURING HUMAN TREADMILL WALKING. M. C. Wetzel, S. A. Olivares* and R. E. Wetzel*. Psychol. Dept., Univ. of Ariz., Tucson, AZ 85721.

In previous studies, through operant conditioning, a large EMG burst was produced during the walking step cycle by a thigh muscle (rectus femoris) that usually has slight activity. Tibialis anterior (TA), in contrast, usually is active throughout most of the cycle. The present study tested the capability of operant conditioning to increase and/or decrease the walking EMG of TA.

Four subjects experienced operant conditioning to alter TA activity at heel strike, heel liftoff, or mid-swing. Later, each person was trained in at least one of the other two temporal positions of the step cycle.

Subjects were required to respond to a green light flash within 700 msec by EMG activity exceeding a specified threshold amplitude for 100-400 msec. If, instead, a red light flashed, it was to be followed by EMG activity below that threshold for the same elapsed time. For each of the three temporal positions, the first threshold setting was at high amplitude. Progression occurred to a moderate setting when a subject either passed a criterion of 90% correct responses in 2 trials, or else continued to fail frequently. A trial was 20 colored-light cycles alternating with no-light cycles. If criterion was met for both green- and red-light trials, then green and red lights were ordered randomly within trials. After training at the moderate threshold, voltage level was reduced to a low amplitude, and the conditioning procedure was repeated.

All subjects met the red light criterion at high threshold without errors at every step cycle position. Only 1 of 4 subjects passed every green light criterion, with TA activity not increased greatly above its normal level. At the moderate threshold, green and red criteria were met in all instances. At the low threshold, which was below usual walking amplitude, all subjects except 1 met the red criterion at the tested position, while only 2 of 4 met the green. With few exceptions, whenever green and red criteria were met in the study, so also was the randomized red/green sequence. In conclusion, experimentally produced operant behavior could extensively modify walking EMG, even for a muscle that normally is highly active. Although conditioning effectiveness was not unlimited, it was powerful even during the stance portion of the cycle, when the limb supports body weight.

183.11 BLENDING OF INTRALIMB SYNERGIES DURING THE COORDINATION OF TWO DISTINCT RHYTHMICAL MOVEMENTS. M.C. Carter and J.L. Smith. Dept. Kinesiology, UCLA, CA 90024.

Intralimb coordination during treadmill locomotion is characterized by flexor and extensor synergies in which homologous muscles at the hip, knee and ankle are coactive. Conversely, the paw-shake response (PSR) consists of a mixed synergy with coactivation between knee extensor and ankle flexor muscles. We previously demonstrated that normal and spinal cats could produce a PSR during the swing phase of locomotion with compensatory responses in the contralateral hindlimb (Carter and Smith, Neurosci. Abst. 107.11, 1983). The present study examined the details of the transition from in-phase activity of homologous muscles during the swing phase to the mixed synergies of the PSR.

Four normal and three spinal cats were chronically implanted with bipolar electrode wires in selected antagonistic muscles crossing each joint. Kinematic data from high-speed film and EMG records were used to characterize intralimb coordination.

At toe-off, the PSR was initiated by augmented activity of the tibialis anterior and iliopoas which resulted in a marked increase in knee and ankle flexion. Activity in the vastus lateralis (VL) was initiated during the last phase of the augmented flexor activity, thus producing knee extension coupled with ankle flexion. The first ankle extensor burst from the lateral gastrocnemius (LG) was accompanied by activity of the gluteus medius, a hip extensor not recruited during slow walking. As the PSR continued, the VL was coactive with hip and ankle flexors and reciprocally active with hip and ankle extensors. The shake ended with flexor activity which was followed by low-level extensor activity in preparation for the stance phase of locomotion. Both normal and spinal cats displayed similar intralimb coordinative patterns, however the number of PSR cycles was greater for the spinal than normal cat, 4 vs 12 cycles/PSR, respectively. Further, neither average cycle period or LG burst duration differed from PSRs elicited without locomotion.

The data suggest that if unique burst generators govern locomotion and the PSR, the CNS can blend the output to produce a smooth transition between two behaviors with distinct rhythms and muscle synergies. Alternatively, the central pattern generator maybe more facultative than previously thought, with the organization of muscle synergies based on behavioral demands. Supported by NS 19864.

183.12 LOCOMOTOR DEFICITS IN THE CEREBELLAR MUTANT MOUSE LURCHER. Pierre Fortier*, Serge Rossignol, Allan M. Smith and Richard Wetts. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec, Canada.

The heterozygous mutant mouse Lurcher (+/Lc) loses virtually all of its Purkinje cells within 60 days after birth. A substantial loss of both granule cells (90%) and neurons in the inferior olive (75%) is probably a consequence of Purkinje cell loss, (2, 3). The overall size and cell density in the deep cerebellar nuclei appear to be comparable to the normal mouse (1). The adult Lurcher is characterized by a severe ataxia and the present study attempted to analyze and compare the locomotion of Lurcher with the normal mouse. Chronic EMG electrodes were implanted in the triceps surae and tibialis anterior of one hindlimb as well as the triceps surae of the opposite side. Simultaneous video and EMG recordings were made during treadmill locomotion at varying speeds. The most striking deficit in the Lurcher is the variability in the coupling of the limbs at each girdle during locomotion. Measurements in the hindlimbs showed a mean phase of coupling of 0.52 ± S.D.30 (N = 131) for Lurcher vs 0.49 ± 0.07 (N = 317) for the normal. Although the mean phase is similar, the much larger SD indicates a great irregularity in coupling resulting in frequent loss of equilibrium. In addition, the step lengths of Lurcher were shorter and often unequal producing the lurching gait. The net effects limited forward progression to about a maximum of 148 mm/sec ± 30 compared to 230 mm/sec ± 42 in the normal mouse under our conditions. The pattern of the average EMG in tibialis and triceps surae did not differ markedly from the normal mouse. These two antagonists were reciprocally active at all treadmill speeds. Surprisingly, in Lurcher the rhythmic hindlimb scratching movements were of a normal frequency (15Hz to 20Hz) and the limb movements in swimming showed a regular alternating phase coupling (0.47 ± .08). The results suggest that although spinal pattern generators may be able to produce the rhythmic movements of swimming and scratching, the coordination of terrestial locomotion may be more dependent upon supraspinal centers which are greatly influenced by the cerebellar cortex.

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183.13 PREDICTION MODEL FOR THE SHARK CEREBELLUM Richard S. Babb, Iona College, New Rochelle, N.Y. 10801

Shark swimming movements are produced by transverse waves traveling along the truncal musculature which are generated by neural oscillators in the spinal cord acting on motoneurons. Higher centers modify the action of these oscillators so as to change the amplitude and frequency of these waves but not their wavelengths (Grillner et al., 1976). Perturbations of swimming movements may be corrected by negative feedback. Such an arrangement would compare intended movement of the higher centers with information about actual movement derived from proprioceptors. This negative feedback is, however, likely to introduce instability for rapid swimming movements, since the correction would then be applied with a significant phase difference. By inroducing a feedforward or predictive component in parallel with the feedback circuit, the problem of instability could be mitigated. Since the cerebellar circuit of the corpus is in parallel with the direct motor circuit, it is a viable candidate for this role. The parallel fibers may well have the property of converting space into a time domain (Freeman and Nicholson, 1970) and hence be able to alter the phase relation between the actual and neurally intended movement. As waves of contraction travel along the trunk, the stretch receptors will be activated and send signals via the spinocerebeller tract producing corresponding neural waves traveling longitudinally within the molecular layer of cerebellar cortex. Command signals to the motoneurons are transmitted by the climbing fibers. These will also produce a wave of activation travelling down the length of the cerebellar cortex. With only negative feedback acting, these two cerebellar waves would be out of phase for rapid swim movements and would not act coincidently on the Purkinje cells. The disinhibition could act to shift the phase in the opposite direction to that produced by negative feedback so that instability does not result at high speeds. Coincidence of the two cerebellar cortical waves on the other hand would produce maximal inhibitory activation of Purkinje cells and no phase shift. The model is consistent with the findings of Tilney (1923) who showed that removal of the dorsal part of the corpus of the cerebellum resulted in an animal which, although it can remain oriented, is unable to swim effectively. This hypothesized mechanism may also underly the control of human posture involving the cerebellar vermis, since this structure is homologous with the corpus of the shark cerebellum.

183.14 **IN VIVO RECORDINGS REVEAL DISCHARGE PATTERNS UNDERLYING DIFFERENT BEHAVIORS IN LARVAL SEA LAMPREYS, PETROMYZON MARINUS.** Galen Eaholtz, Joseph Ayers, and Gail A. Carpenter. Marine Science Center, Nahant MA 01908 and Departments of Biology and Mathematics, Northeastern University, Boston, MA 02115.

Ammocoetes of the sea lamprey, Petromyzon marinus, exhibit several behaviors produced by lateral axial undulations, but the underlying neuromuscular discharge patterns for behaviors other than swimming and that of specimens recovered from spinal transection have yet to be determined. In the present investigation, we employ correlated electromyography and cinematography to relate the motor unit discharge patterns to the resulting behaviors (swimming, burrowing, and crawling). In addition, we have been able to compare the discharge underlying voluntary behaviors with that of IN SITU "fictive locomotion" in ammocoetes with their spinal cords exposed to a d-Glutamate saline.

Lamprey behaviors can be differentiated in terms of frequency and curvature of flexion waves but not in terms of intersegmental phase (Science 221: 1312-1314). Our EMG analysis indicates that these differences, in part, result from a change in the relative proportion of the cycle occupied by the burst of motor neuron discharge. For example, during burrowing, the burst of spikes occupies a greater proportion of the cycle than during swimming. In specimens, with the middle segments of the spinal cord exposed to a d-Glutamate bath, the exposed regions exhibited "fictive locomotion", but this behavior is supressed by voluntary behaviors such as swimming.

Specimens recovered from spinal transection exhibit clear deficits in their recovered behaviors. The EMG analysis of recovered behaviors indicates that a similar relationship exists between the relative proportion of the cycle occupied by the burst as found in normal specimens.

184.1 RETURN OF WEIGHT-SUPPORTED LOCOMOTION IN ADULT SPINAL CATS. C.A. Giuliani, N.C. Carter, and J.L. Smith, Dept. Kinesiology, UCLA CA 90024.

It has been reported by Eidelberg et al. (Exp. Brain Res. 40:247,1982) that adult spinal cats tested daily for 2 mos never achieved weight-supported locomotion, but were capable of hindlimb stepping. Recently, Rossignol et al. (Neurosc. Abst. 47.1,1982) reported one adult spinal cat developed weight-supported locomotion over a 3 mo period. The purpose of this study was to futher examine the return of locomotion in adult-spinal cats.

Twenty-one adult cats were tested on a treadmill at 4-5 mos following spinalization at T-12. None of the cats received any treadmill training prior to testing. Of the 21 cats tested, 7 were capable of full weight-supported treadmill locomotion at 0.2 and 0.4 m/s, and all were capable of weight-supported standing. Cats not capable of weight-supported locomotion exhibited reciprocal stepping without good paw placement.

Four cats were chosen for EMG and film analyses of locomotion. At speeds of 0.2 to 0.5 m/s the mean cycle period decreased from 904 to 668 ms. The soleus burst duration was correlated to cycle period and decreased from 542 to 325 ms, while the burst duration of the tibialis anterior was independent of cycle period. Intralimb EMG synergies were consistent with that of normal locomotion with flexor bursts initiated 71±6% after the onset of extensor activity. Interlimb coordination was 180 degrees out-of-phase, typical of a walking gait.

Kinematic data at two speeds (0.2 & 0.4 m/s) were obtained by digitizing 16 mm film (100 fr/s), and displacement data for hip, knee, and ankle joints were compared to similar data of treadmill data in normal cats (Neurosc. Abst. #47.6, 1982). Spinal cats showed no yield at the ankle and knee during stance. Further, at the end of stance the ankle was more extended, while the hip was more flexed than that of normal cats. Similar differences in locomotion were reported for cats decerebrate as kittens (Smith, et al. Exp. Neurol. 76:393, 1982).

Our data show that weight-supported locomotion will return in some (33%) adult spinal cats without daily training. The results suggest that spontaneous return of hindlimb locomotion in adult spinal cats occurs later than in spinal kittens. This does not preclude the possibility that training may facilitate the recovery process.
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184.2 THE MESENCEPHALIC LOCOMOTOR REGION (MLR) IN THE RAT. I. ELECTRICAL ACTIVATION. R. D. Skinner and E. Garcia-Rill, Dept. Anatomy, Univ. Arkansas, Little Rock, AR 72205.

Following a precollicular-postmamillary brainstem transection in the cat, controlled locomotion on a treadmill can be induced by electrical stimulation of the MLR. This study was undertaken to determine whether or not controlled locomotion on a treadmill could be induced in the rat by electrical stimulation of the brainstem. Brainstem transections were performed on barbiturate anesthetized rats using suction ablation. Previous studies in the cat had revealed that the substantia nigra (SN) appears to modulate MLR activity. Indeed, locomotion could not be induced in rats in which the brainstem transections impinged on the SN. The precollicular-prenigral transection (A 4.5) then, appears to be the rat equivalent of the cat precollicular-postmamillary transection. Electrical stimulation of the posterior midbrain was effected using 100μ wires (50 KΩ). Pulses 1 ms in duration at a frequency of 60 Hz were applied as the stimulating electrode was lowered. Locomotion on a treadmill could be induced from the lateral cuneiform nucleus and the pedunculopontine nucleus (PPN) at posterior levels. At more anterior levels, locomotion could be induced by stimulation of the PPN. The mean threshold for inducing locomotion following electrical activation of these areas was 25.4 ± 13.1 μA (mean and S.D.). Locomotion could be induced for only short periods (1-3 min) of constant stimulation. Electromyographic recordings from one flexor or extensor muscle in each limb revealed that, for a constant treadmill speed, the step cycle could be increased from a walk to a trot to a gallop by increasing the pulse amplitude in 3-5 μA steps.

On a different group of rats, more anterior (A 7.5) transections were performed and the brains studied for the presence of a "subthalamic locomotor region". Stimulation of a site within the fields of Forel was found to induce locomotion on a treadmill. Similar thresholds were evident in this area compared to the MLR, but less control could be exercised over the frequency of the step cycle.

These results reveal the presence of both subthalamic and mesencephalic locomotor regions in the rat brain. Stimulation sites, electrical thresholds, stimulus parameters and characteristics of induced locomotion are similar to those observed in the cat.
Supported by NIH (NS20246) & NSF (IP8011447)

184.3 THE MESENCEPHALIC LOCOMOTOR REGION (MLR) IN THE RAT. II. CHEMICAL ACTIVATION. E. Garcia-Rill and R. D. Skinner, Dept. Anatomy, Univ. Arkansas, Little Rock, AR 72205.

Previous studies from our laboratories have demonstrated that locomotion on a treadmill can be induced by chemical means in the precollicular-postmamillary brainstem transected cat. This study was undertaken to determine whether or not locomotion on a treadmill could be induced in the rat by infusions of putative neurotransmitters, their agonists and antagonists into the MLR. Precollicular prenigral brainstem transections were carried out on barbiturate anesthetized rats using suction ablation. A 35g cannula (internal volume of 0.5 μℓ) with an insulated wire (75 KΩ) stylette which protruded 250μ beyond the tip of the cannula was lowered into the posterior midbrain. Electrical stimulation was applied as previously described. Once controlled locomotion could be induced by low threshold (<50 μA) stimulation, the wire was withdrawn and infusions made into the physiologically identified MLR.

Each infusion was 0.5 μℓ in volume and made at rates <0.5 μℓ/min. Infusions of the GABA antagonist picrotoxin (PIC) were found to induce locomotion. Usually, infusions totalling 0.3-0.8 μg of PIC were necessary for inducing walking movements. The effects of PIC could be blocked, in ascending order of potency, by 0.1 M GABA, 0.5 M GABA, 5 mM muscimol and 1 mM diazepam. The effects of substances like GABA and diazepam were immediate (<1 min) while those of compounds like PIC and muscimol had longer latencies (>5 min). Episodes of locomotor behavior were considerably longer (>10 min) than those possible using electrical stimulation. With increasing PIC administration, walking episodes became shorter while convulsive movements increased in frequency. Infusions of equal volumes of Fast Green and Evans blue were made in order to verify the spread of infusates. Infusion sites coincided with the lateral cuneiform nucleus, pedunculopontine nucleus, lateral brachium conjunctivum and dorsolateral mesencephalic reticular formation (A 0.7-1.2, L 1.8-2.0, H 2.5-4.0).

Our findings demonstrate that chemical activation of the MLR can induce and inhibit locomotion in the precollicular prenigral transected rat. As demonstrated in the cat, the rat MLR seems to be under tonic gabaergic input (which is blocked by infusions of PIC). The substantia nigra appears to be the only afferent to the MLR located posterior to the transection and may be partly responsible for this effect.
Supported by NIH (NS 20246) and NSF (ISP 8011447).

184.4 CHEMICAL EXCITATION OF CELLS IN THE PONS AND MEDULLA PRODUCES LOCOMOTION IN DECEREBRATE CATS. B. R. Noga*, J. Kettler* and L. M. Jordan (SPON: J. Paterson). Dept. Physiol., Univ. Manitoba, Winnipeg, Canada R3E 0W3.

A recent study (Shefchyk et al., 1984, Exp. Brain Res., in press) has demonstrated that the mesencephalic locomotor region (MLR) relays its descending information through the midline reticular formation (MRF) and the ponto-medullary locomotor strip (PLS). The purpose of this experiment was to determine whether locomotion induced by electrical stimulation of these brainstem sites is due to excitation of cell bodies or fibers. A stimulating electrode-30 gauge cannulla assembly was lowered through the cerebellum into the brainstem of precollicular-postmamillary decerebrate cats. Treadmill locomotion was induced by electrical stimulation of either the PLS or the MRF. Controlled injections of the excitatory amino acids glutamic acid (0.01M) or DL-homocysteic acid (HCA) (0.1M), or the GABA antagonist picrotoxin (0.005M), were administered at a rate of 1ul/min for 3-5min following electrically-induced locomotion. Infusion of glutamic acid into the MRF could produce weak stepping or reduce the threshold for electrically-induced locomotion by 50-75%. Injections of picrotoxin or HCA into the PLS produced locomotion in some animals. These results demonstrate that the PLS and MRF contain cell bodies capable of producing treadmill locomotion when chemically stimulated. This supports earlier suggestions that these areas contain neurons which relay neural signals for the initiation of locomotion (Orlovsky, 1970, Biofizika, 15:171-177; Shik and Yagodnitsyn, 1979, Neurophysiol.,9:95-97), and it is consistent with previous findings using reversible cooling of the MRF and the PLS to block evoked locomotion (Shefchyk, et al., 1984, Exp. Brain Res., in press). Increased metabolic activity in these areas has been demonstrated during locomotion using the 2-deoxyglucose method (Kettler and Jordan, 1984, Soc Neurosci. Abst.). Supported by the Medical Research Council of Canada.

184.5 METABOLIC MAPPING OF THE BRAINSTEM DURING FICTIVE LOCOMOTION. J. Kettler* and L.M. Jordan. Dept. Physiol., University of Manitoba, Winnipeg, Canada R3E OW3.

The purpose of this study was to determine which areas of the brainstem are activated by stimulation of the mesencephalic locomotor region (MLR). A modification of the 2-deoxy-D-glucose (2-DG) method (Sokoloff et al., 1977, J. Neurochem. 28:897-916) was employed. Cats were placed in a stereotaxic frame, decerebrated at the precollicular-postmammillary level, then stimulated with a monopolar stimulating electrode placed in the MLR. Once locomotion was induced the animals were paralyzed with gallamine triethiodide and injected with tritium-labeled 2-DG (200uCi/100g) intravenously. Fictive locomotion was then evoked continuously for 45 minutes. Neurograms from the cut nerves to tibialis anterior and lateral gastrocnemius were recorded bilaterally as a monitor of locomotion. Control animals were injected with 2-DG but were not stimulated subsequent to the injection. The brainstem of each animal was frozen and processed for autoradiography using tritium-sensitive X-ray film. Increased metabolic activity was found in both the stimulus site and the contralateral cuneiform nucleus (which corresponds to the MLR). The red nucleus and the superior olivary nucleus showed bilateral labeling. Increased activity was also observed in the midline reticular formation of the pons and medulla, including the raphe complex, as well as in the spinal nucleus of the trigeminal nerve, the ventral tegmental area of Tsai and the substantia nigra. These results are consistent with our earlier suggestion that reticulospinal cells in the pons and medulla are part of a functional relay for the initiation of locomotion by stimulation of the MLR. (Steeves and Jordan, 1984, Brain Res., in press; Shefchyk et al., 1984, Exp. Brain Res., in press). The results also suggest that the ponto-medullary locomotor strip (PLS) corresponds to the spinal nucleus of the trigeminal nerve. Supported by the Medical Research Council of Canada and the Manitoba Health Research Council.

184.6 SPATIAL SEGREGATION OF EXCITATORY AND INHIBITORY SYNAPTIC TERMINALS PRODUCING LOCOMOTOR DRIVE POTENTIALS IN ALPHA MOTONEURONS. S.J. Shefchyk and L.M. Jordan. Dept. Physiol., Univ. Manitoba, Winnipeg, Canada R3E OW3.

Previous work from this laboratory has demonstrated that the "locomotor drive potentials" (LDPs) produced in spinal cord alpha motoneurons during fictive locomotion evoked by stimulation of the mesencephalic locomotor region (MLR) in mesencephalic cats are due to alternating excitatory and inhibitory synaptic input. We have now examined the effects of intracellular hyperpolarizing and depolarizing current injections on the LDPs, and these experiments have revealed apparent spatial segregation of the excitatory and inhibitory synaptic terminals producing the LDP. Intracellular recordings from lumbar alpha motoneurons which displayed well-developed LDPs during fictive locomotion were obtained using potassium citrate-filled microelectrodes. Constant depolarizing or hyperpolarizing currents (5 to 20 nA) were injected through the recording electrode during periods of maintained locomotor activity, and the effects of the injected currents on the LDP were determined. In cases of *impalements which* were characterized by large somato-dendritic components in the action potential and small initial segment components, hyperpolarizing currents reduced the amplitude of the LDP, while depolarizing currents increased it. In cases where the impalement was in the initial segment of the motoneuron, as determined by the absence of an after-hyperpolarization and the presence of a predominant initial segment component in the spike, hyperpolarizing currents increased the amplitude of the LDP, while depolarizing currents decreased it. We conclude that the synapses providing the excitatory component of the LDP are located near the initial segment of the motoneuron, while the inhibitory synapses active during the hyperpolarized portion of the LDP are placed farther away from the firing zone of the cell, at somatic or dendritic sites. Supported by the Medical Research Council of Canada.

184.7 DOES THE AFTER-HYPERPOLARIZATION CONTROL ALPHA MOTONEURON FIRING DURING LOCOMOTION? L.M. Jordan and S.J. Shefchyk (SPON: D. McCrea). Dept. Physiol., University of Manitoba, Winnipeg, Canada R3E OW3.

The amplitude and duration of the after-hyperpolarization (AHP) in mammalian spinal cord alpha motoneurons have been accepted as determinants of "rhythmic" firing produced by step depolarization due to intracellular current injection (Gustaffson,B.,Acta physiol.scand. Suppl.,416,1974). The pattern of motoneuron firing observed during locomotion has also been attributed to the AHP (Zajac et al.,J.Neurophysiol.,43,1980). If this suggestion is true, then it must be concluded that the spinal cord central pattern generator (CPG) for locomotion provides a depolarizing input to the motoneuron sufficient to reach threshold at the appropriate time during the step cycle, and the AHP then regulates the pattern of firing which results. We have tested this suggestion by recording intracellularly from spinal cord alpha motoneurons and comparing the AHPs occurring during fictive locomotion produced by stimulation of the mesencephalic locomotor region (MLR) in mesencephalic cats with those produced by intracellular injections of depolarizing current in the absence of locomotor activity. The AHP was markedly reduced in amplitude during fictive locomotion, and its duration was correspondingly reduced. During locomotion the action potentials consistently arose from excitatory postsynaptic potentials rather than by the decay of the AHP of the preceeding spike, suggesting that the CPG for locomotion, rather than the intrinsic membrane properties of the motoneuron, regulates the pattern of motoneuron discharge during locomotion. It is possible that mammalian motoneurons, like motoneurons in Xenopus embryos (Roberts, A.,et al.,Science, 213,1981), receive an excitatory input associated with the step cycle, and another excitatory input related to each action potential. Supported by the Medical Research Council of Canada.

184.8 CHANGES IN FELINE MOTOR CORTICAL MICROSTIMULATION RESPONSES IN MUSCLES DURING DIFFERENT POSTURES AND LOCOMOTION. C.I. Palmer Institute of Physiology, Fribourg University, Switzerland.

Electromyographic (EMG) responses from intracortical microstimulation (ICMS) are not always stable. Systematic variations in these responses were sought in the awake intact cat during locomotion, while the animal lay quietly and while standing. Cats were chronically implanted with electrodes in the pericruciate cortex for ICMS using 10 to 40μA (40ms trains at 350Hz, 0.2ms pulse duration) also with EMG electrodes and electrodes in the dorsolateral funiculus at C3 to record the descending corticospinal volley from ICMS. EMG signals were rectified and averaged with respect to the onset of ICMS trains. ICMS responses were either increases or decreases in EMG activity or a combination of these. ICMS responses from the same cortical site were reproducible when the animal performed the same movement. However they varied with the posture of the animal and with phases of the step cycle. Forexample ICMS bursts of EMG activity in the long head of triceps were most reliably present when the animal was standing and during the stance phase of locomotion. They were associated with periods when the muscle was active and so the motoneuronal pool was more excitable. Excitability changes also occurred at supraspinal levels as indicated by changes in the descending volley from ICMS during different motor activity. ICMS responses did not always have this association with muscle activity. ICMS responses in extensor digitorum lateralis from stimulation at one cortical site consisted of excitatory and inhibitory EMG responses when the animal was lying, on standing only ICMS induced inhibition was seen, during locomotion the muscle was active both at flexion, when only excitatory ICMS responses were seen, and also in stance, when only inhibitory ICMS responses occurred. In conclusion, modulations and even a reversal from excitation to inhibition of ICMS responses were observed as a consequence of changes in the motor pattern. The underlying excitability changes may occur at segmental and supraspinal levels.

184.9 Suppressive and Facilitatory Effects of Stimulation of the Midbrain on Locomotion Elicited by Hypothalamic Stimulation. H. M. Sinnamon, R. N. Ginsburg, and G. A. Kurose. Laboratory of Neuropsychology, Wesleyan Univ., Middletown, CT 06457.

In anesthetized rats electrical stimulation of the hypothalamus elicits coordinated locomotion. Lesion and pharmacological evidence indicates that the deep tectum and the raphe antagonize locomotion. The principal purpose here was to determine if stimulation of the midbrain would suppress locomotion evoked by hypothalamic stimulation.

Rats anesthetized with intraperitoneal injections of Nembutal were mounted in a stereotaxic apparatus so that their limbs contacted the outer surface of a 30-cm diameter wheel which rotated when locomotor stepping occurred. Locomotion was elicited by stimulation (<6 volts, 40 Hz, 0.5 pulses) through a fixed metal electrode in the hypothalamus. Concurrent midbrain stimulation (25 uA) was applied every 200 um through movable pipettes 50-70 um in diamater, filled with 2M NaCl.

In the dorsal midbrain, stimulation of the deep, but not the superficial, layers of the superior colliculus suppressed locomotion. Stimulation at the most dorsal of these suppressive sites was frequently without any observable direct effect. However, as the electrode approached the central gray, suppressive sites which supported hindlimb responses, flexion or palpable tension, became common. In the central gray and in the lateral tegmentum flexion-suppression sites were typical. More posterior, around the dorsal raphe and cuneiformis, the direct effect of midbrain stimulation was more frequently locomotion and its effect on hypothalamic locomotion was facilitation.

In the ventral midbrain, around the medial raphe and caudalis linearis, suppressive sites were associated with post stimulation movements of hindlimbs which in some cases stepped. At these sites, the suppression of locomotion was followed by a rebound increase in locomotion. In the ventral tegmental area, midbrain stimulation frequently produced locomotion and facilitated hypothalamic locomotion. These results show that stimulation of the origin and course of fibers from the deep tectum and the medial raphe suppresses locomotion. Such suppression is consistent with inhibitory roles for these systems in locomotion.

184.10 CIRCLING ELICITED FROM MEDIAL PONS IS DUE TO STIMULATION OF BOTH CROSSED COLLICULAR AND UNCROSSED AXONS FROM INTERSTITIAL NUCLEUS OF CAJAL. E.J. Mlinar and J.S. Yeomans, Dept. of Psychology, Univ. Toronto, Canada M5S 1A1.

Electrical stimulation of many medial brainstem sites elicits rapid ipsiversive head and body movements at low currents (Hess et al., Mschr. Psychiat. Neurol. 112, 1, 1946). Contraversive circling can be elicited by stimulation of superior colliculus (SC). To determine whether these circling sites are functionally connected we used the collision method of Shizgal et al. (J. Comp. Physiol. Psychol. 94, 227, 1980). Stimulating electrodes were placed ipsilaterally in pons and interstitial n. of Cajal (INC), and contralaterally in superior colliculus (SC). The degree of collision between sites was determined by measuring frequency threshold for circling at short and long interpulse (C-T) intervals. Collision was demonstrated when frequency thresholds were higher at short C-T intervals (below 0.5 msec) than at long C-T intervals (above 4 msec).

Collision was observed between pontine and either collicular or INC sites. Collision of 15 and 60% was observed from two sites in the intermediate laminae of SC and 60% from INC. Conduction time was longer for SC sites (0.3 to 4.0 msec) than INC site (0.2 to 2.5 msec), which is consistent with the longer conduction distance for crossed pathways.

We conclude that both crossed tectal and ipsilateral pathways between pons and interstitial n. contribute to pontine circling. The sites from which collision was obtained are consistent with the anatomical trajectories of these pathways.

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184.11 FACILITATION OF VOLUNTARY MOTOR PATTERNS BY TRANSCUTANEOUS ELECTRICAL STIMULATION IN HEMIPLEGIC HUMANS. R.L. Craik*, B.A. Cozzens*, S. Miyazaki*, Moss Rehabilitation Hospital, 12th Street and Tabor Road, Philadelphia, PA 19141.

This study was designed to determine if non-painful transcutaneous electrical stimulation of a lower limb sensory nerve can alter joint motion during walking in humans with hemiplegia. Early swing phase flexion of the hip and knee was the selected movement pattern.

Fifteen patients with hemiplegia of at least 6 months duration participated in three protocols. Two surface rubber disk electrodes were placed on the skin area posterior and inferior to the lateral malleolus in the region of the sural nerve. Constant current stimulation was provided by a Grass stimulator (Model S-8C) and a constant current unit (Model CCU-IA). The minimal amplitude required to elicit a "tingling sensation" was labelled threshold. The electrical stimulation consisted of a 100 ms train of 1 ms pulses delivered at 250 hz. The current amplitude was at least 3 times threshold. Instrumentation used to measure walking performance included potentiometers mounted to measure sagittal plane hip and knee motion. Contact and release of heel and toe were monitored and walking speed was recorded with a tachometer. A minimum of seven steady state strides was compared for each subject during control and stimulus conditions. Transcutaneous electrical stimulation in the region of the sural nerve was delivered at 3 points in the gait cycle for three subjects: toe strike (S1), mid-stance (S2), heel-off (S3), toe-off (S4). The change in subsequent swing phase hip and knee motion was dependent on stimulus time - S1 decreased knee motion, S3 increased knee motion, and S4 increased ipsilateral hip and knee motion. In the second protocol the effect of S3 on swing phase flexion was examined in 7 subjects; joint motion increased in 4 subjects and decreased in 3 subjects. In the third protocol five subjects showed an increased joint excursion when the stimulus was timed to occur prior to initiation of hip flexion. The electrical stimulation evoked an ipsilateral limb effect rather than a whole body response; walking velocity did not increase and there were no significant changes in contralateral joint excursion.

These data support the hypothesis that the type of limb response evoked by additional afferent input is gait phase-dependent. In addition, the results suggest that careful selection of stimulus timing may facilitate early swing-phase flexion in patients with hemiplegia. (Supported in part by NIHR, Grant #23-P-5518.)

184.12 POSTURAL INSTABILITY IN PARKINSON'S DISEASE: MOTOR COORDINATION AND SENSORY ORGANIZATION. F.B. Horak, L.M. Nashner, and J.G. Nutt. Neurological Sciences Institute, Good Samaritan Hospital & Med. Ctr., Portland, OR 97209.

The nature of trunk-leg dyscoordination was investigated in six idiopathic Parkinsonian patients with marked retropulsion. The pattern of EMG activity in six trunk and leg muscles, body sway, and forces exerted by each foot were recorded during sagittal postural perturbations induced with a movable platform. Adaptation of postural responses under varying conditions of support and with and without L-Dopa therapy were analyzed. The muscle activation patterns of the parkinsonian patients were compared with those of age-matched (59-79 yrs) normal subjects and vestibular-deficient patients.

Normal subjects as well as patients with peripheral vestibular disorders produced postural responses specific to each condition of support: Activation of muscles in a distal-to-proximal sequence corrected body sway by rotation about the ankles (ankle synergy) when standing on a normal surface. Activation of muscles on the opposite side of the body in a proximal-to-distal sequence maintained balance by rotation about the hips when standing on a narrow beam (hip synergy). Activation of abdominals or paraspinal muscles alone corrected trunk sway over the hips when sitting on a stool. Normal subjects used mixtures of these synergies during transitional trials just after changing support conditions.

There were two main differences in the responses of Parkinsonian patients: 1) Postural strategies were fixed, independent of support conditions. Although administration of L-Dopa improved the pattern of postural muscle response, it did not affect the lack of context-specific changes in muscle pattern. 2) The muscle response patterns in parkinsonian patients suggested that they used an unusual, ineffective mixture of both the ankle and hip synergies simultaneously. The temporal and spatial pattern of muscle activation within each synergy was appropriate. The absolute latencies (70-100 ms) and magnitudes of the responses were also normal.

These studies suggest that basal ganglia disruption associated with Parkinson's disease in these patients does not affect the spatio-temporal programming of basic muscle response patterns but it does affect how these patterns are combined and related to environmental contexts. Supported by grants NS12661 and NS06926-02.

184.13 PARKINSONISM AND THE PONTINE TEGMENTUM. R.M. Chesire, J.T. Cheng and P. Teitelbaum. Psychol. Dept. Univ. Hawaii at Manoa, Honolulu, HI 96822, Univ. Texas Hlth. Sci. Ctr. at Dallas and Univ. Illinois at Urbana-Champaign.

In rats, systemically administered haloperidol (5 mg/kg) produces akinesia, catalepsy, and exaggerated defense of static, stable equilibrium (De Ryck, Schallert & Teitelbaum, Brain Res.201: 143-172, 1980). Focal infusion of 200 ng gamma-aminobutyric acid (GABA) into the nucleus reticularis tegmenti pontis (NRTP) reverses haloperidol-induced akinesia and releases galloping forward locomotion (Cheng et al., Proc. Natl. Acad. Sci. USA 78: 3279-3283, 1981; Chesire, Cheng & Teitelbaum, Physiol. Behav. 30: 809-818, 1983). In akinetic, haloperidol-treated rats, GABA infused into the region surrounding, but not into, the NRTP completely abolished the defense and maintenance of equilibrium. Intraventricular infusion of the GABA antagonist picrotoxin reinstated defensive reflexes, but did not reverse haloperidol-induced akinesia. We suggest that the region surrounding the NRTP is organized, in part, to maintain reflexes preparatory to successful forward locomotion. Thus, diffuse damage in this region may contribute to the severe equilibrium deficits observed in some forms of Parkinson's disease.

184.14 EFFECTS OF SODIUM ARSANILATE INDUCED VESTIBULAR DYSFUNCTION ON MOTOR ACTIVITY IN THE RAT. M. A. Hunt*, S. W. Miller and H. C. Nielson*. Dept. of Physiological Psychology, University of Utah, Salt Lake City, Utah 84103.

The effects of sodium arsenilate (an ototoxic drug) induced vestibular dysfunction on motor activity in the rat was investigated. Activity in running wheels was chosen as the baseline measure of "normal" behavior, against which to assess the effects of vestibular dysfunction, for two reasons: 1) the diurnal variation of motor activity of the rat in this situation is well known, and 2) when coupled with a food deprivation schedule the increased motor activity in anticipation of food provides a means for distinguishing between motor and motivational defecits. In addition, the rats were tested in an open fields to assess their motor performance under emotional arousal.

Two groups of adult male Long-Evans rats were maintained under a 12/12 light/dark (LD) cycle. Group one was fed during the middle of the dark cycle and group two during the middle of the light cycle. After 14 days the rats had developed stable diurnal patterns of running, and showed bursts of activity in anticipation of food typical of this paradigm. Half of the rats of each group then received bilateral intratympanic injections of sodium arsanilate. The remaining animals served as controls and received equivalent intratympanic injections of bacteriostatic normal saline.

All groups showed significant decrease in activity for the first 24 to 48 hours after injections although there were no significant differences between experimental and control groups. Furthermore, no significant differences between groups were found when all groups had returned to pre-injection levels. However, motor activity in the open field was dramatically different between rats injected with sodium arsanilate and controls. When the open field was illuminated with an incandescent 25 watt red light the two groups did not differ. However, when the open field was illuminated with standard fluorescent lights, the rats with vestibular dysfunction would not move forward but would only move backward, suggesting an interference in motor behavior produced via an interaction between emotional arousal and visual sensation. Studies are under way to further describe and explain this behavior.

CONTROL OF POSTURE AND MOVEMENT I

185.2 UPPER BODY RESPONSES TO POSTURAL PERTURBATIONS IN MAN. M.H. Woollacott and E. Keshner. Inst. of Neurosci/P.E. and Human Movement Studies, U of Oregon, Eugene, OR 97403.

Previous studies analyzing the characteristics of human postural responses to support surface perturbations have indicated that leg muscle responses are organized into synergies which are specific to the direction of induced sway and radiate from the base of support upward. The maintenance of the synergic organization even when visual and vestibular inputs were altered has supported the hypothesis that proprioceptive input from the ankle was responsible for initiating these leg synergies. If the upper body musculature were included in the ascending synergy, then onset latencies should lengthen as one moves rostrally along the same functional surface. As part of the synergy, neck muscles would also be expected to appear during rotational perturbations that supply stretch inputs to the ankle musculature without significant visual or vestibular stimulation. Alternatively, if upper body muscles are activated via vestibular or visual inputs, onset latencies would be early during platform translations, and would be suppressed during rotations.

Leg, trunk and neck muscle responses were recorded in 10 standing adults during 3cm anterior and posterior horizontal platform translations, and 9° platform dorsi- and plantarflexing rotations of 125ms durations. Analysis of EMG latencies elicited by posterior platform translations indicates that the ankle muscles (soleus) and neck muscles (flexors) are activated almost simultaneously (means of 70.6±6ms and 67.3±14ms respectively) and before the onset of all other muscles tested. Abdominal muscles closely followed the neck flexors in latency (69.2±19ms). Note that neck flexors and abdominals show latencies that are earlier than would be expected as an extension of the ankle synergy, and they act in a direction antagonistic to that of the ankle muscles.

When comparing activation patterns for rotations and translations, it was found that neck and trunk muscles were activated significantly more often for translations ($p<.001$). When they did respond to rotations, they appeared to be part of the ascending synergy (soleus: 54±11; lumbar: 88±29; neck extensors: 100±25ms). No differences were noted between eyes open and closed conditions. We conclude from these results that during platform translations, upper body muscles are activated separately from the ankle synergy, possibly via the vestibular system.

185.3 INFERRING MOVEMENT AND MUSCLE SYNERGIES FROM MULTI-JOINT ARM POSTURE. T. Flash* and F. A. Mussa-Ivaldi* (SPON: W. Richards). Dept. of Psychol., MIT, Cambridge, MA 02139.

The purpose of this work is to investigate the biomechanical and neural factors underlying multi-joint arm posture and movement. The combined spring-like behavior of all the arm muscles results in a local elastic field at the hand during posture and contributes to the stability of the hand in face of unpredictable disturbances. The hand stiffness field was recently measured for planar horizontal arm posture (Mussa-Ivaldi et al., Neurosci. Abst., 1984). Investigating the regularities in the hand elastic field, we found that the maximal hand stiffness is aligned with the radial axis connecting the shoulder with the hand when the arm is in a horizontal plane. In addition, the stiffness field becomes more isotropic for more proximal hand positions and more elongated for more distal positions. To explain these regularities, hand stiffnesses were transformed into joint stiffnesses revealing a fixed relationship between the stiffnesses of the double-joint muscles and of the single-joint shoulder muscles, as a function of shoulder angle. The contribution of each muscle to the net joint stiffness depends on its moment arm and its intrinsic stiffness. Using experimentally derived anatomical data (Wood, in prep.) to calculate muscle moment arms, we were able to obtain a rough estimate of the distribution of neural activity to different muscle groups so as to account for the observed variations in joint stiffnesses. This suggested distribution was compared to EMG records taken from several shoulder and elbow muscles. The above method provides new insights into the complex interactions between anatomical constraints and muscle activation during posture and suggests new means for the quantitative characterization of muscle synergies.

Next we tested the hypothesis that a reaching movement is achieved by shifting the hand equilibrium position along a work-space invariant trajectory. Assuming that the main features of the stiffness field during movement are similar to those of the static field, an equilibrium trajectory was inferred from a particular measured arm movement. Arm movements in other regions of the work-space were simulated using simple rotations and translations of this inferred equilibrium trajectory. The predicted trajectories are in good agreement with measured movements indicating the feasibility of the proposed model and suggesting a unified treatment of posture and movement. (Research supported by NIH grants AM27610 and NS09343 and the Bantrell Fellowship. F.A. Mussa-Ivaldi supported by a CNR Fellowship.)

185.1 WITHDRAWN

185.4 EFFECTS OF STATIC TILT ON HUMAN SPINALLY MEDIATED MYOTATIC REFLEXES ASSESSED BY H-REFLEX RECOVERY CURVES. T.Szturm*, R.M.Jell. Department of Physiology, Univ. of Manitoba, Winnipeg, MB, R3EOW3.

The objective of this study is to determine how electrically-evoked myotatic reflexes are influenced by changing whole body (head) angle relative to the gravity vector. Subjects (9) were placed in a reclining chair apparatus within the frame of a circular electric bed. Care was taken to ensure stable, maintained body and extremity position. The head was fixed relative to the body. Changes in whole body (head) angle varied from 90 (bed frame vertical) to 0 (bed frame horizontal) deg in 30 deg steps. At each angle an H-reflex recovery curve was determined at 2 intensities MT (M Threshold) and .8MT. ANOVA shows significant main effects due to: 1) Angle. In this case, the response (H2/H1) at 60 deg was significantly different from that at 0,30,and 90 deg ($p<.01$). Multiple comparisons showed that the differences occurred during the late depression period of the recovery curve, i.e.300-500ms delay. 2) Intensity. In this case, the response at MT was significantly different from that at .8MT ($p<.001$). Multiple comparisons showed that the responses at the 2 intensities began to diverge at 30 deg with this effect greatest at 90 deg ($p<.01$). Furthermore, at MT, the response at 60 deg was significantly less than that at 0,30,and 90 deg ($p.<.01$); and at .8MT, the response at 0 deg was significantly greater than that at 30,60,and 90 deg ($p<.01$). The results at .8MT show a pattern of a progressive increase in alpha motoneuron excitability (H2 recovery) as the angle changes from 90 to 0 deg. An analysis of H1 amplitude alone shows no significant differences between angles at .8MT. These results are consistent with a physical model of utricle orientation. In our experiment, considering the subjects' head orientation, the utricles are near horizontal at 90 deg bed angle and near vertical at 0 deg bed angle. The results at MT do not show such a pattern. This cannot be explained by changes in H1 amplitude as both the H2/H1 ratio and H1 amplitude increase from 60 to 90 deg. The angle effects occurred during the late depression period of the recovery curve. Thus a facilitation or a disinhibition can account for these effects of angle. This late effect, indicated by H2 recovery relative to H1 with no significant changes in H1 alone (monosynaptic), implies a long loop reflex involvement, possibly with polysynaptic segmental influences. (Supported by Manitoba Medical Services Foundation)

185.5 KINESIOMETRIC ANALYSIS OF THE MOTOR DYSFUNCTION OF PATIENTS WITH SUBACUTE MYELO-OPTICO NEUROPATHY (SMON) AND PATIENTS WITH OTHER NEUROLOGICAL DISORDERS. E. Toyoshima[1], H. Tomi*[2], Y. Mano*[3], K. Ando*[2], and R.F. Mayer[1]. [1]Dept. of Neurol., Univ. of Maryland Sch. of Med. & V.A. Med. Ctr., Baltimore, MD 21201. [2]Ntnl. Ctr. for Nerv., Ment. & Musc. Dis., Tokyo, JAPAN. [3]Dept. of Neurol., Nara Med. Univ., Nara, JAPAN.

Many patients with SMON have difficulty in sustaining the standing posture and in walking. To assess these difficulties quantitatively we have measured both the fluctuation of the center of gravity of the body and the spatial movement of the lower limbs during exercise using a gravicorder (Anima Co., Ltd., JAPAN) and a spatial movement recorder (Takei Kiki Kogyo Co., Ltd., JAPAN).

17 patients with SMON, aged 46-77 years, were studied. Controls consisted of 5 normal volunteers, aged 30-69 years. 15 patients with spinocerebellar degeneration (SCD) aged 18-65 years and 11 patients with Parkinson's disease (PD) aged 36-73 years were also examined.

The length (L, Mean+S.E.) of the movement of the center of gravity of the body of the SMON patients was 50.1+7.1 cm with eyes open (OE) and 100.2+13.5 cm with eyes closed (CE) for 20 seconds. The ratio (rL(CE/OE)) was 2.2+0.3. The results from patients with other neurological disorders were: SCD, $\overline{L(OE)}$=82.9+9.7, $\overline{L(CE)}$=129.1+14.2, and $\overline{rL}$=1.6+0.2; PD, $\overline{L(OE)}$=24.0+3.8, $\overline{L(CE)}$=39.2+4.4, and $\overline{rL}$=1.7+0.1. Control values were $\overline{L(OE)}$=20.3+2.6, $\overline{L(CE)}$=34.2+4.7, and $\overline{rL}$=1.7+0.7. Recordings of the spatial movements showed changes corresponding to these results.

Body sway (L) with eyes open and eyes closed in SMON and SCD was significantly greater ($p < 0.01$) than in normal or PD. The ratio (rL) tended to be greater in SMON than in normal, SCD or PD. These results may reflect the abnormalities of perception in the lower limbs of the patients with SMON. The methods used in this study are helpful in the objective, quantitative and serial assessment of motor dysfunction in patients with neurological disorders. (Supported in part by a grant from the Institute for Health and Welfare, JAPAN, and the Veterans Administration, U.S.A.)

185.6 EFFECT OF ELECTRICAL CUTANEOUS NERVE STIMULATION ON MYOTATIC REFLEX EMG AND VOLUNTARY EMG ACTIVITY IN NORMAL HUMANS. R. Hayashi*, D.G. White*, W.J. Becker*, and R.G. Lee (SPON: H. Sarnat). Dept. of Clinical Neurosciences, University of Calgary, Faculty of Medicine, Calgary, Alberta, Canada T2N 4N1.

Short (M1) and long (M2-3) latency myotatic reflexes are facilitated or inhibited by various segmental and suprasegmental inputs on motoneurons (Hendrie & Lee, Brain Res. 157: 369-375,1978; Lee & Tatton, Exp. Brain Res. 45:207-216, 1982). Cutaneous stimuli cause complex patterns of facilitation and inhibition in flexor muscles as measured by EMG recordings (Meinck, et al. Exp. Brain Res. 43:78-86, 1981). In the present study, the effects of electrical stimulation of the fingers on wrist flexor muscles EMG activity were investigated, including: 1) effect on the EMG activity during voluntary isometric contraction, and 2) effect on the EMG during the M1 and M2-3 reflexes. Seven normal subjects were studied. Pulse trains of 3 electrical pulses at 300 Hz (pulse duration 0.2 msec) were applied to the 2nd, 3rd and 4th digits at 4 times perception threshold. EMG activity recorded from wrist flexor muscles was rectified and averaged.

In the first experiment, subjects used wrist flexor muscles to hold a certain position against a constant load produced by a torque motor. Marked inhibition of EMG activity was observed in flexor muscles with onset of inhibition at 33 msec (33+1.7) after electrical stimulation. The duration of EMG inhibition was greater than 30 msec in all subjects. EMG activity during the interval from 10 to 30 msec after inhibition onset was reduced to 40% of control. To investigate the effect of electrical stimulation on EMG activity during the M1 reflex (30-60 msec after stretch onset) and M2-3 reflex (60-90 msec after stretch onset), electrical stimuli were timed to produce maximum inhibition of EMG activity during the appropriate time interval. M1 and M2-3 reflexes were elicited by wrist perturbations produced by the torque motor. Measurements were made during the first 20 msec of each reflex interval. M1 EMG activity was reduced to 60% (60+ 24) of control, and M2-3 EMG was reduced to 44% (44+23) of control. These results suggest that: 1) cutaneous inputs modify motoneuron excitability not only to segmental inputs but also to long loop supraspinal inputs and inputs associated with voluntary muscle activation, and 2) M2-3 EMG components were more sensitive to inhibition produced by cutaneous stimuli than M1 components.
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185.7 EFFECT OF ELECTRICAL CUTANEOUS NERVE STIMULATION ON MYOTATIC REFLEX EMG AND VOLUNTARY EMG ACTIVITY IN HEMIPLEGIC HUMANS. W.J. Becker*, R. Hayashi*, and R.G. Lee. Dept. of Clinical Neurosciences, University of Calgary Faculty of Medicine, Calgary, Alberta Canada T2N 1N4.

Electrical cutaneous nerve stimulation (ECNS) modifies EMG activity in the voluntarily contracted first dorsal interosseus muscle (Jenner, J. Physiol. 333:405, 1982). In the present study, the effect of ECNS on both voluntary and reflex EMG activity in the wrist flexor muscles was determined in both the normal and abnormal arm of subjects with unilateral cerebral hemisphere lesions. In hemiplegic subjects, both short latency (M1) and long latency (M2-3) reflex responses are abnormal in the affected arm (Lee, Can. J. Neurol. Sci. 6:384, 1979). Comparing ECNS induced EMG changes in the normal and abnormal arm might give some insight into the mechanisms involved in ECNS inhibition of EMG activity.

Five hemiplegic subjects with some voluntary movement in the affected arm were studied. Electrical stimuli were delivered to the 2nd, 3rd, and 4th digits via ring electrodes. EMG activity was recorded from wrist flexor muscles. For experiments on voluntary contraction, the subject opposed a constant force produced by the torque motor. Reflex EMG responses were elicited by sudden wrist perturbations produced by the torque motor. ECNS produced marked inhibition of voluntary EMG activity, with onset of inhibition beginning 32-40 msec after electrical stimulation. Inhibition duration ranged from 75-145 msec, and EMG activity was reduced by 70% or more. No difference was found between normal and abnormal arms. Appropriately timed ECNS also produced marked inhibition of EMG activity during the M1 reflex (30-60 msec after stretch onset) and the M2-3 reflex (60-90 msec after stretch onset) which was similar in degree in normal and abnormal arms. These results indicate that the inhibition of motoneuron excitability produced by ECNS affects motoneuron responses to segmental, long loop, and voluntary inputs to a similar degree in normal and hemiplegic arms.

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185.8 EMG, Ia, AND STRETCH REFLEX ACTIVITY DURING DAMPED HAND OSCILLATIONS IN MAN. A.W. Wiegner and R.R. Young. Lab. of Clinical Neurophysiology, Massachusetts General Hospital, Boston, MA 02114.

Hagbarth and Young's data (Brain 102: 509, 1979) suggest acceleration sensitivity of spindle discharge during damped oscillations (DOs) of the hand. Stiles (J Neurophys 50: 327, 1983) argues for the same concept based on detailed studies of phase relations between wrist oscillations and EMG modulation during DOs, but without the benefit of afferent data. Here, we have used some of the methods of the latter study and added microneurographic data on Ia firing patterns.

Subjects were semi-reclining with the upper arm adducted and forearm supinated. A plastic sandwich (wt: 110 g) immobilized the fingers, while an accelerometer was taped 16 cm from the wrist; a goniometer measured wrist angle. Microneurography (median nerve, 5 cm above the elbow) detected single or multi-unit Ia afferent discharges from wrist flexors (flexor carpi radialis or palmaris longus) whereupon needle EMG electrodes were inserted into that area of the muscle from which the afferents originated. Taps to the palm of the hand yielded DOs. Resonant frequency of the hand was altered by the addition of 200 g and 500 g weights.

(1) During taps, typical single unit recordings demonstrated a group of 3-4 discharges 55-70 ms before maximum extension. (2) During 7 Hz tremor voluntarily enhanced by co-contraction, single Ia discharge lagged peak negative acceleration by 49 ms (123°) and EMG lagged peak negative acceleration by 56 ms (140°), such reflex timing tending to sustain the tremor. (3) By cross-spectral analysis, phase lead of flexor EMG on peak extension increased from 40° at 7 Hz to 145° at 3.2 Hz as weight was added to decrease the DO frequency. Phase lead of single Ia discharge similarly increased from 64° at 7 Hz to 149° at 3.2 Hz. Phase intercept of the Ia phase-frequency regression was 201°, showing the afferent signal to be related in phase to negative acceleration. (4) Adding weight increased the damping factor of DOs from 0.14 (no weight) to 0.21 (500 g), suggesting a reflex contribution to damping. (5) Spindle discharges were not attenuated by five minutes of ischemia induced by inflating a blood pressure cuff on the upper arm to 200 mm Hg.

These data, derived from direct recording of Ia activity, are consistent with Stiles' concept and suggest that phase advance of afferent information in the CNS is not necessary for stability of the wrist during DOs.

185.9 FACTORS INFLUENCING FRACTIONATED SIMPLE REACTION TIME IN RAPID ELBOW FLEXION. J.G. Anson, Department of Physical Education, Oregon State University, Corvallis, OR 97331

Simple reaction time (SRT), premotor time (PMT) and motor time (MOT) were measured in agonist and antagonist muscles during initiation of rapid elbow flexion under two levels of prestimulus voluntary muscle contraction; minimal and maximal. Each subject received two blocks of 20 trials in each contraction condition for a total of 80 trials. A single trial began with the command 'READY' which directed the subject to position the manipulandum against a microswitch - (minimal contraction condition). In the maximal contraction condition, after closing the microswitch the subject was additionally required to maximally contract the agonist/antagonist synergists prior to foreperiod onset. In preparation for each trial the subject positioned his right arm on the manipulandum such that the medial epicondyle of the right humerus was in line with the axis of the manipulandum. Electromyographic (EMG) data were sampled via pairs of Ag-AgCl surface electrodes located on the biceps brachii and lateral head of triceps respectively. The amplified and rectified EMG signals were displayed on a Nicolet 3091 Digital Oscilloscope from which the PMT data were recorded.

SRT was shorter for the maximal muscle contraction condition than for the minimal muscle contraction condition. Within SRT the MOT component was not significantly different between conditions. Thus the difference in SRT was almost totally accounted for by a reduction in PMT. Because PMT is thought to reflect central processing delay, the above results could be indicative of a savings in preparation time due to preprogramming of the synergistic muscles in advance of stimulus onset. An alternative interpretation would be that maximal contraction of the muscles prior to stimulus onset is directly associated with increased synaptic facilitation of the peripheral nerves and heightened activity in the respective motor neuron pools. These events maybe considered peripheral (although they are not represented by changes in MOT) in the sense that their actions occur outside the CNS. Thus changes in PMT may not be restricted just to changes in central processing or programming time.

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185.10 THE UNLOADING REFLEX IS A SET-DEPENDENT TRIGGERED REACTION. M. Robinson, S. Spencer*, J. Hore and T. Vilis. Dept. of Physiology, Univ. of Western Ontario, London, Ont., Canada N6A 5C1.

If a limb is held stationary against a constant force, and the constant force is suddenly removed, an unloading reflex occurs. The EMG recording shows silence in the previously active muscle followed by excitation that may extend above the original baseline (Alston et al., 1967). We investigated the hypothesis that the excitatory phase is a set-dependent, triggered reaction.

Four Cebus monkeys were trained to hold a handle stationary in target against a constant force that loaded triceps and to resist unloading pulses of different durations (20 - 200 ms). When an unloading step (2000 ms) was given an unloading reflex occurred as the arm was initially displaced and was then held stationary in the new position.

Application of unloadings of different durations (20 - 2000 ms) revealed that the onset of the excitation in triceps was synchronized to the onset and not the offset of the perturbation. The minimum latency of the excitation in triceps was 60 ms when only an M2 occurred in biceps and 100 ms when both an M2 and M3 occurred in biceps.

The effects of set were investigated by giving unloadings when the monkey was set for large torque steps that stretched biceps. Expected large torque steps required return of the limb to target which was associated with activity in biceps and silence in triceps. When the monkey was set for large torque steps but got an unloading, the excitatory phase of the unloading reflex was greatly diminished. In contrast, when the monkey was set for unloadings but got a large torque step an inappropriate excitation appeared in triceps. This excitation was smaller and occurred later than that for unloadings.

These results demonstrate that the excitatory phase of the unloading reflex is triggered by the onset of the perturbation, is modulated by afferent feedback, and is dependent on set.

Alston, W. et al., (1967) J. Physiol. 190, 189-202.

(Supported by M.R.C. MT-6773, The Bickell Foundation and The W. T. McEachern Foundation).

185.11 EFFECTS OF DEAFFERENTATION AND ANISOMETRICITY ON REFLEX CONTRACTIONS IN DECEREBRATE CATS. E. K. Stauffer, Dept. Physiology, Sch. Med., Univ. Minn., Duluth, MN 55812.

Superimposed on the static level of force of muscle contractions in decerebrate cats are small amplitude oscillations (Burgstahler and Stauffer, Neurosci. Abst. 8:535, 1982) which reflect the physiological activation of a muscle's pool of motor units. In the present study, the behavior of these oscillations was examined under isometric (ISOM) versus anisometric (ANISOM) conditions, and after removal of homonymous afferent feedback.

Experiments were performed on 12 cats decerebrated at the midcollicular level. The left hindlimb was denervated except for the plantaris muscle. Data were obtained during spontaneous and/or experimentally-evoked reflex contractions. and after cutting or blocking the dorsal rootlets associated with the muscle. Spectral density estimates of muscle force, acceleration, and electromyographic (EMG) activity were calculated from the ISOM and ANISOM records.

Under ISOM conditions, the mechanical records commonly showed relatively small-amplitude oscillations which exhibited a tendency for sustained periodicity, with most of the power contained within a very narrow frequency band. EMG activity was tightly grouped into periodic bursts whose interburst frequency was significantly coherent with the mechanical data. Removal of afferent feedback eliminated the periodicity. Instead, muscle activity was markedly asynchronous with a significantly reduced level of spectral power that was distributed over a relatively broad band of frequencies. In several cases, individual trains of unitary EMG potentials were observed to occur in-phase and out-of-phase with one another, thus forming randomly synchronized groups of muscle action potentials (cf. Taylor, J. Physiol. 162:259, 1962). When a neurally-intact muscle was switched from ISOM to ANISOM and allowed to move, periodic behavior became manifest with greatly increased amplitudes and a shift from a more-or-less randomly synchronized EMG to a regularly synchronized EMG. These results indicate that homonymous afferent feedback plays a significant role in the synchronous grouping of motor unit activity which, in turn, leads to the generation of the rhythmical muscle actions that are so characteristic of tremor-like states.

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185.12 CHANGES IN ACTIVATION OF HUMAN SOLEUS MUSCLE DURING SUSTAINED MAXIMUM ISOMETRIC CONTRACTIONS. CG Kukulka, AG Russell*, MA Moore*. Physical Therapy Research Labs, University of Iowa College of Medicine, Iowa City, IA 52242.

Fatigue induced by sustained, maximum isometric contractions is characterized by a decline in both force output and firing rates of active motor units, prolongation of the muscle's twitch relaxation time, and little indication of neuromuscular junction block. These findings have been obtained almost exclusively from intrinsic hand muscles and therefore generalization to other muscles awaits further study. The purpose of this study was to evaluate changes in electrical activation of soleus muscle during sustained maximum efforts. Confirmation, in soleus, of results obtained previously, would provide two important insights: (1) extend the generalization of fatigue induced neuromuscular alterations to a lower extremity muscle and (2) since motoneuron pool excitabilities are most easily evaluated using H-reflex, this muscle could provide an appropriate model for evaluating spinal cord processes involved in the slowing of motor unit firings observed during fatigue.

Ninteen experiments were conducted on 5 subjects ranging in age from 27 to 36 years. Subjects were positioned prone, knee slightly flexed, ankle at 90^{o} and foot secured to a torque measuring plate. Surface and intramuscular fine wire emg recordings were made from soleus. Single, 1 msec pulses, capable of producing maximum M-waves, were delivered every 5 sec during the contractions. Subjects produced maximum plantarflexion contractions, judged maximum by the lack of force increment occurring during single shock stimulation.

The results were in agreement with reports from small hand muscles - fatigue was characterized by decline in force and unit firings, with no appreciable change in M-wave areas. A consistent difference, was in the time course of emg/force changes. Maximum force was maintained for up to 45 sec, with a decline to 2/3 max occurring between 2-3 min. M-wave areas were remarkably stable during the first 60 sec of effort and increased slightly (120% of resting) by 3 min. Reduction in total number of spike counts to 50% of unfatigued levels occurred after 30 sec of effort, with little additional change occurring up to 3 minutes. Future studies concerned with the slowing of motor unit firings during fatigue may therefore employ soleus and tests of its motoneurons' excitabilities.

This investigation was supported in part by Biomedical Research Support Grant RR05372 from the NIH.

185.13 HAND EFFECTS AND SELECTION GRADIENTS IN TWO-CHOICE REACTION TIME FOR ALL FINGER PAIRS IN HUMANS. C.L. MacKenzie. Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

The structure and functional organization of the motor system exert systematic constraints on selection, memory and organization processes underlying motor programming (Sternberg, et al., 1978; MacKenzie, 1980; Requin, 1983). Specifically, some investigators have suggested that choices between two fingers on the two hands are faster than choices between fingers on one hand (Kornblum, 1965; Glencross, 1975; Annett & Annett, 1979). My own research revealed unexpected results for finger pairings not used by previous investigators, and in terms of mutual, contextual effects (MacKenzie, 1980).

Because of the above anomaly, all possible pairs of fingers (excluding thumbs) were examined in a discrete trials, 2-choice reaction time (RT) paradigm. From the 28 finger pairing conditions, one could ask whether RT for one hand was faster than the other, whether between hand decisions were faster than within hand decisions and whether there were gradients in the RT data. Ten, female, right-handed undergraduates performed two replicates of the 28 finger pairing conditions, the order being randomized for each subject. Blocks of 30 trials were given in each condition. On each trial, after a constant foreperiod of 2 sec., the warning light was followed by an imperative stimulus light, indicating with which finger to respond. Light-finger mapping was counterbalanced across subjects. The required response was a keypress with the correct finger, in the absence of vision of the hands.

The data were conceptualized in matrices, where matrix symmetry mirrored the symmetrical structure of the two hands. Results showed that RT for a finger changes depending on the finger with which it is paired. RT for fingers on the right hand (255 msec) was faster than left hand (260 msec), and that RT between hands (253 msec) was faster than RT within hands (261 msec). The within-between RT differences were more pronounced for the right hand than for the left hand. Finally, there were several significant gradients in the RT data. One gradient revealed that regardless of finger pair, the rightmost finger of the pair had a faster RT. These data will be more fully described and discussed with respect to constraints of the motor system on response selection, attention, and body schema processes for this task.

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185.14 DIFFERENTIAL CHANGES IN LONG-LATENCY ELECTROMYOGRAPHIC RESPONSES DURING MOTOR PROGRAMMING. M. Bonnet*, G.E. Stelmach* and J. Requin (SPON: J.D. Vincent). Dept. Psychobiol. exper., Inst. Neurophysiol. et Psychophysiol., CNRS, Marseille, France.

It has previously been shown that providing partial information about a movement performed in visuo-manual pointing tasks with the temporal constraints of a reaction time procedure, results in differential changes in RT. The partial information may be about the direction, the extent or the limb to be used in the movement, any two or all three. In order to look at the physiological mechanisms that underlie the specification of these spatial parameters of movement, we analyzed the amplitude of different components (M1, M2 and M3) of the EMG responses triggered by a sudden stretch of the right wrist flexor muscles during the preparatory period of task. The task, which was performed by human subjects, consisted of pointing to different targets above or below a starting position, by rotating a handle by either extending or flexing the left or right wrist.

We observed differential changes in the amplitude of the different components of the EMG response, as a function of the nature of the partial information: a) advance information on movement extent results in a modulation of M1 amplitude only, which is larger for a long than for a short extent, b) advance information about the limb involved in the movement did not change M1 amplitude, but did change M2 and M3 amplitudes which were larger when the tested muscle was ipsilateral to the responding side than when it was contralateral, c) advance information on the direction of the movement did not change M1 amplitude, but did change M2 amplitude slightly. The major effect was on the amplitude of M3 which was larger when the tested muscle was the agonist in the movement than when it was antagonist. It was suggested that the different processes responsible for specifying the different parameters of the movement during programming could be carried out by different neural pathways, namely the spinal, the cortical and the cerebellar structures, respectively. This would be consistent with current hypotheses about the neural pathways involved in the long latency EMG responses to muscle stretch.

185.15 FATIGUE INDUCED ENHANCED PHYSIOLOGICAL TREMOR. B.T. Shahani, A.W. Wiegner and R.R. Young. Clinical Neurophysiology Laboratory, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

Fatigue-induced tremor was studied in 6 healthy subjects 22 to 47 years of age. The fingers were immobilized by placing the hand in a plastic "foam sandwich" weighing 110 g. An accelerometer was placed 16 cm. distal to wrist and subjects were asked to point their fingers to a fixed mark. The experimental protocol consisted of 5 trials of 4 minutes each in succession recording a.) without weights, b.) with a 200 g. weight on the hand placed 12 cm. distal to wrist, c.) after removal of the 200 g. weight, d.) with a 500 g. weight, e.) after removal of the 500 g. weight. Five second acceleration epochs were sampled by a PDP 11/23 computer for calculation of fast Fourier transform (FFT) and power spectrum. In addition, root mean square (RMS) displacement was calculated digitally from twice-integrated accelerometer records. Increasing fatigue resulted in increased displacement at the wrist (up to 375 microns RMS). No change was seen in frequency of the underlying physiological tremor except during the period when weights were attached to the hand.

The initial physiological tremor was not monorhythmic but fatigue quickly resulted in the appearance of one peak frequency in these subjects; as with other types of enhanced physiological tremor, that frequency did not change as the amplitude of fatigue tremor increased. One feature differentiating physiological tremor from enhanced physiological tremor is the monorhythmic nature of the latter whereas the former has multiple frequencies in any one subject at any one time.

186.1 PATTERNS OF MOTONEURON PROJECTIONS IN THE EMBRYONIC MOUSE HINDLIMB PRIOR TO CELL DEATH. C. Lance-Jones. Dept. of Anatomy and Cell Biology, Univ. of Pittsburgh, Sch. of Medicine, Pittsburgh, PA 15261.

In the lumbar (L) or hindlimb-innervating segments of the mouse spinal cord, 67% of the motoneurons in the lateral motor column die between embryonic day (E) 13 and E18. To determine if this event is associated with the removal of imprecise axon projections, motoneuron projection patterns to the hindlimb were characterized at E12, a stage shortly after nerve trunk formation, and compared to those of an adult (described by McHanwell and Biscoe, Proc. Roy. Soc. Lond. B., 293:46, 1981). A few E13 embryos were also examined.

Retrograde and orthograde horseradish peroxidase (HRP) labeling techniques were used to define projection patterns. In an isolated hindlimb-spinal cord preparation, muscle regions or lumbar cord segments were injected with HRP. The tissue was processed for light microscopy using standard DAB or cobalt-DAB protocols.

In E12 embryos, HRP was injected into one of the four muscle primordia of the hindlimb: the preaxial dorsal, preaxial ventral, postaxial dorsal, and postaxial ventral primordia. Injections usually filled one of these muscle primordia entirely. Labeled motoneurons were found in the same spinal cord position as those in an adult. For example, injections of the preaxial dorsal region resulted in the labeling of cells in a lateral position in L1-L3, while postaxial ventral injections labeled neurons in a medial position in L3-5. In contrast to the adult pattern, a few labeled cells were found in the posterior part of L5 and in L6 following postaxial ventral injections. Whether these represent incorrect projections or HRP leakage to posterior nonlimb regions is not now known. Discrete injections of preaxial ventral and postaxial dorsal regions were difficult at E12, but injections at E13 gave rise to a clear adult-like pattern of labeling. In embryos (all E12) in which cord segments were injected, the paths of labeled axons were traced and also found adult-like.

Results suggest that motoneuron projections to the mouse hindlimb prior to cell death are largely correct. Cell death does not appear to function to remove large-scale projection errors in the mouse hindlimb. (Supported by NIH grant NS20711.)

186.2 XENOGRAFT SURVIVAL IN RATS TREATED WITH CYCLOSPORIN-A. A.K. Gulati* and A.A. Zalewski. Lab. of Neurochemistry, NINCDS, NIH, Bethesda, MD 20205.

Cyclosporin-A (Cy-A) is an immunosuppressive agent that prevents the rejection of allogeneic nerve and Schwann cells in normal (nonimmunized) or presensitized recipients. Because Cy-A is such a potent inhibitor of allograft rejection (i.e., rejection between genetically different members of the same species), we have investigated whether Cy-A suppresses xenograft rejection (i.e., rejection between different animal species). Xenografts of sensory nodose ganglia were obtained from adult male guinea pigs and hamsters and implanted into the sternocleidomastoid muscle of adult male Fischer (FR) rats. Some FR recipients of xenografts were not immunosuppressed while others received Cy-A (10 mg/kg, ip). Cy-A therapy was started the day before grafting and continued daily thereafter. The xenografts were removed after 4-28 days and examined histologically to determine rejection or survival. Guinea pig and hamster ganglia were rejected by nonimmunosuppressed rats and by those rats given guinea pig ganglia and Cy-A. These rejected ganglia lacked nerve, Schwann and sheath cells, and they were surrounded by an intense mononuclear and polymorphonuclear leukocytic reaction. It is of interest that guinea pig ganglion cells were lost earlier (4-7 days) than hamster cells (7-14 days). Neither higher doses of Cy-A nor prolonged host pretreatment prevented the ultimate rejection of guinea pig xenografts. In contrast, hamster nerve, Schwann and sheath cells were present at all times in all xenografts in rats treated with Cy-A. These accepted xenografts had similar numbers of surviving cells, and were not subjected to any host leukocytic reaction. Our results demonstrated that Cy-A can prevent the rejection of zoologically closely-related (hamster and rat) but not widely-divergent (guinea pig and rat) xenografts of ganglia. We speculate that Cy-A will be effective in preserving xenografts if they are normally rejected by an antigen stimulated cellular, rather than a naturally occurring xenoantibody, immune response. If allogeneic neurons are unavailable to repair injured nervous tissue, xenogeneic neurons could substitute provided that the donor is closely related to the host and the host is immunosuppressed with Cy-A.

186.3 SURVIVAL OF ALLOGRAFTS OF GANGLIA IN THE SPINAL CORD OF PRESENSITIZED RATS TREATED WITH CYCLOSPORIN A. A.A. Zalewski, H.G. Goshgarian+ and A.K. Gulati*.Lab. of Neurochemistry, NINCDS, NIH, Bethesda, MD 20205; and +Dept. of Anat., Wayne State Univ., Sch. of Med., Detroit, MI 48201.

Although an allograft may survive in the central nervous system (CNS) of a nonimmunized recipient, it is rejected if the host is presensitized to the histocompatibility antigens of the donor of the allograft. Moreover, even a long-term surviving allograft in the CNS can be rejected after specific sensitization, regardless of whether it was obtained from an adult or fetal donor. Since we have found that cyclosporin A (Cy-A) prevents the rejection of a nerve allograft in the leg of a presensitized host, we have investigated whether this immunosuppressive agent similarly inhibits allograft rejection (i.e., second-set) in the CNS. Inbred strains of adult male Fischer (FR) and ACI rats were used. In order to presensitize FR rats to ACI antigens, each FR rat was given an allograft of ACI nodose ganglion that was inserted into a sternocleidomastoid muscle. As expected and verified histologically, each FR rat rejected its allograft of ganglion within 6 weeks. At that time, each presensitized FR rat received a second ACI ganglion that was implanted into its cervical spinal cord. Thereafter, some of these regrafted animals went untreated whereas others received Cy-A (10 mg/kg, daily, ip). Six weeks later, histological examination of the spinal cord implant site revealed that all untreated presensitized rats rejected their ACI ganglia whereas all Cy-A treated ones accepted them. The rejected ganglia lacked neurons, Schwann and sheath cells, and they contained scattered remants of mononuclear cell infiltrations. On the other hand, accepted ganglia contained neurons, Schwann and sheath cells, and no mononuclear cell infiltrations were present. It is noteworthy that Cy-A also prevented the rejection of Lewis (LE) ganglia allografts in the spinal cord of ACI rats that were injected intravenously with LE blood 1-4 months earlier. Our results demonstrated that allograft rejection occurs in the CNS of untreated, but not Cy-A immunosuppressed, presensitized hosts. Because allografts in the CNS are subject to rejection if sensitization develops, we suggest that a course of immunosuppressive therapy be given whenever the risk of sensitization arises.

186.4 REDUCTION AND ENHANCEMENT OF NATURALLY-OCCURRING CELL DEATH IN THE CILIARY GANGLION OF THE CHICK EMBRYO FOLLOWING BLOCKADE OF GANGLIONIC AND NEUROMUSCULAR TRANSMISSION. J.L. Maderdrut*, I. Merchenthaler*[1] and R.W. Oppenheim (SPON: W.K. O'Steen). Department of Anatomy, Bowman Gray School of Medicine, Winston-Salem, NC 27103; [1]Hebert Research Center, Tulane University Medical Center, Belle-Chasse, LA 70037.

The ciliary ganglion (CG) of the chick embryo is composed of two distinct populations of cholinergic neurons: the larger ciliary neurons innervate the striated muscles of the iris and ciliary body, while the smaller choroid neurons innervate the smooth vascular muscles of the choroid coat (Landmesser & Pilar, '78). Synaptic transmission between the ciliary neurons and the iris and ciliary body is mediated by nicotinic acetylcholine receptors (AChR), while synaptic transmission between the choroid neurons and the choroid coat is mediated by muscarinic AChR (Nunez et al., '80).

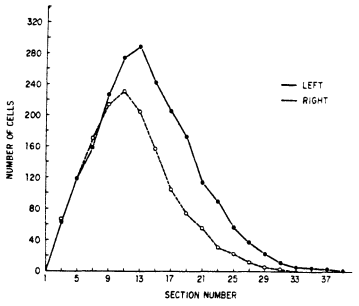
Between embryonic day (E) 8 and E15, there was a reduction in the total number of ganglion cells in the CG of about 50%. The loss of ganglion cells was accompanied by the appearance of pyknotic cells. Substance P- and enkephalin-containing nerve terminals were detected in the CG during the period of ganglion cell loss by immunohistochemistry.

The administration of pempidine, a ganglionic blocking agent, twice per day from E6 to E14½ resulted in a decrease in the number of both ciliary and choroid neurons on E15. The decrease in the number of ganglion cells was accompanied by an increase in the number of pyknotic cells. The administration of levorphanol, an opiate agonist, twice per day from E8 to E14½ resulted in a decrease in the number of both ciliary and choroid neurons on E15, while the administration of naloxone, an opiate antagonist, twice per day from E8 to E14½ resulted in a selective increase in the number of ciliary neurons on E15. The highest dose of naloxone completely prevented the naturally-occurring loss of ciliary neurons.

The administration of scopolamine, a muscarinic antagonist, twice per day from E8 to E14½ resulted in an increase in the number of both ciliary and choroid neurons on E15. The daily administration of curare, a competitive neuromuscular blocking agent, resulted in a decrease in the number of choroid neurons on E15. The administration of decamethonium, a depolarizing neuromuscular blocking agent, twice per day from E8 to E14½ also resulted in a decrease in the number of choroid neurons on E15. The loss of choroid cells was greater following injections of curare than decamethonium. Neither curare nor decamethonium seemed to alter the number of ciliary neurons on E15.

186.5 CELL DEATH IN THE DORSAL ROOT GANGLIA OF ADULT RATS W.J. Gilbertie*, G.W. Lee*, H.L. Vahlsing*, and E.R. Feringa. Neurology Research Lab, San Diego VA Medical Center and Univ. of California, San Diego, CA 92161.

We counted the neurons of L-5 dorsal root ganglia (DRG) of adult rats to determine the effects of central vs peripheral axotomy and to study the central nervous system (CNS) projections. Four groups were studied: 1) spinal cord transection at T-9; 2) right hind limb amputation; 3) both procedures; 4) no treatment - age/sex matched controls. Twenty-five weeks post-surgery, the L-5 DRG were cut and stained with H&E. We counted the DRG cells at 120μ intervals throughout each ganglion. The number of cells in both L-5 DRG in rats whose long CNS processes were axotomized (4122±168) did not significantly differ from non-transected DRG counts (4009±173). Peripheral axotomy, however, yielded significant ($p<0.001$) DRG cell loss; 1639±143 on the amputated side vs 2337±241 contralaterally. Similarity in transected vs non-transected DRG cell counts indicates that either 1) axotomy of the central projection of the DRG cell is not lethal to that cell, or 2) that an insignificant proportion of DRG cells have long central projections passing through T-9.



The distal to proximal distribution of cells in the DRG's after right hind limb amputation. DRG's were sectioned from distal to proximal. Every 20th section (120μ) was counted. No cell loss was seen in the distal 440μ. Proximally we found cell loss at every level.

186.6 INCREASED NEURON DEATH IN THE CHICK EMBRYO CILIARY GANGLION FOLLOWING DEAFFERENTATION. S. Furber* and R. W. Oppenheim (SPON: J. Turner), Dept. of Anatomy, Bowman Gray Medical School, Winston-Salem, NC 27103.

Recent studies have indicated that the afferents as well as the efferent targets of developing neurons play a role in regulating the number of cells that survive the period of naturally-occurring neuron death (Clarke, P., Soc. Neurosci. Abst., 1983, 9:322; Okado, N. and R. W. Oppenheim, J. Neurosci., 1984, in press). By contrast, over 35 years ago, Levi-Montalcini (R. Acad. Naz. dei Lincei, 1947, 3:144) reported that a total deafferentation of the chick embryo ciliary ganglion (CG) resulted in a significant cellular hypotrophy but little or no reduction in cell number during the normal cell death period. In a later report, however, Levi-Montalcini (J. Comp. Neurol., 1949, 91:209) indicated that under the same conditions, cell number in the CG was reduced to almost zero by the time of hatching. Because of this apparent discrepancy and because the chick CG receives afferents from a single source, the accessory oculomotor nucleus (AON) in the midbrain, we have re-examined the effects of deafferentation on this population.

Removal of the anlage of the midbrain was carried out on embryonic day (E)2 (stages 11-12) - which is after the migration of the neural crest derivatives that populate the CG - using the vibrating needle technique (Wenger, B., Bioscience, 1968, 18:226). Naturally-occurring neuron death in the CG occurs between E8 and E15 (Landmesser and Pilar, J. Physiol., 1974, 241:737). Embryos were allowed to survive to E8, 10, 12, 15 and 18. The CG were removed and processed for histology and cell counts. The brains were also examined histologically to confirm the success of midbrain removal.

At present only the CG from E15 embryos (n=6) have been analyzed quantitatively. Cell number in the CG was reduced by 75% (3,500 vs 900) on E15 following deafferentation. The small, choroid cells appeared to be affected to a considerably greater extent than the large, ciliary neurons. Although we are presently examining the ganglia of deafferented embryos sacrificed on E8, 10, 12 and 18, these initial data from E15 show that complete deafferentation of the CG greatly exacerbates naturally-occurring neuronal death in this population.

186.7 THE QUANTITATIVE MATCHING HYPOTHESIS OF NEURONAL CELL DEATH --A TEST USING CHICK-QUAIL CHIMERAS. H. Tanaka* and L. Landmesser. Physiology Section, Biological Sciences Group, The University of Connecticut, Storrs, CT 06268.

Naturally occurring neuronal cell death has been proposed as a means of matching the size of a neuronal population with its target. For the chick, McLennan (Dev. Biol. 92:263) showed a 1:1 correspondence between the number of lumbosacral motoneurons (MN) and the number of myotube clusters during the MN cell death period. Since quail have smaller limbs than chicks and it is possible to create chick-quail chimeras, we decided to determine the quantitative relationship between MN's and myotubes during normal quail development and in chick-quail chimeras.

We found that by the end of the cell death period (Stage 38) quail had only about half the chick number of MN's; 5807 ± 376 (mean ± S.D.) versus, 9409 ± 981. In both species there was a good correspondence between the number of MN's and the number of myotube clusters; the ratio being 1.16 for the chick and 0.80 for the quail. However, the pre-cell death MN population was also smaller for the quail, so that the percent of surviving MN's was similar for both species; 44% for the quail, 49% for the chick.

Thus, the species difference in neuronal population size is not the result of differential MN survival. However, the extent to which MN cell death normally serves to match target size in either species should be clarified by using chimeras. Based on spinal nerve stimulation (being confirmed by MN pool localization), chick MN's appear to innervate homologous muscles in the quail, providing a good test of the quantitative hypothesis.

The size of the chick MN population innervating a quail limb was substantially reduced (81% of the control value), although it still exceeded the quail value by 27%. The reason for this is not yet clear. However, because the distribution of fast and slow muscle fibers changed in some quail muscles innervated by chick MN's, it is possible that the ratio of fiber types also affects MN survival. In the converse experiment, if the matching hypothesis holds, it should be possible to reduce quail MN cell death by 75% by allowing them to innervate a chick limb. These experiments are now in progress.

Supported by NIH grant NS19640. H. Tanaka was supported by the Japanese Ministry of Education, Science, and Culture.

186.8 THE EFFECTS OF CHRONIC NEUROMUSCULAR BLOCKADE AND cGMP ADMINISTRATION ON DIFFERENTIAL MOTORNEURON SURVIVAL IN THE AVIAN CILIARY GANGLION. S. D. Meriney, G. Pilar and R. Nuñez.* Physiology Section, Biological Sciences Group, The University of Connecticut, Storrs, CT. 06268.

Motorneuron (MN) survival in the chick ciliary ganglion was investigated after drug treatments beginning one day prior to onset of cell death in the ganglion (day 7 of incubation) and continuing through completion of cell death (day 14). The ciliary ganglion is composed of two populations of neurons (ciliary and choroid) which are both cholinergic, but which innervate different targets. Ciliary cells make nicotinic synapses with the striated muscle of the iris and ciliary body, but the initial functional contacts are muscarinic. Choroid cells make muscarinic synapses with the smooth muscle of the choroid coat. During the period of normal cell death, both populations of neurons decrease by 50%. The effects of both nicotinic (∞BTX-12.5 μg/day, and dTC-2mg/day) and muscarinic (atropine-0.15mg/d) receptor blockade, as well as cGMP (1mg/day) administration were examined by total MN counts, and differential MN counts by selectively labeling ciliary cells with retrogradely transported HRP from the iris and ciliary body; the remaining choroidal population was counterstained with cresyl violet. Control ganglia showed a total of 3750 cells at day 14, 1397 of which were ciliary and 2476 of which were choroidal. When embryos were treated with nicotinic blockers there was no change in total cell number, but there was a 44% increase in the ciliary cells (1980 vs 1397, $p>0.03$). Chronic blockade of muscarinic receptors also resulted in a 69% increase in ciliary cells (2359 vs 1397, $p>0.01$) with no change in total cell number. When cGMP was administered, the same was observed (cGMP has been shown to save spinal MN's: Weill, Nature 308:452, 1984). The total number of cells was unchanged while the number of ciliary cells increased (51%; 2110 vs 1397, $p>0.05$). In all cases, ciliary cell survival increased while choroid cell survival decreased. It is interesting that different treatments produced this decrease in choroidal cell survival, with a corresponding increase in ciliary cells, such that total ganglion cell number remained at control levels. These observations suggest that local factors in the ganglion intrinsically regulate the total cell number. This possibility is not exclusive of differential drug effects on target development. (Supported by NIH Grant NS 10338 and an MDA Fellowship)

186.9 NEURONAL CELL DEATH IN THE ELECTRIC LOBE OF TORPEDO MARMORATA. G.Q.Fox and G.P.Richardson. Max-Planck-Institut für biophysikalische Chemie, 3400 Göttingen,Fed.Rep.Germany.

Two waves of neuronal cell death have been identified in the electric lobe of Torpedo marmorata during embryonic development(Fox & Richardson, J.Comp.Neurol.,207:183,1983). The first and most prominent occurs early in gestation, well before the differentiation of the target electrocytes and is localized primarily in the posterior pole of the electric lobes. The electromotoneurons of each lobe project to the electric organ by way of four major electromotor nerves which are components of the four branchial arch nerves. At early stages of development, however, the Torpedo has five innervated branchial arches so the 5th nerve must be lost or greatly reduced in size sometime prior to the end of gestation when only the four are recognized. The caudal localization of both cell death in the lobe and the 5th branchial arch nerve suggest a direct relationship with a further implication that the lack of supportive target tissue is the direct causal agent for neuronal cell death.

A series of Torpedo embryos from stages 23 to 75mm(total body length) was therefore prepared for light microscopic examination. Samples of electric lobe and 5th branchial arch were also prepared for electron microscopy. This material revealed that an anlage of electric tissue forms in the 5th arch at about the time it appears in the other arches. The formation of the characteristic myotube columns begins somewhat later though and only 10-20 such columns are generated here. These columns remain separate from those of the first four arches which subsequently fuse to form the main organ. They also appear retarded in development never achieving comparable dimensions with fewer and smaller myotubes. These cells do, however, differentiate into electrocytes beginning at the 53mm stage(compared to 40mm in the main organ columns) and maintain the same morphologic polarity.

A distinct large caliber branchial nerve is present up to the 30mm stage but then becomes greatly reduced in size. This exactly overlaps the period of the first wave of cell death in the electric lobes. Additionally, innervation of the 5th arch electrocytes also occurs indicating that some electromotor nerve remains. Because of the small amount of electric tissue produced in the 5th arch it seems likely that a mismatch between this tissue and the initial projection from the lobe has occurred and that the 30mm stage is the point when developmental dependancy between the two tissue is first expressed.

186.10 THE PREVENTION OF NATURAL MOTONEURON CELL DEATH BY THYROTROPIN RELEASING HORMONE (TRH). C.L.WEILL, Depts. of Neurology and Anatomy, Louisiana State University Medical Center, New Orleans, Louisiana, 70112.

Recently, Brooks et al. (Neurol.,34S:239,1984) reported an increase in cyclic GMP levels in mouse lumbar spinal cord upon treatment with TRH and Weill & Greene reported the prevention of 58% of natural motoneuron cell death in the chick spinal cord by dibutyryl-cyclic GMP treatment. The present study was undertaken to assess the effect of TRH on natural motoneuron cell death.

TRH dissolved in Tyrode solution was administered to chick embryos by dropping 0.1ml of solution onto the chorioallantoic membrane on embryonic days 5-9. Embryos were sacrificed on day 10, staged,and weighed. The lumbar spinal cord was dissected, fixed in Cornoy's solution, and stained with thionin. The tissue was embedded in paraffin, serially sectioned at 10 um, and for every 10th section all large, dark-staining cells of the lateral motor column (LMC) containing at least one nucleolous were counted at 400x.

During normal development a peak of 22,838 +/- 731 (mean +/- S.E.,n=6, counts uncorrected) motoneurons per LMC is observed on embryonic day 6. The number of cells declines by 34% to 15,018 +/- 537 (n=12) on day 10. Daily injections of 0.1847 ug of TRH per embryo resulted in the survival of 17,588 +/- 380 (n=5) motoneurons per LMC on day 10. That is, 33% of those cells that would have died by day 10 survived. Increasing the dose 5-fold to 0.9235 ug resulted in the survival of 17,627 +/- 311 (n=4). The mean body weight of control embryos on day 10 is 2,414 +/- 0.107 g (n=5),while that for the low and high doses of TRH was 2.589 +/- 0.064 (n=5) and 3.396 +/- 0.226 g (n=4) respectively. While there is no significant difference between the values for the controls and the low dose of TRH, the value for the high dose of TRH is significantly higher ($P<0.01$) than that for the controls and the low TRH dose. No significant difference in the number of cells containing 2 nucleoli was observed for either TRH dose relative to controls.

These data show that exogenous addition of TRH can enhance the survival of motoneurons of the chick spinal cord LMC during the period of natural cell death. While it is inviting to suggest that the mechanism of TRH action involves cyclic GMP, further studies will be required for confirmation.

Supported by NIH grant NS18642.

186.11 FIBER TYPE COMPOSITION OF REINNERVATED ADULT AND NEONATAL RAT SOLEUS MUSCLE. W. J. Thompson. Dept. of Zoology, University of Texas, Austin, TX 78712.

Each motor neuron to rat soleus muscle confines its innervation to largely one of the two types of fibers present in the muscle, both in the adult and early in postnatal life when every fiber is polyneuronally innervated (Kugelberg, J. Neurol. Sci. 27:269, 1976; Soc. Neurosci. Abs. 9:321, 1983). To gain further insight into how this segregation of innervation might be established during development, I have investigated the fiber types comprising reinnervated muscles. The right soleus muscle was denervated by crushing the soleus nerve at its entry into the muscle. At varying times after reinnervation, the right (reinnervated) and left (control) muscles were removed, examined physiologically and then sectioned for myofibrillar ATPase histochemistry. In agreement with previous reports (eg. Bearcroft et al., J. Physiol. 338:12P, 1983), muscles denervated at postnatal day 2 were never completely reinnervated: they contained fewer motor units and only a fraction of the normal number of soleus muscle fibers. Nonetheless, two wks after denervation the fibers in these muscles were differentiated into type I and II fibers in roughly the normal proportion. Obvious grouping of fiber types was not present. Further study is required to establish whether this result means a selective reinnervation according to fiber type or a non-selective reinnervation followed by type conversion of fibers mismatched with their innervation.

In contrast to neonatal denervation, muscles denervated in the adult animal were completely reinnervated: they contained the normal numbers of motor units and muscle fibers. Although a mixture of type I and type II fibers was present 8 days after reinnervation, almost 100% of the fibers were type I 6 wks later. Even more surprisingly, the contralateral soleus was likewise transformed to 100% type I fibers, although over a somewhat slower time course. Muscles denervated at postnatal day 2 and the contralateral muscles were also transformed to type I 16 wks later.

These results show that reinnervation leads to a conversion of type II to type I fibers in soleus. This conversion occurs even if apparently all soleus motor neurons return to the muscle or, in the case of the contralateral muscles, even though the normal innervation is intact. This suggests that the conversion of muscle fiber types is due to a conversion of the soleus motor neurons. How this conversion is effected by a lesion to a motor nerve (and how it is effected also on the contralateral side) is unclear.

187.1 DIAZEPAM AND VOLTAGE INCREASE GABA ACTIVATED CL ION CHANNEL OPENING KINETICS IN CULTURED MOUSE SPINAL NEURONS.
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Gigaseal single channel patch clamp recordings were made on cell-attached and excised membrane patches from neurons cultured for 2-6 weeks from the embryonic mouse spinal cord. Recordings were made in Hank's Basic Saline Solution, routinely containing TTX and 20mM TEA. Patch pipettes contained this solution plus .5 uM GABA, with or without 10 uM diazepam (DZM). In both cases, two distinct kinetic populations of open and closed times occurred in most patches. Lifetime histograms were fit by double exponential functions to derive relative amplitude and time-constant values. Neither the single channel conductance (normally 28-30 pS in symmetrical NaCl solutions) nor the fast and slow open and closed time constants were convincingly affected by DZM. However, the ratios of the number of fast to slow events was 2.7± 0.5 (S.E.) for open times and 1.4± 0.3 for the closed times in control patches, and 3.6±0.7 and 5.2± 1.3 for experimental patches (N=9 patches for each case). Diazepam thus acts to increase the relative number of fast closed events with respect to controls, which reflects increased re-openings,or bursts, of a GABA receptor-channel complex. Calculated burst length increased from 22.8± 4.5 ms in GABA to 54.3± 14.9 ms in GABA+DZM. The fractional open time, or percentage of the total recording time during which a channel is open, was 0.28± .05 in GABA, and 0.41± .05 in GABA+DZM. The ratio of the number of fast to slow closed events was voltage sensitive in some of the patches examined, showing an e-fold increase with a depolarization of approximately 78 mV in GABA, and 114 mV in GABA+DZM. The increased number of short closed events with depolarization in these control patches shows an effect of voltage similar to that of DZM, resulting in increased repetitive openings of GABA-activated Cl ion channels. Additionally, the voltage sensitivity with DZM suggests that the relative potentiation of GABA channel opening frequency by DZM may be greater at resting or hyperpolarized membrane potentials. (Supported by F32NS07044-02).

187.2 AN ELECTROPHYSIOLOGICAL STUDY: EFFECTS OF BENZODIAZEPINE ANTAGONISTS IN THE RAT SUBSTANTIA NIGRA
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There are at least two types of benzodiazepine (BZ) receptor antagonists. One type, is exemplified by ethyl-β-carboline-3-carboxylate (β-CCE), which blocks the effects of BZ's while producing effects which are the opposite of BZ's. These effects include an anxiogenic and a pro-convulsant action. Another type of BZ receptor antagonist exemplified by the imidazobenzodiazepine, Ro-15-1788, blocks the effects of BZ's without possessing any intrinsic actions. The substantia nigra zona reticulata (SNZR) has a high concentration of BZ and GABA receptors. BZ receptors are functionally linked to GABA receptors and BZ's appear to act by potentiating the inhibitory action of GABA. It has recently been shown that GABAergic agonists injected directly into the SNZR can suppress seizures, and that diazepam, a BZ agonist, potentiates GABA-induced inhibition of cell firing in the SNZR. Thus BZ anticonvulsant effects may in part be mediated by a potentiation of GABA in the SNZR.

In order to examine the effects of systemically administered BZ antagonists on idendified SNZR neurons we used single unit recording techniques and microiontophoresis. β-CCE (0.2 to 0.8 mg/kg i.v.) produced a significant, dose-dependent increase in the activity of SNZR neurons (n = 10). In contrast, Ro-15-1788 had no effect on SNZR cell firing in doses up to 4 mg/kg (n = 10). However, Ro-15-1788 (2.0 mg/kg) reversed the β-CCE-induced excitation. β-CCE in a dose which had no effect on cell firing (0.1 mg/kg) significantly reduced GABA-induced inhibition of SNZR cell activity while having no effect on the inhibition produced by iontophoresis of glycine (n = 8). Ro-15-1788 (2.0 to 4.0 mg/kg) failed to effect either GABA or glycine-induced inhibition but completely reversed the effects of β-CCE on GABA-induced inhibition.

Thus, the pro-convulsant effects of β-CCE may in part be mediated by an attenuation of GABAergic inhibition in the substantia nigra. It is possible that some component β-CCE's anxiogenic effects also is mediated through a similar mechanism in the SNZR.

187.3 BIDIRECTIONAL EFFECTS OF β-CARBOLINES WITH AFFINITY TO BENZODIAZEPINE RECEPTORS.
L.H. Jensen*, E.N. Petersen*, C. Braestrup* and T. Honoré* (SPON: P. Roland). A/S Ferrosan, Research Division, Sydmarken 5, DK-2860 Soeborg, Denmark.

Benzodiazepine (BZ) receptor ligands inducing direct opposite actions have been discovered among β-carbolines. The full agonist at BZ receptors, ethyl 6-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate (ZK 93423) induces anticonvulsive, anticonflict, antiaggressive and sedative effects while the partial agonist ethyl 5-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate (ZK 91296) induces anticonvulsive and anticonflict effects but lacks sedative properties. Ligands with the diametrically opposite action have been called inverse agonists. The full inverse agonist, methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM), induces convulsions and proconflict effects while the partial inverse agonist, N'-methyl β-carboline-3-carboxamide (FG 7142) produces anxiety in man (Dorow et al., Lancet ii:98,1983) and proconflict and proconvulsive effects in lab. animals.

The antagonist, ethyl 5-isopropoxy-4-methyl-β-carboline-3-carboxylate (ZK 93426) induces almost no effects but inhibits the effects of both agonists (ZK 93423 and ZK 91296) and inverse agonists (DMCM and FG 7142). Due to both BZ receptor occupation and the opposite effects, inverse agonists inhibit the effects of agonists and vice-versa. Furthermore, the partial agonists ZK 91296 may in some paradigms inhibit the effects of the full agonists ZK 93423, while the partial inverse agonist FG 7142 inhibits the convulsions induced by the full inverse agonist DMCM. These results clearly demonstrate that β-carbolines comprise a continuum of BZ receptor ligands spanning from full agonists to full inverse agonists.

These different effects of BZ receptor ligands seem to be induced by facilitatory or inhibitory modulation of the GABA-induced increase of chloride conductance at the GABA receptor-BZ receptor-Cl^--ionophore complex.

187.4 CONTINUOUS RELEASE OF DIAZEPAM: ELECTROPHYSIOLOGICAL, BIOCHEMICAL AND BEHAVIORAL CONSEQUENCES.
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We have previously reported that chronic administration of diazepam (DZ) decreases GABAergic sensitivity (Nature 308: 74). In those studies, DZ was administered as a single daily injection and at the time GABA sensitivity was tested, levels of DZ were not detectable. To determine whether the observed decrease in GABA sensitivity was due to tolerance or possible early withdrawal effects, we have implanted silastic tubing filled with crystalline DZ to provide for continuous release and maintenance of relatively constant brain levels of DZ throughout the 3 week treatment period. Drug-filled tubing was calibrated in vitro to release 5.0 mg/kg rat/24 hr and implanted s.c. under anesthesia the back of the rat. Rats were killed at weekly intervals and brain levels were measured using a radioreceptor assay. Under our treatment procedures, levels were measured to be 72.5, 70.0 and 106 ng/g brain tissue at 1,2 and 3 weeks respectively. Measurements of GABA sensitivity were then done with the pellets remaining in the animal to ensure the continuing release of DZ.

Electrophysiological recordings indicated that despite the continued release of DZ, GABA sensitivity was significantly decreased (GABA I x T_{50}: DZ = 38.0 ±3.4 vs VEH = 26.5 ± 2.8 nA.sec) while 5HT sensitivities were unchanged (5HT I x T_{50}: DZ= 202 ± 43 vs VEH= 239 ± 47 nA.sec). Furthermore, GABA displacement of bicuculline binding was increased in cortical membranes from chronic DZ animals (IC_{50}: DZ= 0.74 vs VEH= 1.05 μM). Finally, as a behavioral test of GABA sensitivity in the continued presence of DZ, the latency of various seizure components was measured after administration of the GABA antagonist, bicuculline. Acute administration of DZ blocks bicuculline-induced seizures. After a 2.5mg/kg s.c. dose of bicuculline, which produced seizures in all animals, generalized myoclonus occurred more rapidly in chronic DZ-treated rats despite the continued presence of DZ (time to myoclonus: DZ= 5.5 min vs VEH= 7.2 min)

Taken together, these data indicate that subsensitivity to GABA occurs in the continued presence of DZ and is therefore associated with tolerance to the benzodiazepines. (Supported by: Klingenstein Foundation, USPHS NS 19655 & MH 14276 and the State of Connecticut).

187.5 THE EFFECTS OF BENZODIAZEPINE ANTAGONISTS ON SINGLE UNIT ACTIVITY IN THE LOCUS COERULEUS. Gene Stoner*, L. Skirboll, D. W. Hommer. Electrophysiology Unit, Clinical Neuroscience Branch, NIMH, Bethesda, MD 20205.

The locus coeruleus (LC) has been proposed as an important neural substrate in the pathophysiology of anxiety (Redmond DE et al., Life Sci. 25: 2149, 1979). The benzodiazepine (BZ) diazepam, a potent anxiolytic, attenuates single unit activity in this noradrenergic nucleus suggesting that increased activity in the LC may be important in the production of anxiety (Grant S. et al., Life Sci. 27: 2231, 1980). There are two classes of BZ antagonists. One class, which includes the imidazodiazepine Ro-15-1788, block BZ effects without having any intrinsic action of its own. The other class, which includes the β-carbolines, do have intrinsic action when administered in the doses which also antagonize the effects of BZ agonists. These active BZ antagonists have been shown to be proconvulsant and anxiogenic in several animal models and to produce severe anxiety in humans (Skolnick, P., et al., J. Clin. Psychiat. 44:12, 1983).

To test the hypothesis that increased activity in the LC is important in the production of anxiety, we administered β-carboline-3-carboxylic acid ethyl ester (βCCE) and Ro-15-1788 intravenously while recording from single units in the LC of the rat. In another series of experiments, diazepam was administered i.v.(in doses up to 2 mg/kg) and βCCE's and Ro-15-1788 ability to reverse diazepam induced attenuation of LC firing rate was evaluated. LC neurons were identified by waveform, firing pattern, response to foot pinch, as well as histologically. Neither βCCE or Ro-15-1788 had any effect on LC firing rates in cumulative doses up to 6.4 mg/kg. In contrast, diazepam (0.5 - 2.0 mg/kg) produced a 30% decrease in firing rate of LC neurons. This diazepam induced decrease could be reversed by either βCCE of Ro-15-1788 (1.0 - 2.0 mg/kg).

Although neither BZ antagonist seems to possess any intrinsic action on LC neurons, both βCCE and Ro-15-1788 reversed the inhibitory action of diazepam. Therefore, anxiogenic and non-anxiogenic BZ antagonists possess a similar action in the LC. In summary, since βCCE is anxiogenic when administered in doses which fail to affect the activity of LC neurons, it appears that activation of the LC is not essential for production of anxiety by BZ antagonists.

187.6 CONCENTRATION-DEPENDENT EFFECTS OF BENZODIAZEPINES ON GABA RESPONSES AND SUSTAINED HIGH FREQUENCY REPETITIVE FIRING IN MOUSE CULTURED NEURONS. J.H. Skerritt*, D.M. Rock*, M.J. McLean* and R.L. Macdonald. Dept. of Neurology, Univ. of Michigan, 1103 E. Huron, Ann Arbor, MI 48104.

Mouse spinal cord and cortical neurons were used to study the effects of the anticonvulsant benzodiazepines (BDZ) diazepam (DZ), clonazepam (CLZ) and nitrazepam (NZ) and the convulsant BDZ Ro5-4864 on iontophoretic GABA responses and sustained high frequency repetitive firing of action potentials.

Mouse neurons were maintained in primary dissociated cell culture for 4-6 weeks prior to experiments. For experiments the cell cultures were bathed either in Dulbecco's phosphate buffered saline or TRIS buffered saline solutions. In studies of repetitive firing or GABA responses, magnesium was elevated (1 mM to 10 mM) to suppress background spontaneous activity. Transmembrane potentials were recorded intracellularly with high resistance (20-50 M) glass micropipettes filled with either 4 M potassium acetate (repetitive firing and spontaneous activity experiments) or 3 M potassium chloride (GABA responses). BDZ were added to the bath for repetitive firing experiments or were ejected onto neurons by pressure pulses (.2-.8 psi) applied to blunt tip (5-10 μm) glass micropipettes.

DZ and CLZ (>1 nM) and NZ (>10 nM) reversibly enhanced GABA responses in both spinal cord and cortical neurons. In contrast, Ro5-4864 reversibly antagonized GABA responses in spinal cord neurons at higher concentrations (>100 nM). DZ (>75 nM), CLZ (>75 nM) and Ro5-4864 (>250 nM) all limited high frequency firing of action potentials.

We suggest that anticonvulsant BDZ including DZ, CLZ and NZ may have dual anticonvulsant actions to enhance GABAergic synaptic transmission and suppress RF while the convulsant BDZ Ro5-4864 may produce seizures by antagonizing GABAergic synaptic transmission.

Supported by NIH Grant R01 NS19692 (RLM).

187.7 LOW DOSE CLONAZEPAM INHIBITON AND CGS-8216 EXCITATION OF HIPPOCAMPAL CA1 NEURONS IN VITRO; A POTASSIUM MECHANISM. N. Gurevich, M.F. Davies and P.L. Carlen. Playfair Neuroscience Unit, Toronto Western Hospital; Addiction Research Foundation Clinical Institute; Depts. of Medicine and Physiology, University of Toronto, Toronto, Ontario, M5T 2S8.

A previous study has shown that clinically relevant nanomolar concentrations of the water soluble benzodiazepine midazolam did not enhance GABA actions but instead inhibited cell excitability by augmenting Ca^{++}-mediated K^+ conductance (gK_{Ca}) (Carlen et al., Brain Res. 271:358-364, 1983). This work has been extended by examining the effects of a water insoluble benzodiazepine, clonazepam, which is also known to specifically bind to the central benzodiazepine receptor. Intracellular recordings were obtained from CA1 cells in rat hippocampal slices. Clonazepam, added to the perfusate or applied focally by pressure ejection (2×10^{-9}M) caused a hyperpolarization, decreased spontaneous activity and increased the amplitude and length of the post-spike afterhyperpolarization (AHP). As well as being apparent with 3M K acetate or 2M K methylsulphate electrodes, these effects persisted when chloride ions were injected into the cell from 3M KCl electrodes, indicating that these drug responses are probably not mediated by chloride conductance. Clonazepam was able to cause its usual affects even when the GABA-chloride ionophore was blocked by picrotoxin (1×10^{-4}M), again suggesting that the effect of clonazepam at this low concentration is not mediated by chloride conductance (gCl).

It has been previously shown that the water soluble benzodiazepine antagonist Ro14-7437 alone has actions opposite to midazolam (Carlen et al., Brain Res., 271: 115-119, 1983). Like Ro14-7437, another benzodiazepine antagonist CGS-8216 (1×10^{-8}M), alone caused an increase in spontaneous activity and a decrease in the size of the AHP, and also antagonized the actions of clonazepam.

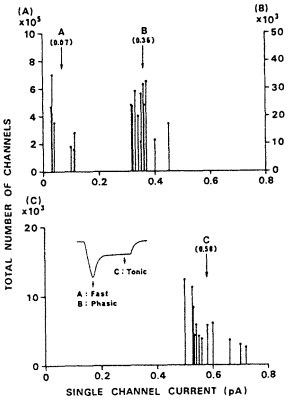
From these data it appears that the central benzodiazepine receptor agonist increases gK_{Ca}, not gCl, at low concentrations, and this can be antagonized by at least 2 central benzodiazepine antagonists. Supported by the Medical Research Council of Canada; Canadian Geriatrics Society and Ontario Mental Health Foundation.

187.8 GABA ACTIVATES THREE TYPES OF THE RECEPTOR-IONOPHORE COMPLEX WITH THEIR RELATIVE DISTRIBUTION. N. Akaike* and Y. Oomura (SPON: H. Kannan). Dept. of Physiol., Fac. of Med., Kyushu Univ. 60, Fukuoka 812, Japan (Dept. of Physiol., Univ. of Occup. and Environ. Health, Sch. Med., Kitakyushu 807, Japan).

The GABA-activated chloride current (I_{Cl}) separated from other ionic currents in internally-perfused and voltage-clamped cell bodies (n=12) of the frog dorsal root ganglia was subjected to noise analysis. The estimated number of the ionophores (N) versus the calculated elementary Cl conductance (γ) was nearly exponential, thus following Zipf's law and thereby implicating an interacting differentiation during the receptor-ionophore ontogenesis. This graph also indicates the existence of three subgroupes (A, B and C) of the receptor-ionophore units; N≃3.6 x 10^3, 2.3 x 10^4, 5.9 x 10^3 (Figure), and γ≃1.6, 8.0, 12.9 pS for A, B and C in that order.

The activation appears to be phasic with A and B and tonic with C, since the given subtype emerged from noise analysis of the selective interval, i.e., either around the peak (for A and B) or the steady state (for C) of chloride responses to GABA. Single channel recordings conformed to the presence of B and C; the failure of detecting A may be accounted for by its small γ value coupled with a rapid desensitization incurred with this subtype. Further, we established 'concentration clamp' technique in which external solution can by completely exchanged by new test solution within 8 msec. Using this technique, we could separate fast, phasic and tonic currents.

DISTRIBUTION OF GABA-ACTIVATED CHLORIDE CHANNELS



187.9 ELECTROPHYSIOLOGICAL ACTIONS OF BENZODIAZEPINES (BZD) AND THEIR PUTATIVE ANTAGONISTS ON RAT DORSOLATERAL SEPTAL NEURONES (DLSN) IN VITRO. D.R. Stevens*, J.P. Gallagher and P. Shinnick-Gallagher, Dept. of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston,TX 77550.

We have reported previously that the BZD, flurazepam (Flu), inhibits spontaneous firing of DLSN (Stevens et al., Soc. Neurosci. Abstr., 9, Pt.1, p.411, 1983). Flu inhibits firing of DLSN by hyperpolarizing these neurons. The mechanism of this inhibition does not involve a GABA-chloride ionophore complex, but rather is due to an increase in potassium conductance (Stevens et al., Int. Cong. Pharmacol. 9, 1706, 1984). The present report further characterizes the actions of BZDs on DLSN neurons.

Using intracellular recording techniques from an in vitro brain slice preparation of DLSN we have recorded inhibitory actions of Flu at concentrations as low as 100 picomolar applied via superfusion. In comparison, diazepam, which also shared this novel hyperpolarizing action, was only active at concentrations of 100 nanomolar or greater.

RO15-1788 has been characterized as a BZD antagonist on the basis of behavioral and binding studies. When applied to DLSN, RO15-1788 induced a membrane hyperpolarization, decreased membrane resistance and inhibited spontaneously firing neurons. This action was similar in potency and mechanism to that seen with the BZD agonists. RO14-7437, a water soluble but less characterized BZD antagonist, also hyperpolarized DLSN and inhibited spontaneous firing.

Our data suggest that the two putative BZD antagonists act as agonists on DLSN.

187.10 INHIBITORY EFFECT OF CHOLESTERYL γ-AMINOBUTYRATE ON EVOKED ACTIVITY IN RAT HIPPOCAMPAL SLICES. G.W. Hesse*, V.E. Shashoua and J.N. Jacob* (SPON: E.D. Bird). Department of Biological Chemistry, Harvard Medical School, Mailman Research Center, McLean Hospital, Belmont, MA 02178

Recently this laboratory has reported the synthesis of a new lipid-soluble candidate GABAmimetic agent (Shashoua et al., J. Med. Chem., in press). This compound, cholesteryl γ-aminobutyrate (C-G), readily crosses the blood-brain barrier and has behavioral effects on rats and mice. It reduces open field activity, delays the onset of bicuculline-induced seizures, and alters operant behavior motivated by rewarding brain stimulation. To determine if the pharmacological effects of C-G are due to specific GABAmimetic properties of the compound, we have analyzed its mechanism of action on evoked activity in rat hippocampal slices. Extracellular field potentials were recorded from the stratum pyramidale of the hippocampal CA1 region and were elicited by stratum radiatum stimulation. Various compounds of interest were dissolved in medium and applied to the surface of the slice near the recording electrode by pressure ejection of small droplets (volume about 300 picoliters) from a micropipette. When C-G was applied to the stratum pyramidale it produced dose-dependent inhibition of the evoked population spike. C-G was somewhat less effective than GABA in inhibiting the evoked population spike. However, the duration of the inhibition produced by C-G was nearly ten-fold longer than that produced by a comparably effective dose of GABA. The inhibitory effect of C-G (and of GABA) was reversibly antagonized by picrotoxin added to the slice medium, and by replacement of chloride in the medium with isethionate. In addition, pretreatment of slices with the esterase inhibitor phenylmethylsulfonylfluoride attenuated the inhibitory effect of C-G, but not that of GABA. These results suggest that C-G has GABA-like actions in the CNS, and that its inhibitory activity is largely dependent upon enzymatic release of GABA from the compound by esterases present in CNS tissue. Thus, cholesteryl γ-aminobutyrate is an effective prodrug for delivery of GABA to the CNS. The characteristics of this compound suggest it may be useful in a variety of clinical applications.

This research was supported by USPHS grant NH 16367 and by NRSA MH 14275-08 (GWH).

187.11 MONOCLONAL ANTIBODIES TO BENZODIAZEPINES. DEMONSTRATION OF BENZODIAZEPINE-LIKE DETERMINANTS IN THE BRAIN. L. Sangameswaran*, H.M. Cherwinski* and A.L. de Blas. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, NY 11794.

Five independent hybridoma lines secreting monoclonal antibodies to benzodiazepines were produced by immunizing BALB/c mice with a benzodiazepine-BSA conjugate. The antibodies compete with the brain benzodiazepine receptors for the binding to flunitrazepam. Preliminary experiments suggest that the binding affinities of the antibodies and the mammalian brain benzodiazepine receptors for flunitrazepam are similar. In contrast β-carboline carboxylate ethyl ester, an inverse agonist and RO15-1788, an antagonist, both of which have high affinities for the brain benzodiazepine receptors do not bind to the monoclonal antibodies.

One of the anti-benzodiazepine monoclonal antibodies binds to brain tissue with high-affinity. Immunocytochemical experiments indicate that the brain antigen is localized only in neurons. The binding of the antibody to the brain antigen is blocked by benzodiazepines but not by β-carbolines. In immunoblots this monoclonal antibody binds to a protein whose molecular weight has not been determined yet. We do not yet know if the brain antigen recognized by this anti-benzodiazepine monoclonal antibody has an endogenous benzodiazepine function.

187.12 AUTORADIOGRAPHIC LOCALIZATION OF BENZODIAZEPINE RECEPTOR DOWNREGULATION. E.I. Tietz, H.C. Rosenberg and T.H. Chiu, Medical College of Ohio, Toledo OH 43699.

Our laboratory has previously demonstrated that chronic administration of flurazepam reliably decreases maximal ^{3}H-flunitrazepam (^{3}H-FNP) binding 15-20% in homogenates from rat hippocampus, cerebral cortex and medulla-pons, but not in remaining areas isolated by gross regional dissection. To further localize these regional differences, autoradiographic techniques were used to quantitate binding site density after chronic flurazepam treatment.

Male rats (125-150 gm) drank .02% saccharin water containing flurazepam for 1 or 4 weeks according to a regimen previously described. A control group received only saccharin water. 16 μM frozen sections mounted on slides were prewashed for 30 min in cold isotonic TRIS-HCl (pH 7.4 at 0°C). The slices were incubated 30 min at 0°C with a near saturating concentration (20 nM) of ^{3}H-FNP, washed 4x15 sec with TRIS to reduce nonspecific binding and rapidly air dried. For nonspecific binding adjacent sections were incubated in the presence of 1 μM clonazepam. Paraformaldehyde vapor-fixed slices were apposed to LKB-Ultrofilm for 3 days. Densities over selected areas measured with a microscope photometer were quantitated using a standard curve generated with ^{3}H-FNP standards. Many areas of localized receptor downregulation were evident, most notably the substantia nigra pars reticulata.

Specific ^{3}H-FNP Binding (pmol/mg protein) (*,P ≤ .05)

	Control(4)	1 Week(4)	4 Week(4)
Hippo., molec. layer	2.41	2.10(13%)	1.96(19%)
Substantia nigra, p.r.	1.24	1.11(11%)*	0.79(36%)*
Superior colliculus	2.97	2.44(18%)*	2.31(22%)*
Cerebral cortex, lay. IV	3.26	2.74(16%)	2.25(19%)*
Lateral amygdaloid nuc.	2.65	2.16(19%)	2.11(20%)
Corpus callosum	0.11	0.08	0.19
Nonspecific binding	0.17	0.21	0.20

It is unclear if the changes in the binding density might be partly due to the presence of residual drug despite the precautions taken to limit residual drug effects. Further experiments are currently being undertaken to rule out this possibility. This technique allows accurate quantitation of localized changes in benzodiazepine receptor number in response to chronic treatment.

Supported by DHHS grants F32-DA05079, RO1-DA02194 and RO1-NS16595.

187.13 AUTORADIOGRAPHIC DETERMINATION OF BRAIN REGIONS WHERE THE DISTRIBUTION OF BENZODIAZEPINE AND LOW AFFINITY GABA RECEPTORS OVERLAP. J.K. Wamsley, D.R. Gehlert* & R.W. Olsen. Depts. of Psych. & Pharmacol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84132 and Dept. of Pharmacol. UCLA Sch. of Med., Los Angeles, CA 90024.

Benzodiazepines are thought to bind to specific receptors which are part of a macromolecular receptor complex associated with a chloride ionophore. An intrinsic part of this chloride-channel coupled receptor complex is the presence of bicuculline sensitive-baclofen insensitive receptors for gamma-aminobutyric acid ($GABA_A$ sites). The distribution of benzodiazepine receptors (Young and Kuhar, 1980), as defined by receptor autoradiography using [^{3}H]-flunitrazepam ([^{3}H]-FLU), does not correlate with that of the high affinity GABA receptors localized by [^{3}H]-muscimol binding (Palacios et al, 1981). This discrepancy has left open the possibility that the low affinity $GABA_A$ sites may be the GABA receptor subtype associated with the actions of benzodiazepines. Low affinity GABA receptors have recently been labeled for autoradiography using [^{3}H]-bicuculline ([^{3}H]-BMC) in the presence of thiocyanate (Olsen et al, 1984). We have compared the distribution of low affinity $GABA_A$ sites with those of the benzodiazepine receptor using a quantitative technique of receptor autoradiography.

Slide mounted sections of rat brain were labeled in vitro with either [^{3}H]-FLU or [^{3}H]-BMC using conditions previously shown to produce a high specific to nonspecific binding ratio. Labeled sections were then opposed to LKB Ultrofilm and the autoradiograms generated were analyzed using computer assisted microdensitometry. Low affinity $GABA_A$ sites were localized to the same regions where benzodiazepine receptor binding took place. This overlap occurred in such areas as: lamina IV of the parietal cortex, cingulate cortex, molecular layer of the dentate gyrus, stratum oriens of the hippocampus, subiculum, several thalamic and hypothalamic nuclei, zona incerta, periaqueductal gray matter, substantia nigra-zona reticulata, superficial layer of the superior colliculus and in the molecular layer of the cerebellum.

The absolute density of low affinity $GABA_A$ sites thus correlated very closely with the baseline binding of [^{3}H]-FLU as well as with areas showing GABA enhancement of [^{3}H]-FLU binding (Unnerstall et al, 1981). These observations, coupled with results of previous binding studies performed in membrane homogenate preparations, support the concept that it is the low affinity $GABA_A$ sites which are associated with benzodiazepine receptors.

187.14 ANTISERUM TO GABA: CHARACTERISATION AND IMMUNOCYTOCHEMICAL USE ON CAT, MONKEY AND HUMAN BRAIN TO IDENTIFY GABA-ERGIC NEURONS. A.J.Hodgson [1], A. Erdei *[2], B.Penke *[3], I.W. Chubb *[1], P.Somogyi *[1,4]. Dept. Physiol. Flinders Uni. of S. Australia, Bedford Park, 5042, Australia [1] ; Dept. Immunol., L.Estvos Uni.,Budapest, Hungary [2]; Dept. Med. Chem., Med. Sch. Szeged, Hungary [3]; 1st Dept. Anat. Semmelweis Med. Sch. Budapest [4].

Rabbits were immunized with a GABA-BSA conjugate. Antisera were characterised by coupling various compounds to nitrocellulose with glutaraldehyde and then immunostaining the paper by the unlabelled antibody enzyme method. Several of the antisera reacted with GABA but not with any of the following: glutamate, glutamine, glycine, l-aspartate, d-aspartate, α-aminobutyric acid, β-aminobutyric acid, taurine threonine, alanine, serine, putrescine, carnosine, homocarnosine, GABA-leucine and δ-aminolevulinic acid. The antisera showed weak reactivity with β-alanine and γ-amino β-hydroxybutyrate(GABOB) but strong reactivity with δ-aminovalerate and ε-aminocaproate which are homologous to GABA but have longer carbon chains. All immunostaining on paper or tissue sections was abolished by solid-phase adsorption of the sera to GABA but was not affected by glutamate and only slightly attenuated by β-alanine or GABOB. We conclude that the antisera localises GABA itself in tissues and not related compounds.

Immunocytochemical staining of rat, cat, monkey or human brain using either a pre- or post-embedding unlabelled antibody enzyme method showed staining of many neuronal perikarya in the cerebral cortex, striatum, cerebellum and hippocampus. Serial sections showed that some GABA neurons in hippocampus and cerebral cortex also contained somatostatin or cholecystokinin. Staining of neurons that had been characterised by Golgi impregnation, from either the cortex, hippocampus or striatum of cat, monkey or human, demonstrated that several types of interneuron could contain GABA. Strongly stained punctae filled the neuropil and surrounded GABA-stained as well as unstained cells. Staining of EM sections by the protein A immunogold method showed that the punctae were boutons making Gray type II synapses.

The results show that the antisera to GABA are useful in identifying the neurotransmitter used by morphologically characterised neurons. Its use in conjunction with other techniques for tracing neuronal pathways will increase our understanding of GABA-ergic circuits and how they may be involved in disorders such as epilepsy.

187.15 CEREBELLAR GRANULE CELLS IN CULTURE CONTAIN GABA-MODULIN AS DETECTED BY A MONOCLONAL ANTIBODY. F. M. Vaccarino, M. J. Dobersen*[1], J. A. Hammer*[1], V. Gallo* and A. Guidotti. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032 and [1]Sec. Myelin and Brain Development, DMNB, NINCDS, NIH, Bethesda, M.D. 20205.

GABA-modulin is a 17000 dalton basic protein that has been purified from rat brain synaptosomal membranes and down-regulates the number of high-affinity receptors for GABA by an allosteric mechanism. GABA-modulin is similar to the small basic protein present in myelin (SMBP), however it can be differentiated because: it is selectively present in the synaptosomal fraction of rat brain, it has a higher molecular weight and differs from SMBP for the amino acid composition. Since polyclonal antibodies raised against GABA-modulin cross-react with myelin basic proteins, monoclonal antibodies have been prepared against GABA-modulin in order to study the physiological role and localization of this protein in brain. NZB/N mice have been immunized and their lymphocytes fused with P3x63 myeloma cells. One of the cloned hybrids was selected because it secretes antibodies specific for GABA-modulin which did not cross-react with a 100-fold higher concentration of SMBP or of large myelin basic protein. These antibodies have been used to detect GABA-modulin in a population of neurons particularly rich in GABA-receptors, the cerebellar granule cells. Granule cells in primary culture are not myelinated and contain less than 5% of astrocyte and oligodendrocyte cells (Dev. Brain Res. 10:227, 1983). These primary cultures represent an excellent model for studying the GABA-BZ-Cl^- receptor/ionophore complex and its regulation. They express, in fact, post-synaptic GABA receptors, BZ receptors as well as Cl^- channel-associated receptors, as detected by ^{3}H-muscimol, ^{3}H-flunitrazepam and ^{3}H-t-butylbicyclophosphorothionate binding respectively under physiological conditions and in undisrupted cells. Granule cells contain a protein that, purified by a standard procedure, is identical for molecular weight and amino acid composition to GABA-modulin purified from all brain synaptosomes. This protein also reacts with our specific monoclonal antibody and its concentration in the granule cells is 0.52 μg/mg protein.

The presence of GABA-modulin in a homogeneous population of neurons receiving a strong GABA input but not using GABA as a neurotransmitter is highly indicative of a post-synaptic localization for this protein. Moreover, the availability of a monoclonal antibody probe could be very important in studying the functional role of GABA-modulin in association with GABA-receptors.

187.16 ONTOGENY OF GABAERGIC NEURONS IN THE RAT BRAIN: AN IMMUNOCYTOCHEMICAL STUDY. J.M. Lauder,*[1] A.C. Towle,*[2] V.K.M. Han*[3] and P. Henderson,*[4] Depts. of Anatomy,[1,4] Pediatrics[3] and the Biol. Sci. Res. Ctr.,[2] Univ. of N.C. Sch. Med., Chapel Hill, NC 27514

We have raised an antiserum in the rat against GABA-glutaraldehyde-hemocyanin conjugates. The specificity of this antiserum has been established in blocking experiments with glutaraldehyde conjugates of GABA, glutamate and β-alanine. For developmental studies, embryos and postnatal rats were perfused with 2% paraformaldehyde, 2% glutaraldehyde and 1% Na meta bisulfite in 70 mM Na-phosphate buffer, pH 7.0 and their heads or brains embedded in paraffin. Sections (10 μm) were stained with the GABA antiserum at dilutions of 1:2000-5000 using the avidin-biotin (ABC) method. GABA immunoreactivity (IR) was clearly seen in the youngest embryos examined (gestational day 12; E12). In the sagittal plane, GABA-IR was localized in long tracts coursing in the marginal zone of the spinal cord and brainstem and extending as far as the diencephalon along the ventral aspect of the mesencephalic flexure. Another striking feature was the presence of thick GABA-IR fibers crossing over the surface of the tectum and filling the fascicles of the posterior commissure. At E14, the long tracts in the brainstem had spread from the marginal zone to the intermediate zone (sagittal plane) and thick fibers could be seen passing in the medial longitudinal fasciculus. Innervation of the thalamus was evident one day later. At E16, for the first time GABA-IR cell bodies of the cerebral cortex were seen in the vicinity of the cortical plate and in the molecular layer. By E18, GABA-IR cell bodies were clearly visible in corpus striatum, hippocampus and diencephalon. Cells in the cerebellar plate, faintly IR at E18, were clearly visible at E20. By P8, Golgi, Purkinje, basket and stellate cells within the cerebellum and cells in the superior and inferior colliculi were immunoreactive. The thick tectal fiber bundles, prominent during early development, were replaced by dense, fine fiber plexuses. The source of the earliest developing GABAergic fibers in the spinal cord, brainstem, tectum and posterior commissure is unclear, but they may represent ascending tracts, which develop separately from the intrinsic GABAergic neurons present later in gestation throughout the brain.

187.17 DISTRIBUTION OF GABA-T INTENSIVE NEURONS IN THE RAT HINDBRAIN. T. Nagai*, T. Maeda*, H. Imai*, P.L. McGeer and E.G. McGeer (SPON: D.W. Paty). Depart. of Pediatrics, Osaka University Medical School, Osaka; Depart. of Anatomy, Shiga University of Medical Science, Shiga, Japan; and Kinsmen Laboratory of Neurological Research, Depart. of Psychiatry, University of British Columbia, Vancouver, Canada.

A pharmacohistochemical method, involving systemic administration of an irreversible GABA transaminase (GABA-T) inhibitor (gabaculine) and the detection, 12 to 15 hours later, of the newly synthesized GABA-T by histochemical means, was previously used to identify the distribution in rat fore-brain and mid-brain of GABA-T intensive neurons. (Nagai et al. J. Comp. Neurol. 218:220, 1983) All known GAD-positive cell groups were GABA-T intensive and known non-GABAergic groups were not. This method has now been applied to a study of GABA-T intensive neurons in the rat medulla oblongata which is well known to contain a relatively high concentration of GABA but where there is little information on the localization of GABAergic neurons or GABAergic systems.

GABA-T intensive neurons were found to be rich in the following hindbrain structures: the inferior colliculus, the nuclei of the raphe system, the nuclei parabrachialis dorsalis and ventralis, the nucleus cuneiformis, the nucleus vestibularis medialis, the nucleus tractus spinalis nervi trigemini, the dorsal motor nucleus of the vagus, the nucleus cochlearis, the nucleus reticularis lateralis, the nucleus ambiguus, the fasciculus cuneatus, the principal nucleus of the inferior olive and the reticular formation of the pons and medulla. Neurons of the deep cerebellar nuclei and the rostral portion of the lateral vestibular nucleus were negative for GABA-T but were surrounded by granular staining indicative of impinging GABA-T-rich nerve endings. The data on the cochlear nucleus, dorsal raphe, inferior colliculus, dorsal motor nucleus of the vagus, nucleus ambiguus and neurons of the deep cerebellar nuclei are consistent with literature reports on GAD and on GABA levels, uptake and release. The present results provide further support for the hypothesis that GABA neurons are far more GABA-T intensive than other neurons in the central nervous system. They also provide evidence on the localization of many previously unreported GABA-T intensive, and hence presumptive GABAergic, cell groups.
(Supported by the Medical Research Council of Canada).

187.18 PRESUMPTIVE GABAERGIC PATHWAYS STUDIED BY A DOUBLE STAINING METHOD FOR GABA-TRANSAMINASE AND HORSERADISH PEROXIDASE. M.Araki*, P.L.McGeer and E.G.McGeer (SPON: V.Singh). Kinsmen Laboratory of Neurological Research, Dept of Psychiatry, Univ. British Columbia, Vancouver, B.C., Canada, V6T 1W5.

The pharmacohistochemical method which has been used to map GABA-transaminase (GABA-T) intensive neurons in rat brain was combined with retrograde tracing by horseradish peroxidase (HRP) to study GABA-T neuronal projections. All known GABA neurons stain intensively for GABA-T (Nagai, T. et al. J.Comp.Neurol., 218:220-238, 1983).

Male albino rats received unilateral injections of HRP by microelectrophoresis into (i) the lateral habenula, (ii) the superior colliculus, or (iii) the substantia nigra (SN). Either ethanolamine-O-sulfate (EOS) or gabaculine, irreversible GABA-T inhibitors, was administered 24 hrs after the HRP injection. The animals were sacrificed 17 hrs after EOS or 13 hrs after gabaculine by perfusion with fixative. Serial vibratome sections, 50 μm thick, were stained by the diaminobenzidine (DAB) method of Graham and Karnovsky, by the tetramethylbenzidine method of Mesulam or doubly stained for GABA-T and for HRP by the DAB method.

This double staining method revealed the precise localization of the GABA-T-intensive neurons which are the presumptive cells of origin of three previously investigated GABA pathways. The results indicate: (i) The afferent projection to the lateral habenula mainly originates from the entopeduncular nucleus (EP) and the lateral hypothalamus and involves in each case both GABA-T-intensive and non-GABA-T-intensive fibers. (ii) The afferent fibers to the superior colliculus are mostly GABA-T-intensive and are mainly from the SN pars reticulata but with some from the zona incerta and the reticular formation of the mesencephalon. (iii) The afferent GABA-T-intensive projection to the SN comes from cells clustered at the lateral borders of both the caudate-putamen (CP) and the globus pallidus (GP). These results correspond closely with previous data on GABA projections from the EP to the lateral habenula and from the SN to the superior colliculus, reinforcing the concept that GABA neurons are GABA-T-intensive. They also explain most of the controversy found in biochemical studies of descending GABA fibers from the basal ganglia to the SN in lesioned animals. The GABA-T-intensive cells account for about 80% of the projection from the GP but only about 20% of that from the CP.
Supported by MRC of Canada.

187.19 GABA RECEPTORS ON CHICK CILIARY GANGLION NEURONS. A.E. McEachern, J.F. Margiotta, and D.K. Berg. Univ. of Calif., S.D.; La Jolla, CA. 92093.

Chemical transmission through the chick ciliary ganglion is mediated by cholinergic synapses. We report here that the neurons have GABA receptors in addition that can mediate inhibitory responses to exogenously applied GABA capable of blocking transmission through the ganglion.

GABA receptors on embryonic ciliary ganglion neurons were first characterized in cell culture. Intracellular recording was used to measure membrane potential and conductance changes induced by GABA that was pressure ejected onto the soma from a pipet. The GABA responses had a reversal potential of -47 +/- 2 mV (mean +/- SE, n=6) and were mediated by Cl^- ions. Muscimol, a common GABA agonist, elicited similar responses from the neurons. The GABA antagonists bicuculline (100 μM) and picrotoxin (150 μM) each blocked greater than 98% of the response to 50 μM GABA.

At 5 days in culture, the neurons displayed conductance changes of 28 +/- 6 nS to 50 μM GABA (n=18). During the second week of culture about 15% of the neurons were found to be insensitive to GABA though they remained sensitive to acetylcholine (ACh). Exposure to 100 μM GABA in the culture medium for 3 days followed by thorough rinsing resulted in a 7-8 fold reduction in GABA responses with little or no change in ACh responses. Conversely, chronic exposure to 25 mM K^+ in the medium followed by rinsing resulted in a 3-4 fold reduction in ACh responses with no change in GABA responses. These results complement findings by J. Tuttle suggesting that GABA and ACh receptors can be independently regulated on the neurons.

The effects of GABA on transmission through the ganglion were tested by bath applying the drug to an intact 15 day embryonic ganglion while stimulating the preganglionic input and extracellularly recording the compound action potential triggered in postganglionic ciliary nerves. GABA at 15 μM completely blocked transmission; half maximal blockade was achieved at 2-5 μM GABA. Picrotoxin (50 μM) caused a 40% reduction in the effect of 10 μM GABA.

These results demonstrate that ciliary ganglion neurons have GABA receptors both in cell culture and in vivo, that GABA and ACh receptors can be independently regulated by the neurons, and that the GABA receptors can be activated to block transmission through the ganglion. (Supported by NS 12601.)

187.20 IN-VITRO DEVELOPMENT OF GABAERGIC NEURONS: AN IMMUNOCYTOCHEMICAL AND AUTORADIOGRAPHIC STUDY. V.K.M. Han*[1], E. Lieth*, A.C. Towle*[2] and J.M. Lauder. (SPON: H. Krebs). Dept. of Anatomy, Pediatrics[1] and Biol. Sci. Res. Ctr.[2], Univ. North Carolina Sch. of Medicine, Chapel Hill, NC 27514

We have raised a polyclonal antibody in the rat against GABA-glutaraldehyde-hemocyanin conjugates (see abstr. Lauder et al.). Using the antibody for GABA immunocytochemistry in combination with ^{3}H-GABA uptake autoradiography, we studied the development of GABAergic neurons cultured by two techniques, above and under coverslips. Dissociated cell suspensions from cerebral cortices of E14 and E18 rat embryos were plated on poly-L-lysine coated plastic tissue culture coverslips at varying cell densities (0.1 to 1.0 x 10^6 cells per coverslip) in 24 well plates. At 18 to 24 hours, half of the coverslips were inverted. Comparisons were made between two types of cultures on 3, 7, 14 and 21 days in-vitro (DIV) by (a) morphology under phase contrast microscopy, (b) neuron specific enolase (NSE) and glial fibrillary acid protein (GFAP) immunoreactivity (IR) and (c) GABA-IR and autoradiography of high affinity ^{3}H-GABA uptake ± β-alanine. The avidin-biotin (ABC) method was used for IR studies. Cultures under the coverslips differ from those above the coverslips in that; (a) glial cells develop less, (b) neurons survive at a much lower plating density (0.1 x 10^6 cells/coverslip), which allows excellent study of neuronal morphology and (c) neurons develop extensive neuritic outgrowth and differentiate earlier. Only nonspecific GABA-IR is seen at 3 DIV. However, the percentage of neurons specifically stained for GABA decreases with time in culture from 25-30% (7 DIV) to 10-15% (14 or 21 DIV). The majority of the stained cells are of small, bipolar interneuron type. Most of the large multipolar neurons are unstained. Extensive arborization of GABA-IR neurites are seen throughout the culture. Combined GABA-IR and ^{3}H-GABA uptake autoradiography shows direct correlation between intensity of staining and uptake capacity. However, there exists a subpopulation of GABA-IR neurons with minimal or no GABA uptake. The reason for nonspecific GABA-IR in young cultures is unclear at present. The more specific GABA-IR in older cultures probably represent IR in differentiated GABA neurons. The cultures grown under coverslips have different growth characteristics when compared to sister cultures grown on coverslips. This should be considered in studies of neuronal differentiation in culture.

188.1 POSSIBLE EFFECTS OF β-CARBOLINES ON MEMORY. G. Chapouthier*, P. Venault*, L. Prado de Carvalho*, J. Simiand* and J. Rossier. Depts. I and II, Physiologie Nerveuse, CNRS, Gif-sur-Yvette and Sanofi Recherche, Toulouse, France.

Methyl-β-carboline 3-carboxylate (β-CCM) is a benzodiazepine (BZ) receptor ligand with pharmacological properties opposite to those of BZ in all situations tested. Thus β-CCM is a convulsant whereas BZ are known anticonvulsants;β-CCM is anxiogenic (L. Prado de Carvalho et al, Nature, 301 : 64, 1983) whereas BZ are known anxiolytics. Since BZ have been shown to be amnesic drugs, we have hypothesised that β-CCM could have the opposite effect i.e memory enhancing effects. This hypothesis was tested in three different situations. 1- Latent learning in mice. Rodents previously deprived of food and placed in a new environment with food, eat only small quantities. When placed in the same environment for a test session a few days later, the same food deprived animals eat more than during the first session. This enhancement in food consumption is interpreted as a sign of retention of the first exposure to the environment. β-CCM (0.2 to 0.3 mg/kg), when administered before the first session, induces a further enhancement of food consumption during the test session; on the other hand, diazepam (DZ) (1mg/kg) reduces food consumption. 2- Passive avoidance learning in mice. Mice were submitted to a one-trial passive avoidance task. During the training session, mice were placed in an illuminated box leading to a dark compartment. Entrance into the dark compartment was punished by an electric shock. The next day animals were placed again in the illuminated box. Latency to reenter the dark compartment was taken as an index of memory. β-CCM (0.2 to 0.3 mg/kg) when administered before the training session, enhanced the latencies the next day : on the other hand DZ (1 to 4 mg/kg) decreased them. 3- Imprinting in chicks. Chicks placed in front of a moving decoy during an acquisition session,learn to follow it. The ability of the chicks to follow the decoy is tested for in the same situation 24h later (test session). In chicks injected with β-CCM (2.5 mg/kg) before the acquisition session, the following of the decoy was enhanced in the test session. On the other hand, DZ (0.25 mg/kg) administered before the acquisition session reduced decoy following during the test session. In the three models, neither β-CCM nor DZ had any effect on the performance during the first session. Our results suggest that β-CCM enhances memory whereas DZ has amnesic effects.

188.2 PROTECTION AGAINST FEBRILE SEIZURES BY γ-VINYL GABA. D.D. Johnson, B. Wilcox*, J.M. Tuchek* and R.D. Crawford*, Depts. of Pharmacology & Animal and Poultry Science, University of Saskatchwan, Saskatoon, Sask. S7N OWO Canada

The high seizure susceptibility in epileptic fowl is due to an autosomal recessive mutation. Epileptiform seizures can be evoked in chicks homozygous for the epileptic seizure gene by elevating their body temperature using microwave diathermy. These seizures precede and differ in motor seizure pattern from a subsequent clonic-tonic seizure produced by hyperthermia in epileptic chicks. The latter seizure type also occurs in non-epileptic heterozygote hatchmates. Two-week-old epileptic chicks have a basal body temperature of 40.6±.1°C. In control epileptics seizures occurred after a temperature rise of 2.8°C. The time of seizure onset was dependent on the absolute body temperature and not on the rate of rise of body temperature. Previous studies have shown that phenobarbital but not phenytoin or valproate afford protection against the febrile seizures in epileptic chicks.

We now report that pretreatment of two-day-old or two-week-old epileptic chicks with γ-vinyl GABA (100 mg/kg) significantly reduces the incidence of the initial epileptiform seizure evoked by hyperthermia and delays the onset of the subsequent clonic-tonic seizure. This effect of γ-vinyl GABA does not begin to appear until 4 hours after its administration and increases over the subsequent 4 hours. The protection against febrile seizures was not due to a reduction in basal body temperature. The anticonvulsant action of γ-vinyl GABA in this model of febrile seizures was associated with a significant elevation of GABA concentrations in the cerebral hemispheres and optic lobes. (Supported by the Medical Research Council of Canada.)

188.3 TOLERANCE DOES NOT DEVELOP TO THE ANTICONVULSANT ACTION OF PK 11195, A BENZODIAZEPINE POTENTIATOR. M.A. Simmonds and Sandra E. File. MRC Neuropharmacology Research Group, The School of Pharmacy, University of London, England.

PK 11195 is an isoquinoline carboxamide derivative that potently displaces the benzodiazepine ^{3}H Ro 5-4864 (Le Fur et al. Life Sci. 32 : 1849, 1983). PK 11195 (30-60 mg/kg) significantly reduced the incidence of convulsions caused by Ro 5-4864 (30 mg/kg). No tolerance developed to this anticonvulsant action even after 25 days of daily dosing with PK 11195 (30 mg/kg). This contrasts with the rapid tolerance found to the anticonvulsant effects of diazepam against leptazol and against Ro 5-4864. There was no cross-tolerance between the anticonvulsant actions of diazepam and PK 11195 which suggests the two drugs act at different sites.

Recent electrophysiological evidence indicates that PK 11195 is a benzodiazepine potentiator. In the rat cuneate nucleus slice PK 11195 (10 μM) potentiated the actions of flurazepam (0.1 μM) to enhance the responses to the GABA analogue, muscimol. In higher concentrations (30 μM) PK 11195 also enhanced the muscimol response per se. Thus PK 11195 does act at the GABA-benzodiazepine receptor complex, but at a novel site. These effects of PK 11195 are compatible with its anticonvulsant action that shows no cross-tolerance with diazepam. It is unlikely that these behavioural and electrophysiological effects of PK 11195 are mediated by the peripheral type of benzodiazepine sites.

188.4 CHLORDIAZEPOXIDE AND Ro 15-1788, BUT NOT CGS 8216, REVERSE THE ANXIOGENIC ACTION OF FG 7142. Sharon Pellow and Sandra E. File. MRC Neuropharmac. Research Group, School of Pharmacy, University of London, Brunswick Square, London WC1N 1AX, UK.

FG 7142 (B-carboline-3-carboxylic acid methyl amide) inhibits 3H-benzodiazepine binding to CNS receptors (Jensen et al, Life Sci.33:393-399,1983) and is anxiogenic in man (Dorow et al, Lancet:98-99,1983), and in a punished drinking test in rats (Petersen et al, Adv. Biochem. Psychopharmacol.:38 1983). Since the social interaction test has proven sensitive to anxiogenic actions of B-CCE (File et al, Neuropharmacol.21:1033-37,1982) and B-CCP (File et al, Neuropharmacol. in press), we investigated the effects of FG 7142 in this test.

Pairs of singly housed male rats were placed in a wooden test arena 20min after injection (i.p.) and their social interaction scored for 7.5min. An anxiogenic profile is most clearly seen when rats are tested in low light and are familiar with the test box. At 5-10mg/kg FG 7142 had a significant anxiogenic effect (shown by reduced social interaction without a concomitant decrease in locomotor activity) that was positively correlated with the plasma concentrations of the drug. The anxiogenic action of FG 7142 (5mg/kg) was reversed by chlordiazepoxide (CDP, 5mg/kg) and Ro15-1788 (10 mg/kg) but not by CGS 8216 (10mg/kg). This pattern of results is similar to that seen with B-CCE in this test (File and Lister, Neurosci.Lett.39:91-94, 1983).

However, the profile of FG 7142 can be distinguished from those of Ro15-1788 and CGS 8216, both of which are also anxiogenic when given alone (File and Lister, ibid.). The anxiogenic effect of Ro15-1788 could only be reversed by chronic treatment with CDP, and neither acute nor chronic CDP, Ro15-1788 nor B-CCE could reverse the anxiogenic effect of CGS 8216 (File and Lister, ibid.; File and Pellow, submitted). It appears that the anxiogenic effects of FG 7142, Ro15-1788 and CGS 8216 may be mediated via different binding sites or mechanisms in the CNS.

188.5 EFFECTS OF THE ATYPICAL BENZODIAZEPINE Ro 5-4864 ON SELF-STIMULATION IN THE RAT. L.J. Herberg*, Sharon Pellow and Sandra E. File. (SPON: J. Liebman). Exp. Psychology Lab., Inst. Neurology, Queen Square, London WC1N and MRC Neuropharmacology Research Group, Brunswick Square, London WC1N, UK.

Ro 5-4864 is a 1,4-benzodiazepine (BDZ) that has high affinity for peripheral-type, but not for classical CNS, BDZ binding sites (Braestrup & Squires, Proc. Natl. Acad. Sci. USA : 3805-9, 1977). It has convulsant (File & Mabbut, Br. J. Pharmac. 78 : 76P, 1982), anxiogenic (File & Lister, Neurosci. Lett. 35 : 93-6, 1983) and sedative (File & Pellow, Psychopharmacol. 80 : 166-70, 1983) properties. We investigated its effects on responding for lateral hypothalamic stimulation on a variable interval (VI) 10 sec schedule of reinforcement; this procedure can identify and distinguish GABAergic compounds, anti-convulsants, BDZs and their 'antagonists' (Herberg & Williams, Pharmac. Biochem. Behav. 19 : 626-33, 1983; Pellow et al., Neurosci. Lett. in Press, 1984).

Rats with lateral hypothalamic electrodes were trained to press a lever for stimulation available on a VI 10 sec schedule, with current fixed at the lowest intensity for each rat that elicited steady responding. During test sessions rats were allowed to self-stimulate for 30 min to provide a baseline, then for a further 75 min after i.p. injection. Drug effects were expressed as a percentage of baseline and compared with controls.

Ro 5-4864 (30 mg/kg) significantly reduced self-stimulation. This effect could be reversed by PK 11195 (60 mg/kg), an isoquinoline that may be an antagonist at peripheral-type sites (LeFur et al., Life Sci. 33 : 449-57, 1983), but that may also have activity at the GABA-BDZ receptor complex in the CNS (Simmonds, in preparation). Chlordiazepoxide (CDP, 5 mg/kg) also reversed these effects, but the facilitatory effect of CDP alone was not reversed by Ro 5-4864. CDP has no affinity for peripheral-type BDZ sites, so an action via the CNS GABA-BDZ receptor complex seems likely. Phenytoin, which can reverse the anxiogenic and convulsant effects of Ro 5-4864 (File and Lister, File & Mabbutt, ibid), was unable to reverse the reduction in self-stimulation seen with Ro 5-4864.

188.6 EFFECTS OF PK 8165, PK 9084, TOFISOPAM AND Ro15-1788 ON THE RAT CORTICOSTERONE RESPONSE. Sandra E. File and Sharon Pellow. MRC Neuropharmacology Research Group, School of Pharmacy, Univ. of London, Brunswick Sq., London WC1, UK.

PK 8165, PK 9084 and tofisopam are putative anxiolytic compounds. The former two displace, and tofisopam enhances, the binding of 3H-benzodiazepines (BDZs) to CNS receptors (LeFur et al, Life Sci. 28 : 1439-48, 1981; Saano & Urtti, Pharmac. Biochem. Behav. 17 : 367-9, 1982). All 3 compounds have a BDZ-like profile in a rat punished drinking test (LeFur et al, ibid., Pellow, Neurosci. Biobeh. Rev. in press, 1985) but not in the social interaction test (File & Lister, Pharmac. Biochem. Behav. 18 : 185-88, 1983; Pellow, ibid.). To further compare these compounds with BDZs we investigated their effects on plasma corticosterone levels in the rat. In the home cage, low doses of BDZs leave unchanged and high doses increase levels; however, both low and high doses decrease the rise in corticosterone induced by exposure to a novel environment. We therefore selected both a low and a high dose of each of our compounds. For comparison we also investigated the effects of Ro15-1788, an anxiogenic BDZ antagonist that acts at BDZ receptors (Hunkeler et al, Nature 290 : 514-6; File et al, Neuropharmacol. 21 : 1033-37, 1982).

Male singly-housed rats were anesthetised with ether and blood samples taken by direct cardiac puncture between 8.00 and 10.00 h. Plasma concentrations of corticosterone were determined using a fluorimetric assay (Zenker & Bernstein, J. Biol. Chem. 231 : 695-701, 1958). Rats in the home cage condition were sampled 40 min after i.p. injection of Ro15-1788, 50 min after other compounds. Rats in the novelty stress condition were sampled after 20 min exposure to a brightly lit novel room or a holeboard, 20 or 30 min after injection, as appropriate.

PK 8165 and 9084 (10 x 25 mg/kg) resembled high doses of BDZs : they increased corticosterone levels in the home cage and reduced them in novelty stress. Tofisopam (10 and 25 mg/kg) increased home cage levels, but had no effect on the stress response. Ro15-1788 was totally unlike BDSz : at 4 and 10 mg/kg it had no effect on home cage levels but increased the response to novelty stress.

188.7 Ro15-1788 PRETREATMENT REVERSES GABA SUBSENSITIVITY INDUCED BY CHRONIC BENZODIAZEPINES. S.F. Gonsalves and D.W. Gallager. Department of Psychiatry, Yale University School of Medicine, New Haven, CT 06508.

Acutely administered benzodiazepines exert their pharmacological effects through selective facilitation of post-synaptic GABAergic neurotransmission. Recent clinical, behavioral and electrophysiological evidence suggests, however, that prolonged exposure to these agents diminishes drug response. We have recently demonstrated a decreased sensitivity to GABA in serotonin(5HT)-containing neurons of the dorsal raphe (DR) nucleus following long term exposure to diazepam (Nature 308:74, 1984). This GABAergic subsensitivity is rapidly reversed by acute administration of the specific benzodiazepine antagonist, Ro15-1788. We now report reversal of diazepam-induced GABA subsensitivity when Ro15-1788 is administered 12-16 hours prior to electrophysiological recording.

Adult male rats were injected with diazepam (DZ; 5 mg/kg/day,ip) or vehicle (VEH) daily for 3 to 4 weeks; they received Ro15-1788 (4 mg/kg,ip) or its Tween 80 vehicle 8 hours after the final injection of DZ/VEH. Twelve to 16 hours after the antagonist or vehicle, microiontophoretic sensitivities to 5HT or GABA were quantified in serotonergic DR neurons by calculating IxT_{50} values, defined as the product of iontophoretic current (nA) and time (sec) required to reduce the spontaneous firing rate of a recorded cell to 50% of its basal rate. As previously observed, chronic DZ alone significantly reduced the sensitivity of DR neurons to GABA (DZ: IxT_{50}= 40.3±5.5; VEH: IxT_{50}= 20.9±2.8, $p<.05$) without affecting 5HT sensitivity (DZ: IxT_{50}= 172.6±33.9; VEH: 151.8±37.5, ns). Pretreatment of chronic DZ rats with Ro15-1788 increased GABA sensitivity values to the vehicle range (DZ+RO: IxT_{50}= 24.4 ±4.4; VEH: 20.9±2.8, ns). Ro15-1788 did not affect GABA sensitivity in VEH animals nor did it modify 5HT sensitivity in either treatment group.

Preliminary data suggest that GABA sensitivity returns to control levels within 48 hours after terminating DZ treatment. The present study shows, however, that control GABA sensitivity can be restored during the course of chronic DZ treatment by administration of a short acting benzodiazepine antagonist. This observation suggests a possible strategy for prolonging the therapeutic effectiveness of benzodiazepines. (Support: Klingenstein Fund, USPHS MH 14276 & NS 19655, Epilepsy Foundation, and the State of Connecticut).

188.8 PROCONFLICT ACTION OF RO 15-1788 A PURPORTED INERT ANTAGONIST OF BENZODIAZEPINE RECOGNITION SITE. M. Ferrari*, M. G. Corda, A. Guidotti and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

Several lines of investigation support the view that RO 15-1788 (ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazol-[1,5]-[1-4]-benzodiazepine-3-carboxylate) curtails the actions of benzodiazepine and β-carboline-3-carboxylate (β-CC) while it is devoid of typical anticonvulsant, muscle relaxant, sedative and anticonflict actions of benzodiazepines or of proconvulsant and proconflict actions of β-CC. This lack of intrinsic activity has been taken to indicate that the benzodiazepine recognition sites are devoid of physiological significance. An alternative hypothesis proposes that transduction of chemical signals at GABAergic synaptic receptors is a multi signal process with additional sites to mediate the action of modulatory cotransmitters. Since benzodiazepine recognition sites are the receptors for a polypeptide cotransmitter (PNAS 80:3531, 1983) modulating GABAergic synaptic function, the action of ligands for benzodiazepine recognition sites endowed of weak intrinsic activity such as RO 15-1788 can be revealed only following a perturbation of the GABAergic receptor function. We have reported (Neuropharmacol. 21:86, 1982) that in rats receiving isoniazid to reduce the GABA content there is a critical need for benzodiazepine receptor availability and injections of RO 15-1788 trigger convulsions. We have adopted a similar strategy to study the action of RO 15-1788 on conflict. Rats received 300 mg/kg s.c. of isoniazid and then were tested with a modified Vogel test (PNAS 80:2072, 1983) to detect anticonflict and proconflict actions of drugs.

The rats treated with isoniazid responded to this test similarly to saline treated rats. When rats received RO 15-1788 (20 mg/kg s.c.) 40 minutes after isoniazid and 10 minutes before the Vogel test there was a very marked proconflict action. This action can be elicited by 10 mg/kg s.c. but not by 5 mg/kg s.c. of RO 15-1788. This benzodiazepine receptor antagonist given to saline treated rats is devoid of proconflict and anticonflict actions. It is concluded that the study of the benzodiazepine recognition site function and the evaluation of specific ligand potency is best performed in presence of a perturbation of GABAergic synaptic function.

188.9 DIAZEPAM BUT NOT CL218872 DECREASES DOPAMINE TURNOVER IN WHOLE RAT BRAIN DURING COLD STRESS. B.A. Meiners*, V. J. Justice* and A.I. Salama (SPON: R.D. Krell). Stuart Pharmaceuticals, Div. of ICI Americas Inc., Wilmington, DE 19897.

Diazepam has been reported to alter the turnover of catecholamines (CA) under conditions of stress. The effects generally required the administration of sedative doses of the benzodiazepines (BZs) and it was speculated that the effects were due to the sedative component of BZ action (Lidbrink, in The Benzodiazepines ed. by Garattini et al., 1973). Non-BZ anxiolytics that are less sedating than diazepam have been developed, for example, CL218872 (CL). It was of interest to determine whether this agent had similar effects on CA turnover as diazepam.

Dopamine (DA), norepinephrine (NE), 5-hydroxytryptamine (5HT), and 5-hydroxy-indolacetic acid (5HIAA) were measured by liquid-chromatography with electrochemical detection. Turnover of DA and NE were estimated by measuring the decline of both amines in whole brain following the inhibition of their synthesis with alpha-methyl-p-tyrosine (AMPT; 250mg free base/kg, i.p.).

Both diazepam and CL were administered at four times their minimum anxiolytic dose (20mg/kg p.o. and 10mg/kg p.o., respectively). At these doses they had no significant effect on the steady state level of DA. However, when the turnover of DA was measured during a 3 hour period of cold stress (5 degrees) the AMPT-induced decline of DA was reduced 30 to 60% by diazepam. In contrast, CL caused no change in the turnover under the same conditions. Likewise, the cold stress alone did not alter the turnover of DA. It may be suggested that the noted difference on DA turnover may reflect a pharmacologically relevant difference between the two compounds and perhaps be related to the relative lack of sedation of CL.

The turnover of NE was increased by cold stress as expected. Furthermore, there was a non-significant trend for this increase in turnover to be reversed by diazepam but not by CL. It is possible that this also represents a relevant difference between diazepam and CL. The effects of BZ and non-BZ anxiolytics on 5HT and 5HIAA will be discussed.

188.10 LACK OF EVIDENCE FOR CIMETIDINE AS A GABA-RECEPTOR ANTAGONIST. J.P. Trzeciakowski*, G.D. Frye. (SPON: H.W. Sampson). Dept. of Medical Pharmacol. & Toxicol., Texas A&M University, College of Medicine, College Station, TX 77843.

The histamine H_2-receptor antagonists metiamide and cimetidine increase mean arterial blood pressure (MAP) when given intracerebroventricularly (icv) to rats or cats. These changes in MAP are inconsistent with histamine receptor blockade, as both histamine and selective histamine receptor agonists produce only pressor effects following icv injection. Recently Antonaccio (Eur. J. Pharmacol., 72:369, 1981) attributed the pressor actions of metiamide and cimetidine to GABA-receptor blockade based in part on the observations that these drugs displaced [^{3}H] GABA in vitro from rat brain membranes. However the extremely high IC50 values reported for GABA displacement (0.15 to 0.26 mM) led us to question whether GABA receptor blockade plays an important role in the actions of these antihistamines.

In this study we tested cimetidine for GABA-blocking activity in vitro and in vivo. Ranitidine, a non-imidazole H_2-receptor antagonist that does not bind to GABA receptors (Lakoski et al., Eur. J. Pharmacol., 88:241, 1983) was used for comparison. In transmurally stimulated guinea pig ileum, GABA and baclofen produce a dose-dependent inhibition of the twitch response that is insensitive to blockade by bicuculline ($GABA_B$-response). These responses to GABA were not antagonized by either cimetidine or ranitidine in concentrations as high as 0.3 mM. Guinea pig ileum longitudinal muscle displays dose-dependent contractions to GABA or muscimol that are blocked by bicuculline ($GABA_A$-responses). Ranitidine did not antagonize these $GABA_A$-receptor mediated contractions. Cimetidine also had little effect on these responses except at very high concentrations (0.3 mM) in which it caused a slight shift to the right in the GABA dose-response curve. Nevertheless, it was not possible to demonstrate GABA blockade by cimetidine in vivo. Bicuculline (0.01 nmole) microinjected into the inferior colliculus of rats caused clonic seizures whereas cimetidine (100 nmoles) was without effect. In awake rats, ranitidine caused 3-fold greater elevations in MAP than cimetidine at equimolar icv doses, yet neither compound affected the hypotensive response to subsequent icv injections of muscimol (10 ug). Thus GABA-receptor blockade does not appear to play an important role in the central actions of cimetidine. Supported in part by the American Heart Association, Texas Affiliate, and PHS AA06322.

188.11 A DIALLEL CROSS GENETIC ANALYSIS OF BICUCULLINE-INDUCED SEIZURES IN MICE. T.J. Phillips* and B.C. Dudek (SPON: S.B. Tieman). Dept. of Psychology and The Neurobiology Research Center, SUNY-Albany, Albany, NY 12222.

Genetically-defined mice have been particularly useful for studying seizure susceptibility, its genetic bases and its neurochemical controls. Greer and Alpern (Life Sci. 21: 385-392, 1977) reported that genetic differences in flurothyl-induced myoclonic seizures are mediated by dopamine and clonic seizures by acetylcholine and gamma-aminobutyric acid (GABA). Non-chemically induced audiogenic seizures (AGS) have not been conclusively linked to any neurotransmitter system(s). However, strains of mice that are highly susceptible to AGS are also generally more sensitive to other convulsant agents than mice resistant to AGS.

We investigated the genetic architecture underlying susceptibility to seizures produced by the GABA-antagonist bicuculline, in a diallel cross of four inbred mouse strains, and in six additional genotypes. Latencies to myoclonic and clonic seizures were measured in male and female mice of the Au/Abg(Au), C57BL/6Abg(B6), DBA/2Abg(D2), and Mus musculus molossinus(MOLD/Abg) strains and their reciprocal hybrids following 4.55mg/kg i.p. bicuculline. This dose was chosen as the minimal dose for production of both myoclonic and clonic seizures in all genotypes and was consequently lethal in most.

A quantitative genetic analysis revealed a primarily additive system of inheritance for both myoclonic (susceptibility: D2=Au>BALB/c=BALB/cBy>MOLD>B6) and clonic (susceptibility: D2=BALB/c>MOLD=BALB/cBy=Au>B6) seizures with some dominance and reciprocal effects.

The susceptibility of B6 and D2 mice to both AGS and other convulsant agents has been widely reported. D2 mice were found to be more susceptible to AGS, electroconvulsant shock and pentylenetetrazol-induced seizures than B6 mice. This pattern occurred in our study with B6 mice having longer latencies to seize than D2 mice. The study also reaffirms genotype-dependent seizure susceptibility and provides a data base for further more specific neurochemical investigations into the involvement of GABA systems in convulsant and other behaviors. For example, the pattern of inheritance seen in the 22 genotypes in this study is similar to the pattern we observed in a study of the biphasic nature of the alcohol dose response curve. These data bases should serve a role in further studies on the role of GABA receptors in a variety of psychopharmacological phenomena and genetic influences on them.

188.12 MIDAZOLAM-ALCOHOL INTERACTIONS AND REVERSAL WITH A NEW BENZODIAZEPINE ANTAGONIST. P. VanGorder, C. Perkezas, S. Guzman, J.M. Cook, D.J. Miletich, R.F. Albrecht, W.E. Hoffman. Dept of Anesth., Michael Reese Hosp & Med Ctr., Chgo, IL 60616.

The purpose of thses experiments was to analyze the cerebrovascular and cerebral metabolic effects of midazolam, to investigate the interaction of these effects with alcohol administration and to determine the ability of a new benzodiazepine antagonist to reverse midazolam-alcohol effects. Six month old Sprague-Dawley rats were anesthetized and saline filled catheters were inserted into both femoral arteries and veins, the left ventricle (for microsphere injections) and the sagittal sinus (for cerebral venous blood samples). Body temperature was maintained at 37°C and $PaCO_2$ was maintained between 35 and 40 mmHg. The rats received either an ip alcohol injection (2.5 mg/kg), an iv midazolam infusion (0.57 mg/kg), an iv infusion of 3-carbo-T-butoxy-B-carboline (B-CCT, 1.15 mg/kg) or a combination of each of the above. Sham treatments were given as a control. Cortical cerebral blood flow (CBF) was measured with radioactive microspheres and cerebral oxygen consumption ($CMRO_2$) calculated. Neither CBF nor $CMRO_2$ were depressed 20 min after ip injection of ethanol. The midazolam infusion alone decreased CBF and $CMRO_2$ 30-40% while alcohol plus midazolam together produced a 70% depression of CBF and metabolism. B-CCT infusion reversed the depression in both CBF and $CMRO_2$ produced by midazolam and midazolam plus alcohol. Alcohol administration produced no depression in CBF or $CMRO_2$ in spite of high blood ethanol levels. This is consistant with the time dependent effects of ethanol that have been reported. Even though alcohol did not depress CBF or $CMRO_2$, it potentiated the cerebral depression produced by midazolam. The fact that B-CCT reversed midazolam-ethanol induced depression suggests that the effect was mediated through the benzodiazepine receptor.

188.13 DBI, A NEUROPEPTIDE PRECURSOR OF THE PUTATIVE LIGAND FOR BENZODIAZEPINE RECOGNITION SITES ENDOWED OF PROCONFLICT ACTION. P. Ferrero*, D. Konkel*, A. Guidotti and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

The availability of specific anxiolytic (benzodiazepines) and anxiogenic (beta-carbolines) drugs acting on the benzodiazepine recognition site has led to discover that high affinity binding sites for benzodiazepines associated to GABAergic transmission are a major factor in the modulation of GABA mediated synaptic activity. This finding has suggested that these sites recognize an endogenous brain modulator whose function is important in the control of anxiety. Recently a neuropeptide which presumably is the precursor for the putative ligand for the benzodiazepine binding site (DBI = diazepam binding inhibitor) has been isolated and purified to homogeneity from rat brain and human brain cortex. This precursor includes 105 amino acid residues, 45 of these residues have been sequenced (PNAS 80:3531, 1983).

An intraventricular injection of 2.15, 4.25 or 8.5 nmole of DBI in rats deprived of water for 3 days elicited a dose-related facilitation of drinking suppression following an electric shock of 0.15 mA which per se fails to modify drinking in rats receiving saline. The peptide induced facilitation has been termed proconflict action. The proconflict action of DBI is blocked by the benzodiazepine receptor antagonist RO 15-1788 and it is mimicked by the injection of beta-carbolines.

There is a consensus of opinion that benzodiazepine recognition sites, when occupied by a ligand, can modulate the function of GABA receptors. Anticonflict benzodiazepines facilitate and proconflict beta-carbolines disfacilitate GABA transmission. Thus the action of anxiogenic ligands such as DBI increases when GABAergic transmission is reduced. Indeed, isoniazid (200 mg/kg s.c.), which reduces glutamic acid decarboxylase activity, enhances by 5 to 10 folds the proconflict potency of DBI and beta-carbolines.

Trypsin digestion of DBI followed by HPLC of the digested material produces 10 major peptide fragments of which only one is active in the proconflict test in rat. This peptide fragment has been partially characterized: it has a molecular weight of approx. 1,000 dalton, contains one threonine, glycine, valine residue, and two aspartic acid and leucine residues. These data taken together suggest that DBI could be a polypeptide functioning as a precursor of the endogenous putative neuromodulator of GABA mediated synaptic transmission.

188.14 MULTIVARIATE ANALYSIS OF A NATURALISTIC MOUSE BEHAVIOR PROVIDES A SIMPLE AND SENSITIVE ASSESSMENT OF ANXIOLYTIC DRUG EFFECTS. A.M. Maio*, T.J. Phillips*, M. Perrone* and B.C. Dudek. Dept. of Psychology and The Neurobiology Research Center, SUNY-Albany, Albany, NY 12222 and Sterling-Winthrop Research Institute, Rensselaer, NY, 12144.

We have explored the use of a naturalistic mouse behavior which requires no aversive stimulation (shock), no food or water deprivation, and no training, for the study of anxiolytic drug effects. A conflict situation is set up by restraining the mouse under a small plastic cup inverted on a dirt substrate, where escape into an open area is accomplished by digging. The procedure previously revealed marked genetic influences on this escape behavior (Dudek, et al., 1983, J.Comp.Psychol. 97:249). Component latencies to start digging (SD), to head out (HO), to body out (BO) were assessed as were number of head pokes (HP) out of the burrow hole prior to full escape. The choice for the mouse is whether to escape from the "safe" but restraining cup, or to stay out of the large unexplored area.

Chlordiazepoxide (CDZ;2mg/kg), Flurazepam (FLU;2mg/kg), Diazepam (DZ;0.2,0.4,0.6mg/kg) and Ethanol (ETOH;0.75 and 1.5g/kg) all had similar effects on this escape behavior. SD latencies were lengthened, reflecting decreased fear of the restraining space. HO latencies were unaffected. BO latencies were markedly shortened and number of HP were dramatically reduced, indicating reduced timidity in completing the escape into the open area. Discriminant and classification analyses indicated that CDZ and FLU had similar effects which were discriminable from the control group (100% correctly classified as drug treated). Both the DZ and ETOH dose response curves indicated discriminability of the doses from each other and from the controls (>85% correctly classified). The benzodiazepine receptor antagonist RO15-1788 antagonized the effects of DZ (0.6mg/kg) on all measures. We interpret these effects as reflecting the anxiolytic properties of benzodiazepines and ETOH.

The clear advantage of the multivariate statistical procedures in these studies is that the analysis can assess drug effects simultaneously on several domains, all of which might be expected to be related to conflict. These analyses demonstrated high discriminability of even sub-milligram doses of DZ. The combination of these statistical procedures with the naturalistic behavior should be useful for assessment of a variety of potential anxiolytic procedures and compounds.

188.15 COMPARATIVE EVALUATION OF THE ROLE OF GABAergic MECHANISMS IN THE INDUCTION OF ELECTRO- AND CHEMO-CONVULSIONS IN RATS. S.K. Rastogi* and M.K. Ticku (SPON: L. Felpel). Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284.

This study compares the anticonvulsant effect of various modulators of GABAergic transmission, alone and in combination, against maximal electroshock seizures (MES) and chemoconvulsions in rats. The subeffective doses of pentobarbital and diazepam were effective against MES, picrotoxin and bicuculline convulsions, but weakly active against strychnine convulsions. A significant protection against MES, bicuculline and picrotoxin but not against strychnine was observed with a combination of subeffective doses of pentobarbital and ethanol. The subeffective dose of pentobarbital, in combination with progabide, also protected against MES and bicuculline convulsions. RO15-1788, at lower and higher doses, antagonized the protective effect of diazepam and pentobarbital against MES, respectively. Pentobarbital alone was effective at low doses against bicuculline convulsions, whereas moderate doses were effective against picrotoxin and MES. Diazepam was most effective against bicuculline and picrotoxin-induced convulsions, weakly effective against MES and ineffective against strychnine convulsions. Ethanol antagonized MES, whereas progabide was effective against bicuculline convulsions. These results suggest that drugs that facilitate GABAergic transmission are effective as anticonvulsants against picrotoxin and bicuculline. This notion is also supported by drug combination studies using subeffective doses of these modulators. Higher doses of these modulators are also effective against MES and strychnine, suggesting a possible role for GABA in these convulsants.

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189 SYMPOSIUM. SENILE DEMENTIA AND ALZHEIMER'S DISEASE. S. B. Prusiner, Univ. of California, San Francisco (Chairperson); R. Katzman, Univ. of California, San Diego; T. F. Budinger* Univ. of California, Berkeley; D. J. Selkoe, Harvard Medical School; C. J. Epstein*, Univ. of California, San Francisco; P. Davies*, Albert Einstein College of Medicine; D. L. Price, Johns Hopkins University; E. Roberts*, Beckman Research Institute of the City of Hope.

Senile dementia is the fourth leading cause of death in the United States. More than half of all nursing home beds in the United States are occupied by patients with senile dementia. The majority of patients with senile dementia suffer from Alzheimer's disease (AD). The cause of AD remains unknown. AD is characterized by progressive memory loss and a decline of intellectual function. No specific diagnostic tests for AD are available, thus the diagnosis is made by excluding other dementias. Examination of cerebrospinal fluid as well as x-ray computerized axial tomography detect no specific abnormalities in AD. Advances in positron emission tomography and nuclear magnetic resonance imaging may begin to provide important information about the brain in patients with AD. Neurofibrillary tangles and amyloid plaques accumulate in the brains of patients with AD and are considered the pathologic hallmarks of this disorder. The neurofibrillary tangles contain paired helical filaments which have resisted detailed chemical analysis due to their insolubility. Even less is known about the composition of the amyloid plaques, but recent studies on the scrapie agent or prion have raised the possibility that these plaques might be composed of prion-related molecules. Scrapie is a slow infection of sheep and goats which has been suggested as an animal model for AD. Scrapie prions have been found to aggregate into amyloid-like birefringent rods and to be deposited as amyloid plaques in the brains of experimental animals. The role of genetics in pathogenesis of AD is unclear, but virtually all persons with Down's syndrome eventually develop AD. In addition, there is an increased incidence of AD in families where there are members with Down's syndrome. The development of a mouse model for Down's syndrome may give new insights into AD. All patients with AD appear to have a cholinergic deficit manifest by low levels of choline acetyltransferase. Cholinergic neurons in the nucleus basilis of Meynert are profoundly altered in AD. Many attempts at developing pharmacological therapeutics for the treatment of AD have been made. To date, no highly effective therapy for halting the progression of AD or reversing its manifestations has been developed.

190 SYMPOSIUM. MODULATION OF ION CHANNELS BY INTRACELLULAR MESSENGERS. R. Eckert (Chairperson), UCLA; K.L. Magleby, Univ. of Miami; R.W. Tsien, Yale Univ.; I.B. Levitan, Brandeis Univ.; S.A. Siegelbaum, Columbia Univ.

Certain membrane channels appear to be modulated through the action of intracellular messenger or regulatory agents. 'Modulation' refers to a modification of the channel that alters the kinetics of its response to the primary stimulus. Modulation of a channel thus differs in concept from its direct activation by an extracellular agonist or by depolarization. Intracellular messengers implicated in modulation of channels include Ca^{2+} and cAMP, and the latter appears to alter channel behavior through a protein kinase system. Several examples of modulatory actions are considered. First, many cells contain a K channel activated by both depolarization and intracellular calcium. A kinetic scheme to be presented accounts for many properties of the Ca-dependent modulation of this channel. Another recently established action of internal Ca^{2+} is the inactivation of certain Ca channels. The properties of Ca-dependent inactivation will be discussed in terms of a binding-site model, along with proposed relations to the state of Ca channel phosphorylation. In heart and DRG cells, Ca channels switch spontaneously between different patterns of gating; namely i) brief openings clustered in bursts, ii) no detectable openings, and occasionally iii) very long openings and short closings. Shifts between these different modes of gating are involved in β-adrenergic action and modulation by dihydropyridine Ca agonists. In other studies, serotonin was found to cause an increase in K conductance of some molluscan neurons, and a decrease in others. These separate responses both appear to be mediated by cAMP-dependent protein phosphorylation. In Aplysia sensory neurons, the effect of serotonin applied via the bath was detected as increased closures of channels located within cell-attached patches isolated from the bath by the patch pipette, thus implicating an internal messenger. Similar, but less prolonged closures were obtained in response to direct application of the catalytic subunit of the cAMP-dependent kinase to isolated patches. Measurement of phosphorylation in Aplysia R15 indicates that two phosphoproteins are associated with a serotonin-induced increase in K conductance. In certain Helix neurons, protein phosphorylation enhances activation of the Ca-dependent K current. Single-channel analysis indicates that cAMP-dependent protein kinase directly modulates the individual Ca-activated channels.

FEEDING AND DRINKING: CENTRAL MECHANISMS I

191.1 BRIEF PREOPERATIVE SALT-TASTE PROTECTS RATS AGAINST DEFICITS IN SALT APPETITE FOLLOWING CENTRAL GUSTATORY DAMAGE. A.K. Hartzell*, R.A. Paulus* and J. Schulkin* (SPON: E. Stellar). Dept.of Biology,Univ.of Pennsylvania, Philadelphia,PA 19104; Dept.of Psychology, New York Univ., New York, NY 10003.

Animals, when non-deficient, can recognize potential sources of essential nutrients required for the regulation of their internal mileu. For example, rats given previous exposure to saline recognize where (and how) the saline was obtained (while not sodium hungry) even though they have never before experienced a salt appetite; later, when sodium hungry, they will return to the location where salt was encountered (Wolf & Krieckhaus, J.C. P.P., 1968,1970). More recently it has been shown that the latent learning component of this effect requires a brief exposure to the salty taste (Wirsig & Grill, Behav. Neurosci., 1982; Bregar et al., Neurosci. Abstract, 1983).

These data help explain why rats with damage to the thalamic taste relay escape the usual impairments in behavioral sodium regulation following natrorexigenic treatment (furosemide & DOCA) if they are given preoperative exposure to saline--with or without accompanying sodium hunger (Ahern et al., J.C. P.P.,1978). Behavioral regulation is possible, in part, because a taste-place association is formed preoperatively; postoperatively, the animal simply returns to where it had previously encountered the salt and ingests it(Paulus et al., Behav. Neurosci., 1984). In the present study we show that a 30 s exposure to saline is sufficient to promote the mnemonic taste-place associations that protect rats against the usual lesion-induced deficits in salt appetite.

Rats (housed individually with chow ad lib) were placed on a 23¾ hr water deprivation schedule followed by a 45 min drinking period in which water was available from a spout that protruded through the top of the cage. After 4 days of this treatment, rats were given a brief taste-exposure test of either 0.5M NaCl or 0.5M sucrose for the first 30 s of the drinking period; water then was returned permanently to the front of the cage. Lesions were induced in the central gustatory system at the level of the thalamic taste relay 24 hrs later. Following 2 weeks recovery, rats were tested for salt appetite (0.5M NaCl ad lib minus baseline intake) following natrorexigenic treatment. A 30 s exposure to the NaCl protected them (4 of 5) from a postoperative behavioral deficit; preoperative exposure to sucrose did not (2 of 6). Our results suggest that a brief preoperative exposure to saline is sufficient to protect rats against postoperative impairments in salt appetite following central gustatory damage.

191.2 THE DIETARY OBESITY SYNDROME AND VASOPRESSIN SECRETION. P.F. Aravich*, C.D. Sladek and G.B. Forbes*, (SPON: R.J. Joynt). Departments of Neurology, Anatomy, Pediatrics and Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

The dietary obesity syndrome has become one of the most popular animal models of obesity and is produced by feeding normal rats a variety of palatable foods and solutions. We now report that the dietary obesity syndrome is associated with changes in vasopressin (VP) content in specific brain regions.

Adult, female Sprague-Dawley rats were fed either a mixed palatable diet (MPD) or Purina lab chow for 8 weeks. The rats were then sacrificed following a 5 hour fast. Truncal bloods were collected and brains microdissected into five fragments containing, respectively, the supraoptic nuclei (SON), paraventricular hypothalamic nuclei (PVN), median eminence (ME), posterior pituitary (PP), and dorsal vagal complex (DVC). Tissue homogenates and blood serums were then analyzed for VP content via radioimmunoassay. Blood plasmas were analyzed for osmolality and hematocrit.

It was found that the MPD treatment significantly ($p<.05$) elevated body weight (456 ± 16 vs. 296 ± 13 g). Associated with this increase in body weight were significant ($p<.05$) reductions in the VP content of the SON, PVN, and PP. VP concentrations in the ME, DVC, and serum were normal. Plasma hematocrit, but not osmolality, was significantly elevated ($p<.05$) in the MPD group (43.0 ± 1.0 vs. 41.0 ± 1.0% cell vol.). This slight increase in hematocrit may have provided a stimulus for VP release, but is probably not sufficient to account for the region specific reductions in VP content, since chronic dehydration is not associated with decreases in SON and PVN VP levels (Sladek et al., Neurobiol. Aging, 2:293, 1981). It is concluded that the MPD treatment produces a chronic stimulation of VP release from the hypothalamo-pituitary axis, and that under these conditions, hormone synthesis is unable to meet hormone demand. These results, combined with other data (Aravich and Sladek, Neurosci. Abs., 9:135, 1983; Crowley et al., J. Endoc., 77:417, 1978), indicate that VP may play a role in the development and/or maintenance of a variety of obesity syndromes.

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191.3 FEEDING INDUCED BY THE CHOLECYSTOKININ ANTAGONIST, PROGLUMIDE, INJECTED IN THE PARAVENTRICULAR REGION OF THE HYPOTHALAMUS. D. Dorfman*, P. Scott and B.G. Hoebel. Dept. Psychology, Princeton Univ., Princeton, NJ 08544.

Cholecystokinin (CCK) in the brain is thought to play a role in satiety. If so, a CCK antagonist injected in the appropriate place in the brain should produce feeding. In nine satiated male rats with cannulas implanted in the paraventricular nucleus (PVN), unilateral injections of the cholecystokinin antagonist, proglumide, induced feeding. In a dose-response analysis in which a volume of 0.3 ul was injected unilaterally, proglumide at doses of 0.03, 0.09, 0.18, 0.75, 1.5 and 3.0 ug induced Purina pellet intake of 1.1, 1.6, 2.9, 3.4, 3.0 and 2.0 gm, respectively, during the three hours post-injection. When sulfated-CCK (0.12 ug) was added to the 0.3 ul injection, proglumide-induced feeding at the 0.18 and 0.75 ug doses was reduced by 51% and 43%, respectively. Baseline food intake following 0.3 ul of saline was stable at 0.8 gm before and after the above tests. As a localization control, 0.18 ug proglumide injected 1.5 mm deeper in the brain induced only 1.3 gm food intake; when later injected at the usual site it induced 3.2 gm intake.

In sum, proglumide injected in the PVN caused feeding, and CCK counteracted it. This suggests that CCK receptors in the PVN normally suppress feeding in satiated rats. (Supported by USPHS grant MH-35740 and Squibb Inst. for Med. Res.)

191.4 FOURTH VENTRICLE INFUSION OF CHOLECYSTOKININ SUPPRESSES FEEDING IN RATS. R.C. Ritter and E.E. Ladenheim.* WOI Regional Program in Veterinary Medical Education, Univ. of Idaho, Moscow, ID 83843 and Department of VCAPP, Washington State Univ., Pullman, WA 99164.

In the rat, suppression of food intake by systemically injected cholecystokinin octapeptide (CCK-8) is mediated by vagal afferent neurons from the gut (Smith et al., 1981). Although lateral ventricle infusions of CCK-8 suppress feeding in the sheep, such infusions do not suppress food intake in rats (Della-Fera and Baile, 1979). The dorsal hindbrain, an area remote from previous CCK-8 infusions in the rat, contains especially rich CCK-8 terminal networks in nuclei associated with visceral function (area postrema, nucleus of the solitary tract, dorsal motor nucleus of the vagus). To determine whether CCK-8 might influence food intake when administered close to these hindbrain terminal areas, we infused CCK-8 (10, 25 or 50 ng) into the fourth ventricle of rats immediately before presenting them with a highly palatable solid food (cookies). Fourth ventricular CCK-8 infusion produced dose dependent reductions of intake which were statistically significant at all doses within the first 15 minutes after infusion. While rats ate 3.4±0.6g of cookies in the first 15 minutes after administration of the infusion vehicle, they ate only 2.3±0.6, 1.6±0.5 and 0.2±0.1g following 10, 25 or 50 ng of CCK-8, respectively. Although suppression of feeding by 10 ng of CCK-8 was significant only during the first 15 minutes following infusion, 25 or 50 ng of CCK-8 caused suppressions which were still significant 2 hours after presentation of food. Suppression of ingestion by fourth ventricular CCK-8 appears specific for feeding because CCK-8 suppressed food intake driven by 17 hours of food deprivation but did not suppress drinking driven by 17 hours of water deprivation. These results demonstrate that CCK-8 can suppress food intake by acting directly on the central nervous system of the rat and suggest that hindbrain CCK-8 could be involved in the control of food intake in this species.

191.5 CHANGES IN CHOLECYSTOKININ (CCK) CONTENT OF SPECIFIC HYPOTHALAMIC AREAS OF SHEEP WITH FEEDING AND FASTING. A.C. Scallet, M.A. Della-Fera and C.A. Baile. Washington Univ. Med. Sch., St. Louis, MO 63110.

There is considerable evidence that brain CCK plays an important role in feeding behavior of sheep. In order to determine its mechanisms and sites of action, however, information on the localization of CCK peptides in the brain must first be obtained. This study was undertaken to determine 1) the regional pattern of localization of CCK in sheep hypothalamus and 2) whether changes in CCK content occur in specific areas with feeding and fasting. Twenty male sheep were randomly assigned to 4 groups of 5 sheep each and were given feed and water ad lib for a 2 wk adaptation period. Sheep were sacrificed between 0800 and 1200 h by an overdose of pentabarbital either immediately after a meal (0 h) or following a 2, 4 or 24 hr fast. Brains were rapidly removed and dissected on ice. The following hypothalamic areas were collected: anterior (AH), dorsal (DH), ventromedial (VMH), lateral (LH) and posterior (PH). Samples were extracted by sonication in 90% methanol and dried under nitrogen. CCK content was measured by RIA. ANOVA was used to determine significance of differences between means for hypothalamic areas and for fasting lengths (overall and for specific areas). CCK content was highest in VMH (57.2 ng/g), followed by AH (44.3 ng/g; different from VMH, $p<.05$); CCK content was lowest in LH, DH and PH (30.6, 28.4, 26.1 ng/g, respectively; all different from AH and VMH, $p<.01$). There was no main effect of fasting on hypothalamic CCK levels; however, there was a significant fasting by brain region interaction, indicating the presence of an effect in one or more subregions. Individual analyses showed that CCK content in AH and PH, but not other areas, were altered with fasting. AH CCK levels decreased after a 2 h fast (68.4 to 26.9 ng/g; $p<.01$) and remained lower after 4 and 24 h fasts (43.8 ng/g and 38.1 ng/ml, P's <.06 and .05 respectively). PH CCK content increased slightly after a 2 h fast, but was significantly lower after 4 and 24 hr fasts than after the 2 h fast (0 h:27.3; 2 h:37.1; 4 h:18.2; 24 h: 22.0 ng/g). These findings support a role for hypothalamic CCK in the control of feed intake in sheep and suggest the involvement of specific areas (AH and PH) in CCK-mediated satiety functions. Supported by NIH 20000.

191.6 VAGOTOMY ABOLISHES OBESITY IN RATS WITH LESIONS OF THE PARAVENTRICULAR NUCLEUS. J. P. Steves and J. F. Lorden. Dept. of Psychology, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

Electrolytic lesions aimed at the paraventricular nucleus of the hypothalamus (PVN) produce hyperphagia and obesity in rats (Leibowitz et al., 1981); however, the cause of the obesity has not been established. Subdiaphragmatic vagotomy blocks feeding elicited by noradrenergic stimulation of the PVN (Sawchenko et al., 1981), suggesting vagal involvement in feeding behaviors associated with this nucleus. The present study examined the effects of complete subdiaphragmatic vagotomy on PVN obesity and insulin levels. Female rats made obese with PVN lesions or sham-operated control rats maintained on wet mash diets were randomly assigned to vagotomy (Vx) or sham-vagotomy (SVx) groups. Vagotomy produced a significant weight loss in both the PVN and Sham lesion groups. Mean body weight in the PVN-Vx group was reduced to that of the Sham-Vx group within 45 days of nerve section. Both groups maintained body weight at similar levels for the remainder of the experiment. Fasting and glucose-stimulated insulin levels were significantly reduced in the Vx groups; however, the PVN and Sham groups did not differ from each other. Nor were any differences obtained between the PVN-SVx or Sham-SVx groups. In a separate group of animals maintained on a high fat diet, the effects of PVN lesions on fasting and glucose-stimulated insulin levels were measured immediately after the lesion but prior to any postlesion food intake or obesity. Insulin levels were measured again at sacrifice 30 days later. In blood samples drawn 24-30 hr after surgery but prior to food consumption, there were no differences in blood glucose or plasma insulin levels between PVN and Sham-operated groups. At sacrifice 30 days later, the PVN group weighed significantly more than the sham group and showed a significant fasting hyperglycemia. Plasma glucose levels did not differ following glucose infusion; however, insulin levels were consistently higher in the PVN group. The results of these experiments suggest that vagally mediated functions are necessary for the maintenance of PVN obesity. However, hyperinsulinemia is not a consistent or primary feature of PVN obesity. (Supported by NS 14755)

191.7 NEUROPEPTIDE AND OPIOID RECEPTOR CHANGES AFTER OBESITY-INDUCING VENTROMEDIAL HYPOTHALAMIC LESION.
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Electrolytic and gold thioglucose (GTG) lesions of the ventromedial hypothalamus (VMH) in rodents cause hyperphagia, hyperinsulinemia, obesity and altered peripheral parasympathetic and sympathetic tone. The neuropeptides β-endorphin (β-END), dynorphin (DYN), met-enkephalin (MENK), somatostatin (SRIF) and vasoactive intestinal peptide (VIP) were measured 12 days after VMH lesion in rats, or ten weeks after GTG lesion in mice.

RAT VMH LESION	β-END	DYN	MENK	SRIF	VIP
hypothalamus	+85%	+54%	+37%	+74%	n.c
midbrain/thalamus	+88%	n.c	n.c	+17%	n.c
cortex	+70%	n.c	n.c	n.c	n.c
medulla/pons	+97%	n.c	n.c	+28%	n.c
striatum	+94%	n.c	n.c	n.c	n.c
hippocampus	+74%	+27%	+38%	n.c	n.c
septal nucleus	-	-	-	n.c	n.c
pituitary	n.c	+66%	-	-	-
MOUSE GTG LESION					
hypothalamus	+80%	+15%	n.c	+30%	n.c.

Extra-hypothalamic peptide reductions presumably reflect efferent projections of hypothalamic peptide cell bodies. Hypothalamic reductions in β-END, MENK, and SRIF in obesity-inducing monosodium glutamate (MSG) arcuate nucleus lesions which spare the VMH have also been reported.

While reduced baseline pain sensitivity and attenuation of stress-induced analgesia (SIA) in VMH-lesioned rats have been reported, no differences were apparent in this study. In contrast, GTG-lesioned mice had attenuated SIA eight weeks after lesioning, with no differential response to morphine or naloxone. Feeding after cold-swim-stress was much reduced in VMH-lesioned rats, while naloxone suppresion of feeding was not affected.

Compensatory up-regulation and down-regulation of neurotransmitter receptors has been shown to occur with chronic transmitter decreases and increases, respectively. Behavioral compensation of initially perturbed behaviors has been postulated to occur in parallel with changes in recep-receptor affinity or number. Examination of ^{3}H-etorphine binding in VMH-, GTG-, and sham-lesioned rodents revealed no change in brain opioid (μ) receptor affinity or number.

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191.8 **THE EFFECTS OF INTRAGASTRIC HYPERALIMENTATION ON PAIR-FED RATS WITH VENTROMEDIAL HYPOTHALAMIC LESIONS.**
Meredith C. Walgren* and Terry L. Powley. (SPON: F.R. Brush). Laboratory of Regulatory Psychobiology, Dept. of Psych. Sci., Purdue University, West Lafayette, IN 47907.

An intragastric hyperalimentation paradigm was used to assess the relative contributions of altered metabolic efficiency and excess food intake to the obesity and hyperinsulinemia of the ventromedial hypothalamic syndrome. This experiment also addressed the issue of whether altered energy utilization serves as a compensatory strategy for reducing energy retention in the face of excess intake. Female rats were equipped with indwelling intragastric catheters. Half of the animals then ingested a liquid diet orally (OR) while the others received the same diet intragastrically (IG). All animals were fed with a protocol that initially yoked both the amount and the timing of their food delivery to the ad libitum consumption of an intact rat (Cox & Powley, AJP, 240, E566, 1981). Half of the animals in each of the two groups were then given lesions of the ventromedial hypothalamus (VM) and the other half were given sham lesions (SH). After the lesion, the SHIG and VMIG animals were hyperalimented by maintaining them with twice the calories ingested by the ad libitum "master." The oral-fed rats, both VMOR and SHOR, were also allowed access to twice-normal quantities of diet. The postlesion period lasted 30 days. Food intake and body weight were monitored throughout, while body composition and hormonal levels were determined at the end of the experiment.

Although both intact and VM hyperalimented rats became obese and hyperinsulinemic, those with VM lesions became 25% more obese. Thus, 75% of the VM obesity could be attributed to excess calories consumed, and 25% to altered metabolism. In order to retain the identical level of energy above maintenance requirements (i.e. 140KJs of retained energy), VMIG rats required 11% fewer calories than shams. Further, intact rats fed excess calories intragastrically did not evidence the caloric wastage that has been reported to occur in rats overconsuming a "cafeteria" diet. (USPHS grant AM27627.)

191.9 A RE-ASSESSMENT OF GREASY DIETARY TEXTURE IN PROMOTING HYPOTHALAMIC OBESITY.
D.V. Coscina, S. LaCombe*, and J.W. Chambers*. Sect. of Biopsychol., Clarke Inst. of Psychiatry, Univ. of Toronto, Toronto, Ont., Canada M5T 1R8.

Rats with medial hypothalamic lesions are well-known to overconsume diets high in digestible fat (HF). A frequently cited study (Corbit & Stellar, J. comp. physiol. Psychol., 58: 63, 1964) showed these animals would also overconsume a normal caloric diet made greasy by addition of non-nutritive mineral oil (MO) to achieve obesity equivalent to that previously induced by exposure to HF. This suggested that texture rather than calories was the stimulus for hyperphagia on greasy diets. However, the design of that study could not preclude prior obesity and/or order of diet presentation as factors in explaining the MO finding. To clarify this, we induced hypothalamic hyperphagia in 22 adult female rats by placing 3 x 3 mm parasagittal knife cuts (KCs) along the fornix using a retractable wire knife bilaterally. Eleven control (C) rats received sham cuts. After 4 wk access to powdered lab chow (3.6 kcal/g), half of each group received a HF diet (5.4 kcal/g) while the other half received a diet of similar greasy texture made with MO to achieve normal caloric density (3.6 kcal/g). Following 4 wk of these diets, all rats received chow alone for another 4 wk, then were switched to the opposite greasy diet for a final 4 wk. Mean group weight changes (top, in g) and intakes (bottom, in kcal) are listed below for each 4 wk diet condition.

Group	Chow	Diet 1	Chow	Diet 2
KC:HF-MO	88 2558	157 3581	-71 1331	88 2424
KC:MO-HF	84 2828	55 2498	16 2357	125 2838
C :HF-MO	10 2243	28 2406	-8 2021	13 1831
C :MO-HF	11 2198	22 2137	2 2208	25 1995

These findings show that the exaggerated response to greasy dietary texture in the absence of high calories (i.e., MO) only occurs if rats have prior exposure to HF foods and/or were previously obese. Such findings argue against a primary role for altered reactivity to the sensory qualities of greasy diets as an important factor in promoting hypothalamic hyperphagia and obesity.

191.10 **REGULATION OF [^{3}H](+)-AMPHETAMINE BINDING SITES IN HYPOTHALAMUS BY FOOD DEPRIVATION, 2-DEOXY-D-GLUCOSE, AND OBESITY.**
B. Hulihan-Giblin*, R.L. Hauger*, I. Angel*, P. Skolnick, S.M. Paul*, Clinical Neuroscience Branch, NIMH, 9000 Rockville Pike, Bethesda, MD 20205.

In previous studies we have demonstrated the presence of stereospecific binding sites for [^{3}H](+)-amphetamine in the CNS that are highly localized to synaptosomal membranes. Structure-activity studies suggest that the sites in hypothalamus are related to the anorectic properties of various phenylethylamines. In order to examine whether [^{3}H](+)-amphetamine binding sites are regulated by physiological and (or) pharmacological changes in food intake and body weight, the following experiments were carried out. Male Osborne-Mendel rats weighing 150-175 g were food deprived for periods of 1, 2 or 3 days. Food deprivation resulted in a time-dependent decrease in the number of hypothalamic [^{3}H](+)-amphetamine binding sites when compared to fed controls. When animals that had been food deprived for 3 days were then allowed access to food for 4 hours the number of [^{3}H](+)-amphetamine binding sites returned to control levels. The food-deprivation induced decrease in [^{3}H](+)-amphetamine binding site density was not observed in cerebellum or in peripheral tissues such as liver or kidney. In related experiments hypothalamic membranes from genetically obese mice (C57BL/6J-ob) and their lean littermates were studied. The ob/ob mice were allowed to obtain a body weight $\geq$ 50% of their lean littermates. The number of [^{3}H](+)-amphetamine binding sites in hypothalamic membranes was significantly greater ($p < .001$) in the obese mice compared to their lean littermates.

The administration of 2-deoxy-D-glucose (2-DG) (10-200 mg/kg) which causes glucoprivic feeding, and hyperglycemia, significantly increased hypothalamic [^{3}H](+)amphetamine binding site density over saline injected controls. This effect was observed within 15 min. of 2-DG administration and was blocked by pretreating the animals with intraventricular alloxan. Since alloxan has been reported to irreversibly alter membrane bound glucoreceptors in peripheral tissues, as well as brain, our results suggest that the [^{3}H](+)-amphetamine binding site(s) may be associated with a large membrane constituent (presumably an enzyme) that is involved in glucose metabolism or utilization in the CNS.

191.11 DESTRUCTION OF THE AREA POSTREMA DOES NOT ABOLISH GLUCOPRIVIC CONTROL OF FEEDING OR BLOOD GLUCOSE. V.K. Nonavinakere and R.C. Ritter. Department of VCAPP, Washington State Univ., Pullman, WA 99164 and WOI Regional Program in Veterinary Medical Education, Univ. of Idaho, Moscow, ID 83843.

Previous results from our laboratory demonstrate that brain glucoreceptors which initiate feeding and hyperglycemia in response to glucoprivation reside in the hindbrain (Ritter, Slusser and Stone, 1981). The precise location of the glucoreceptors is not known, but recent reports have suggested that the area postrema (AP) or the adjacent nucleus of the solitary tract (NST) may contain such glucoreceptors (Contreras et al., 1982). To further explore this possibility, we have examined the effects of intentionally large lesions of the AP and NST on the feeding and hyperglycemic responses to subcutaneous injections of 2-deoxyglucose (2DG) (100, 150, 200, 300 and 400 mg/kg) and fourth ventricular infusions of 5-thioglucose (5TG) (10, 30, 60, 90 and 120 µg/rat). All of our lesions totally destroyed at least 63% of the volume of the NST, at the level of the AP. Despite the extensiveness of the lesions, 8 out of 12 lesioned rats significantly increased their food intake following 2DG doses of 200 mg/kg or more. During the 6-hour post-injection period, lesioned rats increased their intake by 3.2±0.7, 4.4±1.3 and 3.1±0.8g at 2DG doses of 200, 300 and 400 mg/kg, respectively. Sham-lesioned rats increased their intake by 4.4±0.9, 4.1±0.6 and 4.4±0.5g in response to the same doses. There was no significant difference in the magnitude of the feeding or blood glucose responses between lesioned and sham-lesioned rats. After fourth ventricular infusion of 5TG, 61% of the lesioned rats significantly increased their food intake, as compared to 90% of sham lesioned rats. Since there were no relationships between quantitative and qualitative features of the lesions and the reliability of glucoprivically elicited feeding, we conclude that the AP is not the sole location for brain glucoreceptors which mediate feeding in response to glucoprivation.

191.12 AREA POSTREMA MEDIATES TUMOR EFFECTS ON FOOD INTAKE AND PREFERENCE. C.M.Treneer*, J.N.Kott* and I.L.Bernstein. Dept. Psychol., Univ. of Washington, Seattle, WA 98195.

The growth of a number of experimental tumors is associated with declines in food intake and body weight, as well as with the development of learned aversions to the available diet. Learned food aversions appear to contribute to tumor anorexia since prevention of the aversions reduces the severity of the anorexia.

Brain regions mediating effects of tumors on food intake and preference have yet to be identified. We were interested in examining the involvement of the area postrema in tumor anorexia because there is growing evidence that this region has multiple functions in modulating ingestive behavior. Lesions of the area postrema/caudal medial nucleus of the solitary tract (AP/cmNTS) produce long-lasting depressions of food intake and body weight, and interfere with taste aversion learning in response to certain unconditioned stimuli. The area postrema has also been identified as a chemoreceptor trigger zone for nausea and emesis.

In this study Wistar-Furth rats with thermal lesions of the AP/cmNTS (or sham lesions) were implanted with LTW(m) tumors (or received control incisions). They were exposed to a target diet for 8 days and then given a preference test to assess development of diet aversions. We found that AP/cmNTS lesions blocked or greatly attenuated the anorexia and weight loss induced by the LTW(m) tumors. Further, we found that the severe food aversions which arise in association with LTW(m) tumor growth (Preference for target diet: Sham-Tumor=.02; Sham-Control=.92) did not develop in animals with AP/cmNTS lesions (Preference for target diet: AP-Tumor=.67; AP-Control=.81). These results suggest that the aversions and anorexia produced by LTW(m) tumors may be associated with the detection of a blood-borne chemical by cells within the AP/cmNTS. It remains to be determined whether the role of the AP/cmNTS in tumor anorexia is largely due to its detection of a chemical unconditioned simulus in aversion conditioning, or whether direct effects on the regulation of food intake and body weight are involved. (Supported by USPHS Grant CA26419).

CHEMICAL SENSORY SYSTEMS I

192.1 CO-EXISTENCE OF CALCITONIN-GENE-RELATED-PEPTIDE- AND SUBSTANCE-P-LIKE IMMUNOREACTIVITIES IN NERVE FIBERS ASSOCIATED WITH LINGUAL TASTE BUDS IN THE RAT. T. E. Finger and J. C. Kinnamon. Dept. of Anatomy, Univ. Colorado Medical School, Denver, CO 80262 and Dept. MCD Biology, University of Colorado, Boulder, CO 80309.

Taste papillae are innervated by two classes of nerve fibers. Intragemmal fibers penetrate the taste bud from its base; perigemmal fibers surround the taste bud, but according to the usual descriptions, do not contact the chemosensory cells of the taste bud. Nerve fibers exhibiting substance-P-like-immunoreactivity (SP-LI) and calcitonin-gene-related-peptide-like-immunoreactivity (CGRP-LI) have been reported in association with taste buds. The SP-LI fibers appear to comprise the perigemmal plexus while the disposition of the CGRP-LI fibers is unclear. Accordingly, we undertook a combined light-(LM) and high voltage electron-microscopic (HVEM) analysis of SP-LI and CGRP-LI fibers associated with the circumvallate and fungiform taste buds in the rat.

Rats were perfused with 4% paraformaldehyde in phosphate buffer. The tongues were removed and placed in fresh fixative for 4-24 hours. The tissue was then cryoprotected and sectioned at 50-100 µm on a sliding freezing microtome. Sections were exposed to primary antisera directed against the C-terminal portion of SP or against CGRP. Sections being prepared for combined LM and HVEM analysis were processed according to a modified PAP protocol and flat embedded in Spurr's resin. Double label studies with two different fluorochromes were also undertaken.

The double label studies indicate that all of the CGRP-LI fibers also exhibit SP-LI. The CGRP-LI fibers comprise a portion of the perigemmal plexus in both fungiform and circumvallate papillae. In the circumvallate papilla, CGRP-LI fibers penetrate the basal lamina between taste buds and ramify freely near the surface of the epithelium. Occasionally, some of these CGRP-LI processes penetrate a taste bud from its side and come to lie immediately adjacent to one or more taste bud cells. In some cases, these close contacts appear to involve putative gustatory cells of the taste bud, especially those cells situated in the outer third of the taste bud. No CGRP-LI fibers were seen to penetrate the taste buds from below as do the classic intragemmal fibers. HVEM studies are underway to characterize the nature of the close contacts between the CGRP-LI fibers and the taste bud cells.

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192.2 NEW MONOCLONAL ANTIBODY PROBES REVEAL NOVEL ASPECTS OF OLFACTORY NEURON REGENERATION AND ORGANIZATION. J.I.Morgan* and J.L.Hempstead* (SPON:R.Chizzonite).Dept. of Phys. Chem. and Pharm. Roche Inst. of Molec. Biol., Nutley, N.J.07110.

A monoclonal antibody library to the rat olfactory mucosa has been developed. The antibodies have been classified by the primary cell-type stained in immunocytochemical studies. Thus, the library consists of the following antibody groups: LUM, antibodies reactive with the luminal boundary of epithelium (sustentacular cell brush border and respiratory cilia); SUS, sustentacular cell-reactive antibodies; NEU, antibodies staining neuronal cell bodies and nerve bundles; GLA, clonal antibodies to Bowman's glands and secretory cells of the respiratory epithelium; BCL, basal cell layer-reactive antibodies; NIS, antibodies staining unidentified elements of the neuroepithelium.

The properties of the neuronal-reactive antibodies have been studied in detail. Amongst the neuronal antibodies are those (e.g. NEU-4) that give an immunocytochemical picture similar to olfactory marker protein (OMP), although none of the neuronal clones could be shown to be directed against OMP. Other members of the library react not only with OMP-positive cells but also with a series of OMP-poor nerve bundles (e.g. NEU-5). The OMP-poor nerve tracts may be the vomeronasal nerve. If this is so then the NEU-5 antibody may indicate an olfaction-associated antigen since it does not stain nerves or cell bodies in any other region of brain.

Following unilateral olfactory bulbectomy there ensues a long-term reduction in the number of OMP-positive neurons on the lesioned side of the neuroepithelium. This is paralleled by an even more dramatic loss of staining with NEU-9 and NEU-14 antibodies. In contrast, NEU-1 and NEU-5 antibodies appear unaffected. This would point to the regulation of expression of some neuronal antigenic determinants by the olfactory bulb. In addition cells appear at the level of the sustentacular cell layer that are OMP-negative but that react with some neuronal antibodies (e.g. NEU-9). Such cells are not reactive with antibodies to sustentacular cells or basal cells and may be neurons that have undergone abnormal or alternative differentiation as a result of bulbectomy. Occasionally we have observed large OMP-positive structures in the mucosa of bulbectomized rats. These may be neuromas since they seem acellular and are reactive with almost all neuronal monoclonal antibodies but not those to other cell types.

192.3 SELECTIVE MODIFICATION OF PROTEINS IN THE OLFACTORY EPITHELIUM. Thomas Hellman Morton, Department of Chemistry, University of California, Riverside, CA 92521, and J. Russell Mason*, Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104.

Affinity labelling for selective modification of Schiff base-forming proteins has been developed: a blocker binds reversibly to the active site lysine, and then a fixer converts the reversible complex to an irreversible adduct. The chemistry of this procedure has been probed using the bacterial enzyme acetoacetate decarboxylase (AAD). The extent of covalent modification in vitro follows a well defined quantitative relationship (Mason and Morton, Tetrahedron 40: 483, 1984). Other types of proteins are not detectably modified by the same sequence of blocker plus fixer.

This same affinity labelling procedure induces selective anosmia in tiger salamanders when applied to their olfactory epithelia. Animals conditioned to avoid cyclohexanone (CH) or cyclopentanone at 2% vapor saturation exhibit significant decrements in responding after treatment, while, at the same time, responding to dimethyl disulfide (DMDS) at 2% vapor saturation is unimpaired. Olfactory thresholds to CH and DMDS were determined to be the same (>1.5% of vapor saturation), within experimental uncertainty.

The following model for olfactory detection is suggested. It assumes substantial redundancy in coding; e.g. Schiff base-forming receptors (which would bind ketones and aldehydes) transmit only a portion of the identifying characteristics of these odorants. Information from these receptors cannot be utilized if their signal falls below some set limit. When Schiff base-forming sites are blocked, information from other receptors provides partial identification of a ketone-containing odorant. Thus, detection of ketones remains at a constant level, significantly below DMDS, for periods as long as 1 week following chemical treatment of the olfactory epithelium.

According to this model, dose-response effects ought to show up as changes in the duration (rather than the profundity) of selective anosmia. The chemistry of affinity labelling predicts slight effects from changes in blocker concentration, but large effects from variation of fixer concentration. Experimentally, the duration of selective anosmia is unaffected when blocker concentration is lowered from 0.5 mM to 0.2 mM ethyl acetoacetate, but a marked, regular increase is observed when fixer concentration is raised in steps from 10 mM to 50 mM sodium cyanoborohydride.

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192.4 PROTEINS OF OLFACTORY CILIA THAT MAY BE INVOLVED IN CYCLIC NUCLEOTIDE-MEDIATED SENSORY TRANSDUCTION. Doron Lancet and Umberto Pace*. Dept. of Membrane Research, The Weizmann Inst. of Science, Rehovot, Israel.

Olfactory reception is analogous to neurotransmitter reception: the binding of odorants to membrane receptors leads to changes of membrane conductance that result in spike initiation. The detailed mechanism of vertebrate olfactory transduction is not know, but there is considerable evidence suggesting that cyclic nucleotides may act as second messengers. In this, olfactory transduction mechanisms may be similar to those of several neurotransmitter and hormone receptors (e.g. β adrenergic receptor) and of the sensory receptors in the vertebrate retina. The molecular constituents of such transduction mechanisms are now known with considerable detail. They include a membrane receptor, a transductory enzyme which generates or breaks down cyclic nucleotides and a guanine nucleotide binding protein (G protein or transducin) that serves to couple the other two. The coupling protein may be irreversibly activated by toxins that cause covalent modification by an ADP ribosyl group. The same proteins (as well as other components of the cyclic nucleotide dependent transductory mechanism) undergo irreversible inactivation by specific covalent modifiers of sulfhydryl groups such as N-ethyl maleimide (NEM). Interestingly, olfactory transduction is blocked by sulfhydryl modifying reagents.

In conjunction with our mapping of proteins in a preparation of isolated olfactory cilia (Z. Chen et al., this volume) we decided to investigate whether the sensory cilia contain a specific G-like or transducin-like protein that may be important for olfactory transduction. Isolated frog olfactory cilia were incubated with cholera toxin in the presence of ^{32}P labelled nicotinamide adenine dinucleotide (NAD). ADP ribosylation of proteins was monitored by SDS polyacrylamide gel electrophoresis followed by autoradiography. A polypeptide with molecular weight of about 40kDal was found to undergo a toxin-dependent modification. In control respiratory cilia no proteins appeared to be labelled in a toxin-dependent way. Additional components that may be important in olfactory transduction were studied by labelling cilia with ^{3}H-NEM. Two specific polypeptides (of about 36k and 42kDal) were found to be labelled. These biochemical investigations of candidate transductory proteins are currently being complemented with functional studies, probing the effect of the specific covalent modifiers on odorant induced electrophysiological responses.

192.5 METHYL XANTHINES ENHANCE TASTE: EVIDENCE FOR MODULATION OF TASTE BY ADENOSINE RECEPTOR. S. S. Schiffman, J. M. Gill, II* and Cymantha Diaz*. Dept. of Psychiatry, Duke Medical Center, Durham, NC 27710.

The methyl xanthines (MX), potent inhibitors of adenosine receptors, were found to potentiate taste in both humans and rats. Theophylline, caffeine, and theobromine at concentrations ranging from $10^{-5}M$ to $10^{-2}M$ were applied directly to the tongue. In the human studies, pieces of chromatography paper cut in the shape of half tongues were soaked in a MX solution or deionized water (control) for 10 min. Then two pieces of paper, one soaked in a MX solution and one water control were placed on the tongue for a total adaptation time of 4 min. Next, a standard concentration of a taste stimulus (.02M acesulfam-K, .20M NaCl, .40M NaCl, .30M KCl, .002M quinine HCl, or 1.5M urea) impregnated in a 1.27 cm circle of chromatography paper was placed on the side of the tongue to which MX had been applied. Test stimuli also soaked in 1.27 cm circles were placed on the non-MX side and the concentrations were adjusted to match the perceived intensity of the standard. The greatest potentiation was found for acesulfam-K, an artificial sweetener with a bitter component. With MX at $10^{-5}M$, a concentration known to inhibit adenosine receptors but below that required to inhibit phosphodiesterase, acesulfam-K was potentiated by approximately 100%. Increasing the concentration of MX as high as $10^{-2}M$ did not appreciably increase the degree of enhancement. Addition of $10^{-5}M$ adenosine reversed the potentiation. At $10^{-5}M$, all three MX had their second greatest effect on quinine HCl. Potentiation of neural responses in rat were found in single unit extracellular recordings made from the nucleus of the solitary tract. Ingestion of similar concentrations in humans did not produce any taste potentiation. These findings strongly suggest that an inhibitory adenosine receptor exerts an important local modulatory effect at the level of the taste buds themselves.

192.6 ION CHANNEL ACTIVITY IN ISOLATED MURINE OLFACTORY RECEPTOR NEURONS. R.A. Maue* and W.E. Dionne* (SPON: L.P. Henderson) Division of Pharmacology, University of California, San Diego, La Jolla, California 92093

The mechanism underlying the response of olfactory receptor neurons to odorant molecules is unknown. In an attempt to understand the physiology of these neurons, we have begun to characterize their membrane ion channels and define the role of these in the odorant response. We are studying freshly-dissociated olfactory receptor neurons from 3-4 month old heterozygous athymic mice with the patch clamp technique. Neurons are isolated by incubating the olfactory epithelium in trypsin and DNAase solutions followed by gentle trituration. The isolated cells are then plated on concanavalin A-coated glass coverslips to hold them in place and studied in HEPES-buffered saline solutions containing 9.4 mM glucose. When viewed under Nomarski optics the neurons retain their morphological characteristics, and many exhibit fine cilia attached to the distal knob of their dendritic process. Excised patches of membrane from both the dendritic knob and the soma show several kinds of spontaneously active ion channels. Three of these spontaneously active channels appear to be K^+-selective and in elevated K^+ salines (145 mM) at room temperature (23°C) they have conductances of approximately 140 pS, 80 pS, and 30 pS. In addition to differences in conductance, these channels appear to have different gating kinetics. Changes in Ca^{++} concentration at the cytoplasmic surface of the membrane does not appear to alter the activity of any of these channels. With elevated K^+ salines on both sides of the membrane, neither 30 mM TEA nor 10 mM 4AP have any effect on the activity of these channels. Exposing the cytoplasmic face of the membrane to solutions containing 50 mM Cs^+ ions does appear to block outward currents through the 140 pS and 30 pS channels. The roles these channels have, if any, in the neuronal response to odorants is unknown.

192.7 CHEMORECEPTION IN THE LEECH. Ellen J. Elliott, Dept. of Zoology, Univ. of Maryland, College Park, MD 20742.

Studies of the chemical sensory physiology of the leech Hirudo medicinalis have been undertaken with two long range objectives: to obtain a more complete description of leech behavior and to gain insight into mechanisms of processing and coding of chemical sensory information.

A band of about 100 sensory mounds, or sensilla, line the edge of the dorsal lip of H. medicinalis and can be distinguished in the light microscope as light colored patches of skin. Examination of the dorsal lip by scanning and transmission electron microscopy shows these sensilla, 25-30 um in diameter, to be ciliated, and reveals a number of smaller (7-8 um) ciliated mounds that are not readily detectable in the light microscope. The cilia of both of these types of structures are about 2-3 um long and 0.15 um in diameter. The smaller sensillum has 50-100 cilia, while the larger has 500-1000. Both of these structures are candidates for chemosensory receptors. Neither structure has the longer (10 um) solitary cilia, believed to be involved in mechanoreception, that are found on larger sensilla elsewhere on the leech body.

The band of large and small sensilla is innervated by eight nerves from the "head brain" ganglionic complex of the leech. Four of these lead ventrally to the subesophageal ganglion and four lead dorsally to the supraesophageal ganglion. Extracellular recording from each of these nerves has detected axon spikes in response to stimulation of the dorsal lip with whole mammalian blood, the normal food of H. medicinalis. In these experiments, the nerves were maintained in leech Ringer's solution while the outer surface of the skin was maintained in the more ionically dilute spring water in which the leeches are kept, since perfusion of the dorsal lip with Ringer's solution abolished or masked the response to blood. Experiments in progress seek to determine what components of blood are effective stimuli and, eventually, what neurons are involved in the response. (Supported by NIH grant #20324.)

192.8 THE ULTRASTRUCTURE OF BIMODAL MECHANO- AND CALCIUM SENSITIVE CRUSTACEAN SETAE. K.A.MESCE. Dept. of Biology, Univ. of Oregon, Eugene, OR 97403.

The hermit crab, Pagurus hirsutiusculus h., uses shell-surface calcium and tactile cues to recognize and select gastropod shells. It has been proposed that the "smooth" setae on the minor chela are used to transduce such information. Electrophysiological examinations demonstrate that these setae are both mechano- and calcium sensitive. The ultrastructural study presented here provides additional evidence supporting the chemosensory nature of the chelar hairs as well as contributing to the understanding of modality-specific structures that may underlie stimulus transmission.

The sensory dendrites of these bimodal hairs are continuous throughout the hair lumen, extending up to the hair tip. Each sensillum contains 20-23 dendrites in the lumen. One dendrite in particular contains an extremely dense array of microtubules and may function as the mechano-transducer in each hair. Structures characteristic of mechanoreceptors, such as tubular bodies at the hair base, were not found.

In insect chemosensory setae the route of stimulus entry is via a pore system, often a terminal pore. The tips of the crustacean setae examined here with SEM reveal tongue-like projections whose terminals contain a pore-like depression. However, dye penetration studies indicate that these pores are not necessary for stimulus entry. When the terminal pores are experimentally blocked, mid- to distal regions of the smooth setae are readily stained. Correlative ultrastructural examination shows the presence of minute canals transecting the hair shaft in these regions. The presence of these canals appears specific to the readily staining chelar hair-type. Other setal types which do not readily stain were found not to contain canals. These observations suggest that dye, and possibly stimulus entry, occur via these canals.

192.9 PROPRANOLOL BLOCKS ISOPROTERENOL-INDUCED SECRETORY GRANULE DEPLETION FROM SALAMANDER OLFACTORY GLANDS. M.L. Getchell* and T.V. Getchell. Department of Anatomy, Wayne State University School of Medicine, Detroit, MI 48201.

The β-adrenergic agonist isoproterenol (IPR, 30 mg/kg i.p.) caused a significant reduction in the secretory granule content of acinar cells of superficial (sBG) and deep (dG) olfactory glands.[1] We now report that the reduction in granule content is dose-dependent and can be blocked by the β-adrenergic antagonist propranolol. Preliminary studies have demonstrated that IPR-induced secretion affects the neurophysiological responses recorded from the olfactory mucosa to odorants. I.p. injection of 3 mg/kg IPR caused significant decreases in granule area and the ratio of granule to cell area in sBG and dG acinar cells. Granule area was reduced from 120.5 ± 48.9 μm^2 to 92.4 ± 59.3 μm^2 (p=0.05) in sBG acinar cells, a 23% reduction, and from 113.4 ± 57.2 μm^2 to 46.4 ± 29.7 μm^2 (p=0.001) in dG acinar cells, a 59% reduction. The ratio of granule to cell area decreased by 41% and 39% in sBG and dG acinar cells respectively. The secretory granule content of acinar cells from glands exposed to 3 mg/kg IPR was intermediate between those from control glands and glands exposed to 30 mg/kg IPR. Propranolol (42 mg/kg) injected 10 min prior to 30 mg/kg IPR significantly reduced the effects of IPR. Granule area in sBG acinar cells was reduced by 65% with 30 mg/kg IPR; with propranolol, granule area was reduced by only 35%. In dG acinar cells, granule area was reduced by 77% with 30 mg/kg IPR; with propranolol, granule area was increased by 11%. Propranolol (42 mg/kg i.p.) alone had no effect on the granule area in sBG acinar cells but increased it by 28%, from 113.4 ± 57.2 μm^2 to 157.1 ± 69.1 μm^2 (p=0.05) in dG acinar cells. Coupled with the observation that olfactory nerve section increases the PAS reaction in dG, this suggests that these glands receive a tonic β-adrenergic input. The electroolfactograms evoked by citral and cis-1,2-dichloroethylene recorded at regular intervals after i.p. injection of 30 mg/kg IPR showed systematic decreases in amplitude over time. Injection of 42 mg/kg propranolol 10 min prior to IPR blocked the effect. These results suggest that IPR is a specific β-adrenergic secretagogue for olfactory glands and that secretion from these glands can affect the responses of the olfactory mucosa to odorants. Support: NIH-NS-16340. [1]Getchell, T.V. & M.L. Getchell (1983) Neurosci. Abs., 9 463.

192.10 CHEMICAL STIMULATION EVOKES IMPULSES IN TASTE CELLS OF THE MUDPUPPY. S. Roper and M. McPheeters. Univ. Colo. Med. Sch., Denver, CO 80262.

Recent studies (Roper, 1983; Kashiwayanagi, et al., 1983) have shown that taste cells in some species generate action potentials in response to direct electrical stimulation. These impulses have a substantial Ca^{++} component and it has been postulated that Ca influx may play an important role in chemosensory transduction. Missing, however, has been the demonstration that chemical stimulation of taste cells also evokes impulses. We report here on our investigations of whole nerve activity and intracellular responses elicited by chemical stimulants in intact animals and in isolated lingual epithelia, respectively.

The activity of the glossopharyngeal nerve was recorded with bipolar Ag-wire electrodes while tastants were applied to the lingual surface in anesthetized mudpuppies. Tastants were injected into a continuous stream of distilled water over the tongue. The following tastants elicited brisk discharges from the nerve: 0.005-0.2 M KCl; 0.02-05 M NaCl, LiCl, $CaCl_2$; 0.03 M nicotine; 0.05 M l-arginine, l-tryptamine. The supernatant from solutions of minced earthworms, minnows, and dissolved Purina Trout Chow were also effective. No responses were elicited by: 0.5 M sucrose, glucose, urea; 0.2 M l-alanine; 0.05 M l-proline, l-cysteine, or l-glutamine. Pre-adapting the tongue to 10^{-4} M amiloride, a potent epithelial Na-channel blocker, did not alter the responses to NaCl, LiCl, or $CaCl_2$.

Intracellular recordings showed that when KCl was applied by pressure ejection from a micropipette situated 10-50 μ from the taste cell, it produced a depolarizing receptor potential in all taste cells. If sufficient doses (estimated to be 10-100 mM) KCl were applied, taste cells generated impulses. Similar doses of KCl had no effect on the membrane potential of surrounding surface epithelial cells. L-arginine and NaCl, applied in the same fashion, also depolarized and excited taste cells. In contrast with KCl, however, these tastants were effective only on some, but not all, taste cells. Furthermore, the sensitivity to these substances varied a great deal from cell to cell. We are continuing to apply other stimulants from the above menu of tastants to correlate extra- and intracellular sensitivity.

These data show that chemical stimulation of single taste cells evokes action potentials, just as is the case for electrical stimulation. Future experimentation will focus on establishing what role impulses play in taste transduction.

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192.11 ACTIVITY PATTERNS REVEALED BY [^{3}H]2-DEOXYGLUCOSE IN THE MAIN OLFACTORY BULB OF THE RAT AFTER STIMULATION WITH ODOR MOLECULES OF VARYING POLARITY. Graham A. Bell*& David G. Laing*(SPON: F.H.Güldner). CSIRO Div.Food Res., AUSTRALIA 2113.

[^{3}H]2-DG injected rats were stimulated with octane(very non-polar), limonene (low polarity), ethyl acetoacetate (medium polarity) propionic acid (very polar), or with purified air. All odors were in air dilutions that, to a human panel, had moderate & approximately equal perceived intensity. Autoradiographic maps of the glomerular layer of the main olfactory bulb showed patterns of activity for each odor. Air produced few or no foci.

The compounds of low and medium polarity gave foci spread medially and laterally and were mainly in the mid & posterior regions of the layer.

By contrast, the highly polar & non-polar odors stimulated glomeruli mainly on the medial surface.

Octane produced the least foci, excepting air, suggesting that there is a low probability that the nasal receptors entrap non-polar compounds, and correlating with the known low stimulus efficiency of simple hydrocarbons.

High polarity propionic acid produced a large cohesive cluster of active glomeruli located with remarkable consistency on the medial surface, 3mm from the anterior pole. We have named this: 3MC. We propose that it represents a topographical projection from areas in the nasal epithelium which entrap highly polar compounds.

Since the medial glomerular region of small mammals is known to project predominantly to the medial & septal surfaces of the nasal epithelium, the 2-DG activity produced by all the odorants in the medial bulbar regions, indicates that entrapment of odor molecules is most probable in the medial regions of the nasal receptor surfaces, and that these surfaces contain the highest density of receptors and/or receive the greatest proportion of the airstream.

Similarly, since there are known projections from the lateral glomeruli to the lateral recesses of the nose, the 2-DG patterns suggest that the lateral recesses are more accessible to odorants of low to moderate polarity.

192.12 SEROTONERGIC-RAPHE TERMINATIONS IN THE MAIN OLFACTORY BULB OF THE RAT. S. Schumacher, J. McLean and M.T. Shipley. Dept. of Anatomy and Cell Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0521.

At last year's meeting we reported that the raphe nuclei have surprisingly heavy projections to the main olfactory bulb (MOB) in the rat. However, the precise terminal-organization of these projections has not been clearly established. Because the laminar cytoarchitecture of the MOB make it an attractive model system for studies of connectional organization and development, we have delineated the precise termination pattern of the raphe projection to the MOB in the adult and have begun to study how this specific pathway develops. To determine how the dorsal raphe neurons terminate in the bulb, injections of 1% WGA-HRP have been placed stereotaxically in the dorsal raphe of anesthetized rats. Following survival of one day, the animals were anesthetized and sacrificed by perfusion-fixation. Frozen sections were processed to demonstrate the presence of anterogradely transported WGA-HRP. Intense anterograde labeling was observed in the glomeruli of the MOB and label was sometimes weakly present in the external plexiform layer. The label appeared to enter the MOB via the olfactory nerve layer.

To gain further insight on the input from the dorsal raphe to the MOB true blue (TB) was injected into the MOB of one side in male albino rats. Following survival of 7-10 days, the animals were anesthetized and sacrificed by perfusion-fixation. Frozen sections of the brain were cut and processed for immunofluorescence to demonstrate the presence of 5-HT and TB in raphe neurons and 5-HT in the uninjected bulb. With the aid of fluorescence microscopy and an image analysis computer, it was determined that neurons in the dorsal raphe nuclei which were retrogradely labeled with TB were also serotonergic. Bead-like serotonergic terminals were also observed in the glomeruli and to a much lesser extent in the external plexiform layer of the MOB.

Thus, it has been demonstrated that serotonergic neurons in the dorsal raphe nuclei project very specifically to the glomeruli in the MOB. The profuse projection of the dorsal raphe neurons to the glomeruli suggests that these neurons may have a pronounced influence on neural activity at the earliest site of processing in the olfactory bulb.

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CNS NEURONS I

193.1 VOLTAGE-CLAMP ANALYSIS REVEALS TWO TRANSIENT OUTWARD CURRENTS IN HIPPOCAMPAL CA3 PYRAMIDAL NEURONS. Kerry L. Zbicz* and Forrest F. Weight (SPON: G. Cordingley). Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

Membrane currents were studied in hippocampal CA3 pyramidal neurons using the single microelectrode voltage-clamp technique. Thin slices of guinea pig hippocampus (450 um) were held submersed in a transverse flow chamber and superfused with artificial CSF warmed to 33°C and equilibrated with a 95% O_2/5% CO_2 gas mixture. Neurons in the pyramidal layer of CA3 were impaled with microelectrodes (15-30 megohms) filled with 3 M KCl. Step depolarization of CA3 pyramidal neurons produced outward current which activated rapidly and subsequently declined during maintained depolarization. This transient current was found to be composed of two distinct currents separable by differences in time course, voltage dependence, and pharmacological sensitivity. The slower of these two currents decayed over a period of several hundred milliseconds and was selectively blocked by 0.5 mM 4-AP. A second, much faster current was also observed. This very fast transient current activated and decayed within 20 msec and was insensitive to concentrations of 4-AP which blocked the slower current. The very fast transient current was greatly reduced by 4 mM Mn^{2+} and 10 mM TEA, substances which had little effect on the 4-AP-sensitive current at the concentration used. The very fast transient current was also reduced by bathing the neurons in solutions in which Ca^{2+} was omitted. The activation threshold for the 4-AP-sensitive current was near -60 mV, and the current was completely inactivated when the holding potential of the neuron was positive to -60 mV. In contrast, the fast transient current was found to first activate near -50 mV, and did not show appreciable inactivation at holding potentials negative to -45 mV. The 4-AP-senstive transient current resembles I_A in molluscan neurons in pharmacological sensitivity and timecourse (Connor and Stevens, J. Physiol. (Lond.) 213: 21, 1971; Thompson, J. Physiol. (Lond.) 265: 465, 1977). The fast transient current appears to be similar to a Ca^{2+}-sensitive transient outward current observed in sympathetic neurons (MacDermott and Weight, Nature 300: 185, 1982).

193.2 WHITE NOISE ANALYSIS OF CABLE PROPERTIES OF CNS NEURONS L.E. Moore and B.N. Christensen. Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550

The analysis of the cable and resting properties of single neurons is usually based on the assumption of a passive membrane, that is, the usual active and voltage dependent conductance mechanisms are inactive. It has been shown in a variety of excitable cells that the above assumption is incorrect and that the resting membrane contains partially activated conductances such that the linear electrical properties reflected in the membrane impedance must include a consideration of these specific processes. The approach used in the experiments described here involved a current or voltage clamp in combination with a small white-noise signal that only slightly perturbs the system. The method relies on the principle of piecewise linearization of an inherently nonlinear system and provides kinetic rate constants for different quasi-steady state membrane potentials. The measured impedance functions were fitted with a neuron model consisting of an isopotential soma plus a single equivalent dendritic cable which contained sixteen elements. The frequency domain characteristics of both the passive and active conductances were used to estimate the dendritic to soma areas, the electrotonic length of an equivalent dendrite, the membrane time constant, and the relaxation time constants associated with the voltage dependent conductances. The effect of differing degrees of synaptic input was simulated by localizing the synaptically activated conductances to either the soma, a point at the end of the dendrite, or the entire dendritic membrane. Impedance transfer functions were markedly dependent on the membrane potential changing from a predominantly passive filter at hyperpolarized levels to an active filter showing resonating band pass characteristics. The resonance frequencies shifted to higher frequencies as a function of increasing depolarization consistent with the expected voltage dependency of the activation of the potassium conductance system. Supported in part by NSF BNS 8316704.

193.3 α_2-ADRENERGIC AND OPIATE INDUCED HYPERPOLARIZATIONS OF LOCUS COERULEUS NEURONS: EVIDENCE FOR A SHARED MECHANISM INVOLVING ADENYLATE CYCLASE INHIBITION. R. Andrade and G.K. Aghajanian. Departments of Psychiat. and Pharmacol., Yale Univ. Sch. Med., Conn. Mental Health Center, 34 Park St., New Haven, CT 06508 USA.

Previous studies have indicated that opiate[1] and α_2-adrenergic[2] receptors hyperpolarize locus coeruleus (LC) neurons through an increase in K^+ conductance. Interestingly, in neuroblastoma x glioma cells both receptors have also been shown to decrease intracellular levels of cAMP through a common mechanism[3]. In the present study we investigated the possibility that the selective α_2-adrenergic agonist clonidine (CLON) and the opiate agonist morphine (MS) might hyperpolarize LC neurons through a common mechanism possibly involving the inhibition of adenylate cyclase.

Pontine rat brain slices were cut using a Sorvall Tissue Sectioner and incubated as previously described[4]. Drugs were administered dissolved in the bath at known concentrations.

Administration of CLON and MS both resulted in hyperpolarizations which were accompanied by decreases in input resistance and exhibited identical reversal potentials. Under manual voltage clamp both CLON and MS administered individualy elicited the appearance of large outward currents which exhibited significant non-additivity with respect to those elicited by MS and CLON coadministered. Consistent with the hypothesis that the effects of MS and CLON might be mediated by adenylate cyclase inhibition, the permeable cAMP analogs 8-Bromo-cAMP or dibutyryl-cAMP reversed the CLON or MS induced hyperpolarizations. This reversal did not simply reflect a non-specific activation of these cells as 8-Bromo-cAMP administered by itself was largely devoid of any effect. No reversal of the CLON or MS-induced hyperpolarizations was seen when equivalent concentrations or adenosine were administered. These results suggest that opiate and α_2-receptors hyperpolarize LC neurons through a common mechanism leading to a final increase in K^+ conductance and possibly involving a decrease in intracellular cAMP levels.

1. Williams et al., Nature, 299: 74, 1982
2. Aghajanian and VanderMaelen, Science, 215: 1394, 1982
3. Sabol and Nirenberg, J. Biol. Chem., 254: 1921, 1979
4. Andrade and Aghajanian, J. Neurosci. 4: 161, 1984

193.4 SYNAPTIC COMPONENTS OF THE CENTRAL PATTERN GENERATOR FOR SWIMMING IN AN AMPHIBIAN EMBRYO SPINAL CORD
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Spinal preparations of late pre-hatching Xenopus embryos paralyzed with curare respond to electrical or mechanical stimulation of the skin with rhythmic, contralaterally alternating motor activity. This pattern is similar to swimming activity in intact paralyzed and unparalyzed preparations although of shorter duration.

Intracellular recordings from spinal motor- and interneurons reveal that events involved in the central program for swimming include 1) a depolarizing input with a much slower decay than rise-time; 2) phasic, impulse-generating EPSPs; 3) mid-cycle IPSPs; 4) inhibitory inputs at the beginning of each swim episode; and 5) IPSPs early in at least occasional swim cycles.

Several of these excitatory and inhibitory events are inputs that are closely interlinked in the arousal of swimming; they are equivalently gradable by variations in intensity of electrical skin stimulation. The inhibitory event at the beginning of the episode is strychnine blockable; the slow excitatory components are unaffected or enhanced as inhibition is blocked. Because these inputs persist in preparations transected as far caudally as the 7th post-otic myotome, and because of pharmacological similarities to swim initiation in intact embryos (Dale and Roberts, J. Physiol. 348:527, 1984) the spinal cord includes the essential network for swim generation.

The rhythmic, contralaterally alternating pattern of ventral root activity evoked by skin stimulation is disrupted in strychnine, converting irreversibly to a tonic, uncoordinated pattern. Mid-cycle IPSPs are blocked with similar pharmacological treatment but because they are contralateral in origin, (Soffe and Roberts, J. Neurophysiol. 48:1279, 1982) and because rhythmicity persists in surgically separated sides of the cord (Kahn and Roberts, Phil. Trans. Roy. Soc. B, 296:229, 1982), additional inhibitory circuitry is suspected. One such component may be indicated by 5) above; this IPSP merges with the spike and phasic EPSP and persists following elimination of the mid-cycle IPSP. Tests for a possible role of this inhibitory component will be presented.

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193.5 THE OLFACTORY RECEPTOR CELL: ELECTROPHYSIOLOGICAL PROPERTIES OF A SMALL NEURON. B. Hedlund, L.M. Masukawa and G.M. Shepherd. Sects. of Neuroanatomy and Neurosurgery, Yale Univ. Sch. Med., 333 Cedar St., New Haven, CT 06510

Information about neuronal properties has been derived mainly from studies of large neurons. The applicability of this information to small neurons needs to be tested. We have therefore examined some of the electrophysiological properties of the olfactory receptor cell. With a soma diameter of 8-12um, a single dendrite (length 50-150 um and diameter 1-2 um), and a fine unmyelinated axon (diameter 0.2 um), this is one of the smallest neurons in the vertebrate nervous system.

Intracellular recordings of receptor neurons have been obtained in an in vitro preparation of the salamander olfactory epithelium. We first tested the threshold for single spike initiation in response to injected current. The threshold ranged from 11 to 144 pA (mean 74 ± 46 pA). This is 1-3 orders of magnitude lower than corresponding values for large neurons.

Increasing depolarizing currents elicited repetitive impulse discharges at increasing frequencies. The average frequency-current relation was calculated to be 1.0 ± 0.4 impulses/sec/pA. This is 1-3 orders of magnitude more sensitive than reported values for other neurons.

The maximal impulse frequency was 25-50 impulses/sec. This is significantly lower than for most large neurons.

Previous results indicating that the olfactory receptor dendrite is relatively electrotonically compact have permitted estimates of the conductance change that would give rise to sensory receptor currents equivalent to the injected currents. This gave estimates of only 180-240 pS, implying a relatively limited number of conductance channels open during the receptor response to an odor stimulus at threshold and above. The results suggest the possibility that synaptic integration in small neurons and neuronal processes may be similarly controlled by relatively few conductance channels. The olfactory receptor neuron may thus provide a model for properties of other small neurons and neuronal processes.

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193.6 FUNCTIONALLY INDEPENDENT ARBORS IN AN INTERNEURON.
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The second-order cell of the barnacle's visual pathway, the I-cell, is a unipolar interneuron with two bilaterally symmetrical arbors in each hemiganglion. The arbors are connected by a long (200 um) thin (1-2 um) crossing process. Previous observations suggested that each arbor potentially could operate as a functionally separate unit. Voltages spreading along the crossing process can be severely attenuated (Stuart, et.al., Neurosci. Abst. 9:679). In addition, the output synapses of the I-cell are located on the same arbors as the synapses made between receptors and I-cell (Schnapp & Stuart, J. Neurosci. 3:1100, 1983).

The photoreceptors of the each of the barnacle's three eyes drive the I-cell. While the median receptors bifurcate to contact both arbors, the lateral receptors terminate only on one arbor (Hudspeth & Stuart, J.Physiol. 272:1, 1977; Oland, et.al., J.Neurophysiol. 49:516, 1983). If the arbors are functionally separate, a signal from a lateral eye could traverse one arbor, independent of events in the other arbor, to drive third-order cells in the same hemiganglion.

In a preparation consisting of the ganglion, the median eye and one lateral eye, we tested whether the I-cell's arbors could operate independently. The I-cell responds to receptor input with a transient large increase in conductance which causes a peak hyperpolarization that decays to a plateau. At the offset of light, the cell gives a depolarizing response which overshoots dark rest. This depolarization drives the third-order cell whose spikes can be recorded extracellularly. When we placed both eyes in constant background illumination, the I-cell hyperpolarized to a plateau potential. If the median light was then turned off, the normally large overshooting off-response in the I-cell was reduced to a small depolarization. The conductance increase underlying the plateau phase of the lateral light response partially shunted the response to median input. When the offset of light over the median eye was timed to coincide with the peak hyperpolarization elicited by the onset of light over the lateral eye, the response to median off was completely shunted. Nevertheless, a clear burst of spikes to median off was recorded extracellularly. This response could have been mediated only by the I-cell's distal arbor which receives median, but not lateral input. Thus, the two arbors of the I-cell can operate independently when the eyes are differentially illuminated. Supported by USPHS EY03347.

193.7 SIMULTANEOUS INTRACELLULAR SOMATIC AND DENDRITIC RECORDING FROM PURKINJE CELLS IN VITRO; DYNAMIC SOMA-DENDRITIC COUPLING. R. Llinás and M. Sugimori. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York 10016.

Simultaneous recordings were obtained from soma and dendrites of Purkinje cells in guinea pig cerebellar slices. Following somatic impalement, the dendrite of the same neuron was then penetrated. The distance between somatic and dendritic penetrations ranged from 150 to 250 μm in the different neurons studied, the distance between the soma and surface of the cerebellar cortex (ℓ) being 350 μm. The passive electrical properties were determined by square pulse current injection at somatic or dendritic level. Potentials were recorded at both somatic (V1) and dendritic (V2) sites simultaneously. A third electrode was placed in the most peripheral part of the dendritic tree and glutamic acid iontophoretically applied at that point. The following conclusions were obtained. First, full size (80 mV) Na-dependent somatic action potentials and Ca-dependent spikes were evoked by both somatic or dendritic depolarization. At V2 the fast Na spikes were recorded as small amplitude potentials (10-15 mV) indicating passive invasion of the dendrites. Following tetrodotoxin poisoning, the Ca dependent spikes were large at V2 but much diminished in amplitude at V1, indicating that these dendritic spikes are in turn electrotonically conducted to the soma. Determination of length constant (λ) by inward and outward current pulses indicated that λ changes as a function of voltage. In the depolarizing direction, graded electroresponsive properties in the dendrites and somata, produced by non-inactivating g_{Na} and g_{Ca}, reduced the difference between V1 and V2 as the voltage steps were increased in amplitude. Thus, for depolarizing voltage step of 5 - 15 mV, "length constant" measurements progressively increased from 500 to 1500 μm (ℓ/λ from 0.7 to 0.2). Similar results were obtained when the voltage changes were produced by iontophoresis of glutamate at the most peripheral portion of the dendritic arbor. These findings indicate that besides Na-dependent and Ca-dependent all-or-none spikes, Purkinje cell dendrites and soma are capable of graded voltage-dependent responses which serve to equalize differences in potential along the soma-dendritic length. This property of "dynamic soma-dendritic coupling" facilitates the conduction of synaptic potentials towards the soma of Purkinje cells. Supported by grant NS13742 from NINCDS.

193.8 NORADRENERGIC NEURONS FROM THE NUCLEUS LOCUS COERULEUS IN DISSOCIATED CELL CULTURE: CULTURE METHODS, MORPHOLOGY AND ELECTROPHYSIOLOGY. S. Masuko, Y. Nakajima and S. Nakajima. Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907, U.S.A.

The nucleus locus coeruleus consists of noradrenergic neurons. In the past this nucleus has been organ cultured; however, dissociated cell culture of this nucleus has not been reported. We have succeeded in making functionally active dissociated primary cell cultures of the nucleus locus coerules neurons and have conducted physiological and pharmacological experiments on these neurons. The regions of the nucleus locus coeruleus were dissected from 300 μm thick vibratome slices of the brain stems of newborn mice or rats. These tissue fragments were dissociated and cultured up to 3 weeks on a layer of non-neuronal cells taken from the brain stem. The cultured cells could be classified into three types: (1) multipolar cells (about 55% of the cells) possessing large perikarya with intracellular granules and many varicose processes, (2) fusiform cells (20%) possessing medium-sized ovoid cell bodies with two prominent arborizing varicose processes, and (3) spindle-shaped cells (25%) characterized by small cell bodies with a few relatively straight processes. Almost all of the multipolar as well as fusiform cells showed the glyoxylic acid induced monoamine fluorescence. Electron microscopy with permanganate fixation revealed small granular vesicles characteristic of noradrenergic neurons in the variosities. These results suggest that multipolar and fusiform cells are noradrenergic neurons of the nucleus locus coeruleus. Intracellular recording from these cells revealed that many of them produced spontaneous spike firing and occasional bursting. This behavior seems to be inherent to these cells, since some cells with no obvious contact with neighboring neurons produced spontaneous firing and bursting. When GABA (0.5 mM) or glycine (0.5 mM) was applied by the puff method, the cells either depolarized or hyperpolarized depending on the conditioning membrane potential. These effects were antagonized by picrotoxin and strychnine, respectively. Substance P application produced depolarization or hyperpolarization. A high concentration of D-Ala^2-D-Leu^5-enkephalin (10-30 μM) sometimes produced hyperpolarization, while somatostatin (1 μM) and β-endorphine (1 μM) hyperpolarized the majority of cells. This dissociated cell culture will serve as a useful model system to investigate the central noradrenergic neurons. (Supported by NIH Grant NS-10457).

193.9 MAGNOCELLULAR CHOLINERGIC BASAL FOREBRAIN NEURONS IN DISSOCIATED CELL CULTURE: MORPHOLOGY, PHYSIOLOGY AND PHARMACOLOGY. Y. Nakajima, S. Nakajima, K. Obata and C. G. Carlson*. Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana 47907.

Recent investigations have disclosed that magnocellular cholinergic neurons in the basal forebrain, particularly in the nucleus basalis of Meynert, show specific degenerations in Alzheimer's disease and senile dementia of the Alzheimer's type. However, almost nothing is known about the electrophysiological and pharmacological properties of these cholinergic neurons. We now report results of physiological and pharmacological experiments utilizing intracellular recording on magnocellular cholinergic basal forebrain neurons in dissociated culture.

From 400 μm thick vibratome sections of the forebrain of newborn rats, the following two regions were dissected; (1) the medial and ventral aspects of globus pallidus, which are homologous with the nucleus basalis of Meynert of primates and humans and (2) the medial septum and diagonal band of Broca. These materials were then dissociated after incubation in 0.25% trypsin solution, plated on non-neuronal cells from the forebrain, and grown mostly for 2-3 weeks in a modified Earle's minimum essential medium containing 10% heat inactivated horse serum and 10% fetal bovine serum (or 10% heat inactivated rat serum). In these cultures most of the large cells with processes were positive with choline acetyltransferase (ChAT) immunoreactivity as well as with acetylcholinesterase staining.

The resting potential of these cultured magnocellular cholinergic neurons was about -70 mV. The neurons seldom produced spontaneous firing, but tetrodotoxin-sensitive action potentials were observed following depolarization. The neurons were depolarized by L-glutamic acid and, depending upon the membrane potential, were either hyperpolarized or depolarized by glycine (strychnine sensitive). GABA produced effects similar to glycine, while norepinephrine produced a β-adrenergic (propranolol-effective) hyperpolarization or depolarization. The neurons responded with depolarization (sometimes with hyperpolarization) by substance P. (The monoclonal antibody against ChAT was supplied by Dr. P. M. Salvaterra and Dr. J. E. Vaughn, City of Hope Research Institute; supported by NIH Grant NS-10457).

193.10 SYNAPTIC INTERACTIONS AND NEUROTRANSMITTER RESPONSES OF TURTLE VISUAL CORTEX. A.R. Kriegstein and B.W. Connors (SPON: P. Suppes). Dept. Neurol., Stanford Univ. Sch. of Med., Stanford, CA 94305.

We have investigated some of the synaptic relationships and neurotransmitter sensitivities of the visual cortex of the turtle, P. scripta. The cortex was removed and either sliced transversely (500 μm) or left intact, and bathed in 21°C saline in a recording chamber. Single electrical stimuli to the subpial layer (which is densely invested with specific thalamic afferents) evoked short latency EPSPs in pyramidal cells, followed by large and long duration IPSPs. In double shock experiments the EPSP was potentiated by a second stimulus given 20 to 800 msec later. Stimuli to the subcellular zone (which contains pyramidal cell efferents) evoked small antidromic spikes in some pyramidal cells, followed, in all cases, by a prolonged IPSP. Interneurons responded to subpial and subcellular zone stimuli with EPSPs and trains of action potentials. Responses resembling those to electrical shock were also obtained to a flash of light in a preparation in which the brain, optic nerves and eyes remained intact. We have obtained evidence for at least two types of IPSPs impinging on pyramidal cells. One is Cl^--dependent, bicuculline-sensitive and reverses at -70 to -80 mV. The second is non-Cl^--dependent, bicuculline-insensitive and reverses at -85 to -95 mV. Cortical sections were stained using an antibody specific for glutamic acid decarboxylase (GAD), the synthetic enzyme for γ-aminobutyric acid (GABA). Many of the interneurons and a small proportion of cells in the pyramidal layer stained positively for GAD. Applications of GABA onto pyramidal cells yielded two different responses. One was hyperpolarizing with a reversal potential similar to the Cl^--dependent IPSP, the other was depolarizing and reversed at -45 to -60 mV. GABA evoked both responses in most cells, and both were depressed by bicuculline (50 μM). Focal application of acetylcholine (ACh) generated a biphasic response with a short latency hyperpolarization reversing at -85 to -95 mV, followed by a longer lasting depolarization.

We conclude that the turtle cortex possesses both feedforward and feedback inhibition, largely GABA-mediated. In addition, an unidentified neurotransmitter, possibly ACh, seems to mediate a K^+-dependent conductance. The intrinsic circuitry of the reptile general cortex displays striking functional parallels to that of mammalian neocortex. Supported by a Klingenstein Fellowship (ARK) and by NS 12151 from the NIH (BWC).

193.11 NEURONAL INTERACTIONS UNDERLYING SYNAPTIC INHIBITION IN THE GUINEA PIG HIPPOCAMPUS. R. Miles and R.K.S. Wong Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

We have examined inhibitory synaptic mechanisms by making simultaneous recordings from pairs of neurones in the CA3 region of hippocampal slices from guinea pigs. Two types of unitary inhibitory interactions have been observed.

1. A discrete ipsp of time to peak 3-10 ms. is elicited by each action potential in a presynaptic inhibitory neurone. Pre-synaptic action potentials are followed by a depolarizing afterpotential which can lead to the firing of bursts similar to those generated by pyramidal cells.
2. A slower hyperpolarization results from the summation of inhibitory events elicited by a train of pre-synaptic action potentials. Unitary ipsp's are difficult to discern. Action potentials in these inhibitory neurones are followed by a pronounced after hyperpolarization.

Most spontaneous ipsp's appear to be unitary events of the first type. Their synchronous occurrence in recordings from pairs of pyramidal cells demonstrates the divergence of synaptic contacts made by inhibitory neurones. Connections from pyramidal cells to inhibitory neurones appear to be strong since single action potentials in one pyramidal cell can initiate di-synaptic ipsp's in another pyramidal cell. Ipsp's activated in a feedback manner by single action potentials in the same pyramidal cell demonstrate the recurrent nature of some synaptic inhibition in the hippocampus.

The properties of 17 identified unitary ipsp's activated mono- or di-synaptically or occurring synchronously in two neurones have been studied in detail. Their rise time ranged from 3-10 ms. and they decayed with time constant similar to that of the post-synaptic cell (17-40 ms.). They reversed, uniformly throughout their time course, at potentials between -72 and -80 mV. The peak conductance change associated with these ipsp's was 5-9 nS., corresponding to inhibitory synaptic potentials of -1.2 to -3.1 mV. at post-synaptic resting potentials of -60 to -65 mV. A comparison with the peak conductance change associated with the maximal IPSP elicited by electrical stimulation of fibre pathways suggests that, in a slice, up to 15 inhibitory neurones may synapse on a pyramidal cell.

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193.12 NOREPINEPHRINE DISINHIBITS HIPPOCAMPAL PYRAMIDAL CELLS IN VITRO. D.V. Madison and R.A. Nicoll. Departments of Pharmacology and Physiology, University of California, San Francisco, CA. 94143.

Norepinephrine (NE), applied to the rat hippocampal slice preparation by iontophoresis or in the bathing medium, causes an increase in the amplitude of the extracellularly recorded population spike and the appearance of multiple population spikes. This occurs without any change in the extracellular dendritic field potential, which is a reflection of the excitatory postsynaptic potential (EPSP) current. This suggests that NE increases the excitability of hippocampal pyramidal cells (HPCs) by decreasing synaptic inhibition rather than by directly enhancing excitatory transmission.

Intracellular recordings from a large number of HPCs has revealed that NE reversibly reduces the amplitude of inhibitory postsynaptic potentials (IPSPs) evoked by orthodromic and antidromic stimulation. The sizes of both the fast and slow orthodromic IPSPs are reduced. This is accompanied by an apparent increase in the size of the EPSP which is probably secondary to the decrease in the overlapping IPSP, since the extracellular EPSP field is not increased by NE.

NE does not change the response of the HPC to iontophoretically applied gamma-aminobutyric acid (GABA), the inhibitory neurotransmitter. Neither does NE affect the reversal potential of these GABA responses or of the IPSP itself, suggesting that NE acts presynaptically to reduce inhibition. NE, contrary to expectations, causes an increase in the frequency of spontaneous IPSPs recorded in HPCs with KCl-filled microelectrodes suggesting that NE increases the spontaneous firing of interneurons. Since NE does not appear to decrease the excitability of interneurons, some other mechanism must account for the disinhibitory action of NE. Possibilities will be discussed.

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193.13 THE ACTION OF ENKEPHALIN ON INTERNEURONS IN THE HIPPOCAMPUS. R.A. Nicoll and D.V. Madison, Departments of Pharmacology and Physiology, University of California, San Francisco, CA 94143.

Previous studies have shown that the opioid peptide, enkephalin, facilitates hippocampal pyramidal cell (HPC) discharge in the slice preparation. This effect occurs in the absence of any change in membrane potential of the HPC and appears to result from a selective reduction in inhibitory postsynaptic potentials (IPSPs). Since the sensitivity of HPCs to the inhibitory transmitter, gamma aminobutyric acid (GABA) is unchanged, the inhibitory pathway must be blocked at some step prior to the action of GABA.

To determine the mechanism of the disinhibition, we have recorded from presumed interneurons. These cells have been identified using the physiological criteria of Schwartzkroin and Mathers (Brain Res. 157: 1-10, 1978). The stable enkephalin analogue, D-Ala^2-Met^5-enkephalin-amide (DALA) was applied either by iontophoresis or by addition to the bathing medium. DALA was found to hyperpolarize interneurons. This hyperpolarization could be as large as 10 mV and was associated with a decrease in input resistance, as determined by applying constant current hyperpolarizing pulses. The extrapolated reversal potential for the response was approximately -85 mV. The EPSP evoked by stimulating afferents in stratum radiatum was increased in size in the presence of DALA, even when the membrane potential was shifted back to control values by passing steady depolarizing current. This action was associated with a large decrease in the size of the IPSP that follows the EPSP in these cells. Despite the increase in EPSP size, the hyperpolarizing action of DALA could inhibit spike discharge by the EPSP. All of these actions were blocked by the opiate antagonist, naloxone.

The present results suggest that opiate receptors are specifically localized on inhibitory interneurons and that activation of these receptors results in a hyperpolarization of the soma and inhibition of spike discharge. The negative reversal potential for the hyperpolarization suggests that enkephalin increases potassium conductance in these neurons. This action is entirely consistent with the effects of opiate receptor activation in other systems. However, since the opiate receptors are present on the inhibitory interneurons and not on the HPCs, the net effect of enkephalin action is a marked facilitation of HPC discharge.

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194.1 NEURONAL CELL CULTURES AND GM_1 MONOSIALOGANGLIOSIDE: A MODEL FOR COMPREHENSION OF MECHANISMS UNDERLYING CENTRAL NERVOUS SYSTEM REPAIR. A. Leon, R. Dal Toso*, D. Presti*, D. Benvegnù*, A. Consolazione* and G. Toffano. Department of Biochemistry, Fidia Research Laboratories, 35031 Abano Terme, Italy.

It is known that survival and growth of embryonic neurons in vivo and in vitro is regulated by specific neurono-trophic factors (NTF) supplied to the neurons by their cellular associates or immediate humoral microenvironment. Functionally equivalent influences have been suggested to be also necessary in adult CNS neural tissue for successful restoration of functional neuronal activity following damage (Nieto-Sampedro, M. et al., J. Neurosci., 3:2219, 1983). Studies from our own and other laboratories have documented the capability of GM_1 monosialoganglioside to enhance functional recovery following specific lesions of adult mammalian brain (Toffano, G. et al., Brain Res., 261:163, 1983; Agnati, L.F. et al., Acta Physiol. Scand., 119:347, 1983). We have recently also reported that GM_1 addition to culture medium of dissociated fetal mesencephalic neurons, grown in serum-free conditions, enhances neuritogenesis and dopamine (DA) cell maturation in vitro. In this context, ^{3}H-DA uptake of control cultures and the stimulatory effect of GM_1 quantitatively increase per DA cell with increasing cellular density. At adequate cell densities, GM_1 increases, in a time- and dose-dependent manner, specific ^{3}H-DA uptake and delays DA cell mortality. The effect of GM_1 on ^{3}H-DA uptake is associated with an increase of the apparent Vmax. Similarly GM_1 increases the specific ^{14}C-γ-aminobutyric acid uptake in both mesencephalic and striatal cell cultures and ^{3}H-choline uptake in striatal cultures indicating its capability to affect various neuronal cell populations. Asialo GM_1, sialic acid or oligo GM_1 are totally ineffective. From the above data one may infer that (a) neuronotrophic molecule(s) capable of affecting neuronal cell maturation and survival are produced in culture; (b) the addition of GM_1 to culture medium and its subsequent incorporation on the neuronal cell surface may play a major role in determining the properties of neuronal cells in response to NTF. It may affect the acquisition and/or maintenance of the acquired differentiated state. Verification of the possibility that similar mechanisms may be operative in the effects of GM_1 in vivo is currently under investigation.

194.2 DIFFERENTIAL INDUCTION OF NEURONOTROPHIC ACTIVITY FOLLOWING MECHANICAL OR CHEMICAL CENTRAL NERVOUS SYSTEM LESIONS. POSSIBLE CORRELATIONS WITH GM_1 MONOSIALOGANGLIOSIDE EFFECTS IN VIVO. G. Toffano, A. Leon, R. Dal Toso*, L. Facci*, G. Savoini*, O. Giorgi, K. Fuxe*§ and L.F. Agnati*°. Fidia Research Laboratories, Department of Biochemistry, Abano Terme, Italy, §Histologisk Institutionen, Karolinska Institutet, Stockholm, Sweden, °Institute of Human Physiology, University of Modena, Modena, Italy.

Although it is now clear from lesion and transplantation experiments that the adult mammalian CNS has an innate capacity for functional recovery following damage, it has been suggested that the intensity of functional repair may depend on the induction at the lesion site of specific neuronotrophic factors (NTF) (Nieto-Sampedro, M. et al., J. Neurosci., 3:2219, 1983). However the capacity of the adult brain to produce adequate titers of, or functionally respond to, such factor(s) may be limited and may vary with extent, localization and type of neuronal injury. We have recently documented the capability of GM_1 monosialoganglioside to facilitate dopaminergic (DA) reinnervation of the striatum after unilateral hemitransection (Toffano, G. et al., Brain Res., 261:163, 1983) but not when lesion was induced by 6-hydroxy-dopamine (6-OHDA) injected directly in the substantia nigra of adult rats (Toffano, G. et al., submitted). A comparative study conducted utilizing dissociated fetal mesencephalic neuronal cultures as bioassay for NTF detection indicated that (a) extracts of striatum and substantia nigra of adult unlesioned rats contain trypsin and heat-labile molecule(s) capable of increasing, in a dose- and time-dependent manner, neuritic outgrowth and specific ^{3}H-DA and ^{14}C-γ-aminobutyric acid uptake; (b) titers of such NTF increase following unilateral hemitransection in both ipsilateral and contralateral striatum and substantia nigra; (c) similarly, NTF activity increases upon 6-OHDA-induced lesion. The only exception was that no NTF activity could be detected in the 6-OHDA injected substantia nigra; (d) simultaneous addition of GM_1 (10^{-7} M) to cell cultures results in a significant amplification of the NTF activity. The above data provide further support to the hypothesis that recovery from brain damage may involve NTF induction. GM_1 in vitro is capable of augmenting their potency. In addition, as the NTF induction varies with the different lesion techniques, we postulate that the lack of GM_1 effects in 6-OHDA lesioned animal may be related to the absence of NTF in the injected area.

194.3 CRITICAL STAGES IN THE DEVELOPMENT OF CORTICAL NEURONS. K. Kalil and L. Ramirez*. Dept. of Anatomy and Division of Neurosurgery, University of Wisconsin, Madison, WI 53706.

A number of studies have focused on the role of sensory afferent input in the development of the mammalian CNS. In contrast, there exists little information about the reciprocal question, i.e. how does efferent connectivity affect the development of CNS neurons. In order to address this issue, we studied the growth of pyramidal tract neurons within the hamster sensorimotor cortex during normal development and after early postnatal lesions of their axons within the pyramidal tract. Using both Nissl and retrograde HRP techniques, we first determined that two cell populations exist within the lumbar representation of cortical layer 5B. A large-celled population (40% of the total) projects to the spinal cord and a small-celled population (60% of the total) projects to targets rostral to the medulla and spinal cord. We charted the growth in cross-sectional area of the large cell (corticospinal) population from 7 days postnatal to adulthood. The cells continue to grow until 51 days. However, the most rapid growth rate occurs between 7 and 14 days, during which time the area of the cell body triples in size. The period of rapid growth coincides with the time of arrival of corticospinal axons in the lumbar cord (8 days) and the onset of target innervation (10-11 days).

We then examined the growth of corticospinal neurons after the pyramidal tract was cut on one side in the medulla at various ages. Early lesions of the tract (4-8 days postnatal) interrupt lumbar projection fibers before they establish synapses in the spinal cord. However, the cortical cell bodies continue to grow normally after axotomy until 11 days after birth. At this critical stage, corticospinal cells are arrested in development and remain at the 11-day cell size (which is 50% of normal adult cell size) indefinitely. If lesions of the tract are made in adult animals, the corticospinal neurons undergo a 65% shrinkage in cell size. There is no evidence for cortical cell death after lesions of the pyramidal tract at any age.

These results suggest a critical turning point in the development of corticospinal neurons. Before the age of 11 days, the cell bodies have an independent program of growth that proceeds despite axotomy. After this critical time, which coincides with the establishment of corticospinal connections, the cortical neurons require the integrity of their axons for further growth and, in the absence of normal efferent connectivity, they become arrested in development.

Supported by NIH grant NS-14428 (K.K.).

194.4 SOLUBLE, MUSCLE-SPECIFIC TROPHIC ACTIVITY ENHANCES CHOLINE ACETYLTRANSFERASE LEVELS IN CULTURES OF ENRICHED SPINAL MOTORNEURONS. T.P. Flanigan*, J.G. Dickson* and F.S. Walsh*. Institute of Neurology, Queen Square, London WC1N 3BG, U.K.

Recent evidence from in vitro studies suggests that skeletal muscle may supply soluble retrogradely-acting factors which influence the survival and development of spinal motorneurons. We have previously described the survival and cholinergic development in vitro of a motorneuron-enriched cell population from E7 chick spinal cord. When grown on hydrated collagen gels, these cells extend neurites and continue to express choline acetyltransferase activity in the absence of exogenous sources of trophic influence. In addition, since these cultures are virtually devoid of non-neuronal components, this in vitro cholinergic expression occurs in the apparent absence of endogenous feeder cell activity.

To examine the possible existence of retrograde, soluble factors exerting a trophic influence on motorneurons, primary cultures enriched in motorneurons were established on collagen gels in the presence and absence of soluble extracts from a variety of E12 chick tissues, including skeletal muscle. Cultures were harvested after 5 days and assayed for CAT activity. In the presence of whole embryo extract (CEE, 2%) or a soluble fraction from hind limb muscle (50 µg/ml) cultures exhibited a 2.5-fold increase in CAT level compared to controls. Soluble extracts from other tissues e.g. skin and liver, did not influence significantly the level of CAT in the cultures. All tissue extracts, at levels above 200 µg/ml, did however exert a generalised cytotoxicity in vitro.

This study shows that while significant levels of CAT activity are maintained in the motorneuron-enriched cultures in the apparent absence of any exogenous trophic influence, a soluble factor(s) from skeltal muscle can produce a further enhancement in the level of the enzyme. It remains as yet to be defined whether this trophic activity increases the survival of motorneurons or exerts an inductive effect on CAT expression. The tissue-source specificity of this soluble neurotrophic activity is compatible with a muscle-derived retrogradely-acting growth factor, and in view of the more generalised cytotoxicity of crude tissue extracts, it will be of importance to attempt a partial purification of this activity.

194.5 **NEURON-TARGET CELL INTERACTIONS MAY INVOLVE PROTEASE-INHIBITOR INTERACTIONS.** Randall N. Pittman. Div. of Biology, California Institute of Technology, Pasadena, CA 91125.

Dissociated cultures of rat sensory and sympathetic neurons spontaneously release a calcium-dependent metalloprotease and a urokinase-like plasminogen activator (Pittman, Soc. Neurosci. Abstr. 9:5, 1983). These proteases are preferentially secreted from distal processes and/or growth cones of sympathetic neurons. The release is spontaneous and neither acute nor chronic depolarization significantly alters the amount of protease released from sympathetic neurons. However, growing sympathetic neurons in the presence of heart cells results in decreased quantities of plasminogen activator activity in the culture medium compared to the amount present in the medium in neuron-alone cultures. Mixing conditioned medium from heart cells and proteins released from sympathetic neurons also results in a decrease of plasminogen activator activity. This suggests that an inhibitor of plasminogen activator is being released by heart cells.

A gel electrophoresis method has been developed which allows the properties of inhibitors of plasminogen activator to be indirectly determined. Cultures of heart cells release two inhibitors of plasminogen activator. Both form irreversible complexes with the plasminogen activator, urokinase. In addition, both inhibitors appear to be on the surface of heart cells and therefore could serve as "receptors" for the plasminogen activator being released by neurons. The inhibitors are currently being purified from heart cell conditioned medium in an effort to determine their significance in neuron-target cell interactions.

Supported by a fellowship from the MDA to RP and grants from the McKnight Foundation and NIH (NS 20917) to Paul Patterson.

194.6 SPECIFIC NEURONOTROPHIC INFLUENCES IN EXPLANT CULTURES OF OCCIPITAL CORTEX. A. Repka, F. Haun and T.J. Cunningham (SPON: B. Payne). Dept. of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

Relatively little is known about the trophic interactions which affect the survival and differentiation of specific neuronal populations in the mammalian neocortex. We have investigated this problem using explant cultures of visual system structures of rats. Occipital cortex, diencephalon, and optic tectum were dissected from embryonic day 14 or 15 rats, and placed in plastic petri dishes coated to inhibit fibroblast growth. A piece of occipital cortex was co-cultured with either diencephalon (C+D), tectum (C+T), or other cortex (C+C) in a defined medium. The cultures were terminated after 5 or 7 days and processed for plastic embedding. Semi-thin and thin sections were taken at equally spaced intervals through the cortical explants from each type of culture, and detailed stereological analyses performed. Some of the 5-day cultures had also been exposed to tritiated thymidine at 3 days in culture, and sections were processed for radioautography. After 5 days in culture, cortex pieces from the C+D and C+T cultures appear to survive equally well, with only scattered necrotic cells seen throughout the explant. C+D cortex contained a smaller, more heterogeneous distribution of nuclear diameters than cortex from the C+T cultures. Furthermore the volume fraction of growth cones relative to neurites was greater in the C+D cultures. Preliminary thymidine labeling data indicate that there is a greater percentage of heavily labeled neurons in the C+D explants than in the C+T explants. After 7 days in culture, there is widespread necrosis in the cortex from C+T cultures, but in the cortex from C+D cultures there are significantly more surviving neurons with little necrosis. Taken together, these results suggest that at 5 days in culture the diencephalon supports smaller, later generated neurons in the cortical explants, while the optic tectum supports a larger more homogeneous population of earlier generated neurons. The C+C explants examined so far are similar to the C+D cultures in terms of survivability and heterogeneity of nuclear diameter. It is possible that specific neuronal populations are supported to a greater or lesser extent in the different culturing conditions. The degree to which the cultures are biased toward one population may depend on whether or not that population would normally be directly connected to particular visual structures in vivo.

Supported by NS16487 from NINCDS.

194.7 SPECIFIC NEURONOTROPHIC INFLUENCES IN EXPLANT CULTURE ALTER CORTICAL TRANSPLANTS' NEURONOTROPHIC EFFECT IN VIVO. F. Haun, T. Phillips* and T.J. Cunningham. Dept. of Anatomy, The Medical College of Pennsylvania, Phila., PA 19129.

Transplants of embryonic rat posterior cortex into damaged visual cortex of newborn rats exert a neuronotrophic influence (i.e., promote neuron survival) in the dorsal lateral geniculate nucleus (dLGN) of the host animal. This trophic effect is specific to late-generated dLGN neurons. We now have evidence that specific populations of neurons in the transplant are the source of much if not all of that neuronotrophic influence. Explants of posterior cortex were co-cultured with pieces of either diencephalon, tectum, or other cortex from embryo rats 14 or 15 days of age. After 5 days in culture there is evidence that the cortical explants from cortex + diencephalon cultures contain more smaller, later generated neurons than cortical explants from the cortex + tectum cultures (Repka et al., this volume). We then transplanted these cortex explants into newborn rats with large right visual cortex lesions. Five days later we found a greater than 50% increase in both the volume occupied by, and the number of, surviving late-generated neurons in the dLGN of animals with cortical transplants previously co-cultured with diencephalon, compared to the tectum co-cultured transplants. In animals with cortical transplants previously co-cultured with other cortex pieces, the effects are similar to transplants co-cultured with diencephalon. Tectal co-culturing produces transplants with dLGN effects not significantly different from animals with non-cultured, direct cortical transplants. The combined in vitro-in vivo results suggest that the cortical transplants' trophic influence on dLGN arises predominantly if not exclusively from a particular population of embryonic cortical neurons, most likely those destined to be either targets or sources of afferent input for dLGN neurons.

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194.8 A SOLUBLE FACTOR FROM SPINAL CORD NEURONS MODULATES A CALCIUM ACTIVATED POTASSIUM CONDUCTANCE IN RAT MUSCLE CELLS IN CULTURE. B.A. Suarez-Isla, J.W. Cosgrove and S.I. Rapoport. Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, Maryland 20205.

We had shown (Suarez-Isla et al., Neurosci. Abstr., 8; 1982) that the incidence of slow hyperpolarizing after-potentials decreases significantly in rat myotubes, following innervation in vitro by 6-day chick spinal cord neurons that form stable cholinergic neuromuscular synapses. The slow HAP is associated with an increased Ca-dependent K-conductance that may be responsible for spontaneous contractile activity prior to innervation in vivo. Patch-clamp recordings of single Ca-activated K-channels (Suarez-Isla & Rapoport, Neurosci. Abstr., 9, 63; 1983) have shown that the neurotrophic effect of in vitro innervation is expressed as a change in the channel conductance properties, namely as an increase in the sensitivity of the voltage dependent block of the channels, probably due to an intracellular cation.

We have isolated a fraction (Biogel p 10 column, estimated M.W. < 4,000 D) from the conditioned medium obtained from spinal cord neurons isolated from 6-day chick embryos and kept in culture for 3 days. This fraction significantly decreased the percentage of slow HAPs after 24 h incubation of non-innervated rat myotubes ($p < 0.05$), from 70.0 + 8.2 in control paired dishes (70/100 cells tested, 4 different primary dissociations) to 42.0 + 3.7 in treated cells (21/50 cells, mean + S.E.M.). In addition, the isolated fraction significantly decreased the amplitude and the duration at 50% amplitude (t_{50}), of slow HAPs still present in treated cells:

	Control	+4,000 fraction
HAP amplitude (mV)	8.9 + 0.7 (38)	5.7 + 0.4 (21)*
t_{50} (ms)	139.1 ∓ 15.8 (38)	75.2 ∓ 13.1 (21)*

*$p<0.005$; mean ± S.E.M. (number of cells tested)

This suggests that the slow conductance still present in a subset of the treated cells is partially blocked by the 4,000 D fraction.

We conclude that a soluble factor contained in the 4,000 D fraction of chick spinal cord conditioned medium can block the slow hyperpolarizing after potential in rat myotubes which is generated by a Ca-activated K-conductance. These results suggest also that nerve-mediated electrical activity is not needed for rapid expression of this modulatory effect.

194.9 GANGLIOSIDE GM_1 INDUCES NEURONAL DIFFERENTIATION IN ESTABLISHED DRG CULTURES INDEPENDENTLY OF NGF. P. Doherty*, J.G. Dickson*, T.P. Flanigan* and F.S. Walsh*.(SPON: A. Raines). Institute of Neurology, Queen Square, London WC1 3BG

Neurite outgrowth can be objectively quantified by use of an ELISA assay for neurofilament protein (Doherty *et al.*, 1984; J. Neurochem. 42, 1116).In an attempt to elucidate the molecular action of gangliosides on neuronal cell differentiation we have utilised the assay to quantitate GM_1 associated increases in neurofilament protein levels in dissociated culture of E9 chick DRG. Primary cultures and the ELISA assay protocol were essentially as previously described (Doherty *et al.*, 1984). Cells grown on a collagen coated 96-well microtitre plate in media supplemented with 10% foetal calf serum were fixed at day five *in vitro* and the binding of RT97, a monoclonal antibody reactive against neurofilament protein, was determined.

Significant ($p<5\%$) increases in RT97 binding were found in cultures grown in the presence of 0.04 ng/ml NGF, with a maximal 7 fold greater response apparent at 5 ng/ml NGF. Under identical culture conditions GM_1 (100 µg/ml) failed to induce neuronal cell differentiation as assessed both by the ELISA assay and morphological criteria. In contrast, the addition of GM_1 at 48 hrs *in vitro* to cultures initially established in the presence of 5 ng/ml NGF substantially increased the level of RT97 binding (Table 1). This response was both independent of and not potentiated by NGF. The increases were associated with a GM_1 induced increase in the density and complexity of the neuritic network, with immunocytochemical techniques showing localization of RT97 binding to the neuritic processes. Thus GM_1 cannot initiate a program of neuritic outgrowth, however GM_1 can enhance the differentiation of established cultures with this response being independent of the trophic factor that originally stimulated neuronal growth. We would therefore conclude that GM_1 acts at the level of signal execution rather than signal reception.

GM_1 (µg/ml)	NGF (ng/ml) 0	0.5	5
0	0.19 ± 0.04	0.25 ± 0.03	0.13 ± 0.03
10	0.38 ± 0.04	0.35 ± 0.07	0.21 ± 0.05
50	0.53 ± 0.04	0.47 ± 0.09	0.32 ± 0.05
100	0.64 ± 0.04	0.68 ± 0.01	0.73 ± 0.10
200	0.58 ± 0.06	0.64 ± 0.06	0.40 ± 0.11

Table 1. The absolute increase in RT97 binding between day 2 and five of culture (O.D. units). Each value is the mean ± S.E. of 6 independent determinations.

194.10 HOST-GRAFT INTERACTIONS FOLLOWING CEREBELLAR TRANSPLANTATION IN RAT. M.J. Perlow, G. Nilaver, M.C. Beinfeld, and E.A. Zimmerman. Univ. of Illinois & Augustana Hosp., Chicago, IL 60612; Columbia Univ., Coll. of Phy. & Surg., New York, NY 10032; and St. Louis Univ. Med. Sch., St. Louis, MO 63104.

Interactions between developing neurons and their enviornment have been shown to be critical for the establishment of cerebellar neuronal circuits. The simultaneous development of both pre- and post-synaptic elements in cerebellar ontogeny however, makes it impossible to determine their relative contributions to the formation of normal anatomical connections. The poor viability of Purkinje cells in culture precludes use of in vitro systems for such analysis. Transplantation of embryonic neural tissue into adult brain has been employed to study development and plasticity. We therefore examined the role of neuronal milieu in the ontogeny of Purkinje neurons by studying their development following implantation into various regions of adult rat brain. Fetal cerebellar tissue from different gestational stages (16,18,20 & 22 days) were stereotaxically allografted into the following regions of adult rat brain: lateral, 3rd and 4th ventricles, lateral hypothalamus and parietal cortex. Development was allowed to progress for various times ranging from 30 to 50 days. Neuronal migration and connectivity were analyzed in Bouins fixed, paraffin embedded sections, employing motilin immunoreactivity as a marker for Purkinje cells. All grafts were viable and contained all the cellular elements of intact cerebellum. A close correlation between age of donor tissue and extent of its post-transplant development was seen, neuronal migration and cerebellar lamination being more frequent in donor tissue from earlier gestational periods. The cytoarchitectural pattern of lamination in lateral and 4th ventricular transplants was inverted, with the molecular layer oriented towards the center of the transplant. In contrast, normal lamination was evident in lateral hypothalamic and cortical transplants. Purkinje cells were motilin reactive and showed variable dendritic arborization. On several occasions motilin-ergic Purkinje axons could be traced over considerable distances into adjacent regions of the host brain. The transplants in turn appeared to receive peptidergic inputs from the host as evidenced by oxytocin and neurophysin fiber staining. The absence of graft rejection in this study points to the unique immunoprivileged nature of the CNS. It also illustrates the versatility of this model system wherein the relative importance of both pre- and post-synaptic determinants in cerebellar ontogeny can be independently assessed. (Supported by NIH grants NS18324 and NS18335).

194.11 THE DEVELOPMENT AND MATURATION OF TRANSPLANTED MOTOR NEURONS IN VIVO REMOTE FROM THE INFLUENCE OF SKELETAL MUSCLE. R.C.Yu, M.J.Perlow, F.D.Brown*, R.G.Fessler, S.Woo*, B.H.Wainer and B.G.W.Arnason. The Brain Research Institute, University of Chicago, Chicago, IL 60637, and School of Medicine, University of Illinois, Chicago, IL 60612.

Survival of motor neurons during development is believed to depend upon trophic factor(s) originating from within the target tissue. However, motor neurons can be maintained in vitro for extended periods in the absence of any muscle tissue as long as the culture contains glial cells. In the present study, we have examined the development and maturation of motor neurons transplanted into adult cerebral ventricle, a site remote from any influence of its target tissue. Fourteen to 15-day old rat embryonic spinal cord sections were stereotactically injected into the right lateral ventricle of adult Sprague Dawley rats ranging from 5 to 24 months in age. The host rats were subsequently sacrificed at 10-day intervals for histological and immunocytochemical analysis. The survival rate of post-surgical animals was greater than 90%. Viability of the implants appeared to be independent of host age. Ten days post transplantation, the neuronal elements appeared as immature neurons and reacted poorly with Nissl stain. A few fragmented fine fibers could be seen in silver stained preparations. Early grafts of independent cross-section cord fragments showed evidence of fusion between them, which was further evidenced by the presence of multiple, ependyma-lined, central canals within a single, large intraventricular cord mass 20 days post transplantation. At this stage, the majority of neuronal elements appeared to have the morphology of mature cells. Within each large multipolar soma, Nissl bodies were present in the cytoplasm surrounding a clear, spherical nucleus with a distinct nucleolus. Neurofilamental protein was increased and emerged as a fine network which continued into the cytoplasmic processes. By 30 days post implantation, the transplanted tissue had grown in size to occupy more than half of the ventricular space but no hydrocephalus was seen. Motor neuron-like cells reacted positively with mono-specific antibodies for choline acetyltransferase(CAT). Our results confirm early observations(Devel.Br.Res.10:201)that embryonic spinal grafts can survive in adult brain. We further demonstrated that motor neurons within these grafts can mature and develop certain functional properties(i.e.,synthesis of CAT)in the absence of intimate contact with their target tissue.

(Supported by ALS Society of America)

194.12 RECEPTOR MEDIATED AUTOREGULATION OF SEROTONERGIC GROWTH IN PRIMARY DISSOCIATED CELL CULTURE. E.C. Azmitia and P.M. Whitaker-Azmitia. Dept. of Biology, New York University, New York, NY, and Dept. of Psychiatry and Behavioral Science, SUNY, Stony Brook.

We have previously demonstrated that serotonergic growth in primary culture of dissociated fetal mesencephalic raphe is stimulated by a variety of fetal target tissues (Azmitia et al., 1983, Soc. Neurosci. Abst.). In order to determine if 5-HT receptors are involved in regulating serotonergic growth, both pre- and post-synaptically active drugs were tested in fetal raphe and hippocampal (hipp) co-cultures.

Fetal pups (15-16 days) were removed from pathogen free Sprague-Dawley rats (Hill Top Labs). The mesencephalic raphe and hipp tissue was dissected and dissociated in Versene 1:5000 (GIBCO) by gentle repipetting. The cells were centrifuged (500 x g) twice, and the final pellet resuspended in complete medium containing 5-10% fetal calf serum. The initial cell plating density was 0.5 to 1.0 x 10^6 cells/cm^2 for raphe and 1.0 x 10^6 for hipp cells in 200 ul final volume added to 96 multi-well plates coated with collagen. The cultures (3-6 wells per condition) were incubated at 37°C for 5 days.

5-methoxytryptamine (5-MT, a pre-synaptic agonist), mianserin and ketanserin (both post-synaptic antagonists at low conc. and pre-synaptic antagonists at high conc.) were tested between 10-10,000 nMolar. The growth of serotonergic neurons was assessed biochemically (specific high-affinity ^{3}H-5HT uptake) or immunocytochemically (using an antiserum raised against 5-HT).

The results indicate that both types of drugs can suppress the growth of serotonergic neurons at low concentration (10-500 nM), but the presynaptic agonist (5-MT) appears to be the most potent (30-60% inhibition). If the postsynaptic antagonists are used at high conc. (10 uM) no effect or stimulation of growth is seen. Furthermore, this high dose of mianserin can effectively block the inhibitory effects of 5-MT.

These results suggest that stimulation of a 5-HT presynaptic receptor may suppress the growth of serotonergic neurons in tissue culture.

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194.13 TETRODOTOXIN BLOCKS THE ACTION POTENTIAL IN EXTRAJUNCTIONAL ACETYLCHOLINE SENSITIVE MUSCLE FIBRES FROM AMYOTROPHIC LATERAL SCLEROSIS HUMAN BIOPSIES. O.D.Uchitel and A.L. Dubrovsky (SPON:A.Suburo). Inst. Biol. Celular, Fac. de Medicina, UBA, Paraguay 2155, (1121) Bs.As. and Centro Neurologico, Hosp. Frances, Rioja 951, (1121) Bs As, Argentina.

In amyotrophic lateral sclerosis (ALS) there is a progressive wasting and atrophy of skeletal muscles. The underlying pathological process is characterized by muscle denervation and reinnervation by sprouting of the surviving motoneurons. In mammalian muscles, experimental denervation induces the appearance of extrajunctional acetylcholine (ACh) receptors and tetrodotoxin (TTX) resistant action potentials (AP). However, evidence of full TTX resistant AP in denervated human muscle has not been reported.

We investigated ACh sensitivity and TTX effect on AP of cut muscle fibres obtained from routine limb muscle biopsies of patients with ALS. Histochemical analysis of the electrophysiologically investigated bundles were carried out to estimate the degree of denervation. Muscle fibres from one patient with a short time course of the disease showed no extrajunctional ACh sensitivity . In 3 out of 4 biopsies from patients with long lasting disease including a patient with complete clinical paralysis and electromyographycal denervation all 46 muscle fibres investigated were sensitive to ACh (0.2 to 225 mV/nC). The cut muscle fibres had low resting potentials (-70 to -30 mV). When hyperpolarized to -90 to -100 mV they were always capable of eliciting an AP. In the presence of 5×10^{-7} g/ml of TTX the AP of 28 from 31 fibres studied was blocked. In contrast, a biopsy of a human muscle denervated by nerve section showed extrajunctional ACh sensitivity and also all the fibres were capable of eliciting fully developed AP in the presence of 10^{-6} g/ml of TTX.

Histochemical analysis of the biopsies showed neurogenic changes with no direct correlation between the frecuency of ACh sensitive fibres and that of the angulated atrophic histochemically denervated muscle fibres.

The disociation between extrajunctional ACh sensitivity and lack of TTX resistant AP together with the presence of histochemically non denervated muscle fibres may indicate the presence of functional denervation in ALS and also indicates that denervation in ALS may not be entirely equivalent to denervation due to nerve section.

Supported by CONICET and Fundacion Cherny.

194.14 PHARMACOLOGICAL EVIDENCE FOR SODIUM CHANNELS IN NORMAL TONIC FIBERS OF THE FROG. M. Huerta*, J. Muniz*, and E. Stefani.(SPON: D. J. Chiarandini). Dept. Physiology, CIEA of IPN, Mexico DF 07000, AP 14-740.

Frog skeletal muscles have two different types of extrafusal muscle fibers: twitch and tonic. Normal twitch fibers produce Na action potentials and generate a transient contracture in high K while tonic fibers lack action potentials and give a maintained K contracture. However, after denervation frog tonic fibers display propagated action potentials. To have some clues on the mechanisms of appearance of voltage-sensitive Na channels in tonic fibers after denervation, we tested whether normal tonic fibers possess voltage insensitive Na channels. To this end we studied the effects of veratridine on the membrane potential and tension development. The specificity of veratridine was demonstrated by the reversal of its action by tetrodotoxin (TTX). Muscle fibers of the cruralis muscle were current-clamped. Tension was isometrically recorded from bundles of tibialis anticus longus and cruralis muscles. All salines contained d-tubocurarine (5 ug/ml). Tonic and twitch fibers were identified by their passive electrical properties. Tonic fibers have a large input resistance (6.2±0.33 megohms, n=7) and a long membrane time constant (560±20 msec, 7). The corresponding values for twitch fibers are 0.80±0.05 megohms and 21±3 msec (12). Both fiber types were depolarized by veratridine (44 uM). The resting potential (RP) in twitch fibers was 81±2 mV (14) in control saline and 51±7 mV (53) with veratridine. In tonic fibers the RP was 74±8 mV (20) and -54.1±8 mV (20) after veratridine. A similar depolarization by veratridine was recorded 2-4 weeks after denervation in both fiber types. The depolarization induced by veratridine was reversed by TTX (0.63 ug/ml). As expected from the electrical studies, veratridine (100 uM) induced a transient tension in twitch fibers and a maintained contracture in tonic fibers. The tension was abolished by TTX. These observations indicate that normal tonic fibers possess TTX sensitive Na channels which can be opened by veratridine. One may speculate that after denervation biochemical changes in these channels can be related to the appearance or the unmasking of the voltage gating sensor. Supported by grants: Supported by CONACyT of Mexico, Grants PCCBNAL-790022 and PCCBBNA-020187.

NEURAL PLASTICITY IN ADULT ANIMALS I

195.1 NEUROGENESIS IN ADULTHOOD: ULTRASTRUCTURAL CHARACTERIZATION OF NEW NEURONS IN THE FOREBRAIN OF ADULT CANARIES. G.D. Burd and F. Nottebohm. Rockefeller University, New York, NY 10021

New neurons are born throughout the year in the forebrain of adult male and female canaries. Cells adjacent to the lateral ventricle divide and then migrate into the underlying forebrain (Goldman & Nottebohm. 1983. Proc. Natl. Acad. Sci.; Nottebohm, in prep). The present study is focused on characterizing the ultrastructure of new neurons present in the hyperstriatum ventralis, pars caudalis (HVc). Adult male or female canaries received 1, 4, or 28 IM injections of ^{3}H-thymidine (50 uCi; 6.7 Ci/mmol) over 1, 2, or 14 days, respectively, and were sacrificed at various survival times. Labeled cells were identified in 1 um thick sections by ^{3}H-thymidine autoradiography, photographed, and re-embedded for ultrastructural examination. At short survival times (1 and 6 hrs), only some cells lining the lateral ventricle and, in the forebrain, cells lining blood vessels and a few small, darkly stained cells (presumed glial cells) were labeled. Some labeled cells located in the ventricular zone were in mitosis at 6 hr. At longer survival times (23, 30, and 45 days after the first ^{3}H-thymidine administration), cells in the ventricular zone and some glial cells were still labeled, but now there were also many labeled cells that had distinct neuronal morphologies. In HVc, the ultrastructural characteristics of the labeled cells identified as neurons (n=16) included spherical or ovoid nuclei containing diffuse chromatin and prominent nucleoli, long dendritic processes filled with microtubules and polyribosomes, and synaptic contacts on the cell body and dendrites. The synaptic contacts were formed by terminals that contained 1) spherical, agranular vesicles, 2) large dense core vesicles and smaller spherical, agranular vesicles, and 3) pleomorphic or flattened vesicles. The physiological action of the first two types of terminals is thought to be excitatory and the third type is thought to be inhibitory. The source of these terminals has not been determined. The new neurons were distributed throughout the HVc. The average soma diameters of new neurons labeled after 14 days of ^{3}H-thymidine and 44 days survival ranged from 5 um to 13 um. The overall goal of these experiments is to characterize the life history of the new neurons from their birth in the ventricular zone through their migration, differentiation, and incorporation into existing neural networks.

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195.2 NEUROGENESIS OF INTERNEURONS IN A NUCLEUS OF ADULT CANARY FOREBRAIN. John A. Paton, B. O'Loughlin* and F. Nottebohm. The Rockefeller University, New York, NY 10021.

Neurogenesis in the adult central nervous system is unusual, but not without precedent. One robust example occurs in adult canaries, where neurons are added to nucleus hyperstriatum ventralis pars caudalis (HVc) at rates of up to 1.5% per day (Goldman & Nottebohm, Proc. Natl. Acad. Sci. 80(1983):2390), and are inserted into preexisting neural circuits (Paton & Nottebohm, Science, in press). The addition of these new neurons in canaries adds a form of plasticity to the adult brain, but one whose limits may be set by the types of neurons produced and incorporated.

The purpose of the present study was to determine whether the new neurons in HVc have axons which project out of this nucleus. Adult canaries, both males and testosterone treated females, received injections of ^{3}H-thymidine (50 uCi, 6.7 Ci/mmol) every 12 hours for 14 days. Thirty or sixty days after the last ^{3}H-thymidine injection HRP was injected into robustus archistrialis (RA) and area X of the lobus parolfactorius (X), the two nuclei known to receive projections from HVc. Two days later, the birds were deeply anesthetized and perfused with 1% paraformaldehyde and 1.25% gluteraldehyde. Then 100 um Vibratome slices of the brain were reacted with diaminobenzidine and H_2O_2 to visualize HRP containing cells in HVc. These slices were then embedded in paraffin, cut into 6 um sections, and processed for autoradiography to detect ^{3}H-thymidine labeled cells in the same tissue.

Data were obtained from both male and female canaries 30 or 60 days after the last ^{3}H-thymidine injection, with varied HRP injection sites within RA and X, and sections taken from throughout HVc. In each case more than 2% of the neurons in HVc were ^{3}H-thymidine labeled; but of the thousands of HRP labeled cells examined, none were radioactively labeled. Furthermore the distribution of cell body sizes was such that the median for X-projecting cells was significantly greater than that of cells labeled with ^{3}H-thymidine, and the median for ^{3}H-thymidine labeled cells was significantly greater than that of RA-projecting cells. The simplest interpretation of these results is that the new neurons incorporated into nucleus HVc in the adult canary are interneurons which are intermediate in size between neurons that project to RA or X.

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195.3 IN VITRO NEUROPLASTICITY OF THE ADULT CANARY FOREBRAIN. Steven A. Goldman and Elaine G. Diacumakos*. Rockefeller University, New York, NY 10021.

The vocal control nucleus of the canary forebrain, HVc, has been shown to undergo spontaneous neurogenesis in adulthood (Goldman and Nottebohm, Proc. Natl. Acad. Sci. 80:2390-94, 1983). Neuronal precursor cells, located in the ventricular zone overlying the adult HVc, divide at a relatively rapid rate, after which the resultant daughter cells migrate into the HVc. These neuroblasts then differentiate into mature neurons. These in vivo phenomena, including adult neuronal production, neuroblastic migration, and neuronal differentiation, all suggested to us the feasibility of studying adult neuroplasticity in vitro, using the HVc as a model system.

We have developed a set of culture conditions by which both adult and embryonic canary forebrain can be successfully maintained for prolonged periods of time in vitro (J. Cell Biol. 95:42a, 1982). Using this technique, we have obtained extensive outgrowth of neurons and glia from both explanted adult HVc, and from the adjacent neostriatum. Approximately 20% of adult HVc explants have generated such neuronal outgrowth, resulting in mixed glial and neuronal cultures similar to those we have obtained from embryonic forebrain. The neurons have typically survived for three to six weeks in vitro, while their parent explants and associated glia have been maintained for up to five months in culture. Several neuron-specific immunochemical markers, including neuron-specific enolase, neurofilament, and tetanus toxin binding, were used to verify the neuronal identity of the neuron-like cells in the culture outgrowth. Many of the neurons that were observed to migrate out of the adult explants did so as immature precursor cells, which then differentiated in vitro into neurons. By ^{3}H-thymidine autoradiography, it was determined that over 50% of the outgrowing neurons represented cells which had been born in vivo during the 72 hours prior to explantation. These new neurons were presumed to have been in the process of migration through HVc at the time the nucleus was put into culture. Accordingly, only forebrain areas displaying neurogenesis in vivo manifested neuronal outgrowth in vitro. Non-neurogenic control areas, including the cerebellum and optic tectum, did not display neuronal outgrowth in culture. These observations are indicative of the impressive degree of adult neuroplasticity intrinsic to the songbird forebrain, both as displayed in vivo, and retained in vitro.

195.4 BEHAVIORALLY CONTROLLED DIFFERENTIAL USE OF RESTRICTED HAND SURFACES INDUCE CHANGES IN THE CORTICAL REPRESENTATION OF THE HAND IN AREA 3b OF ADULT OWL MONKEYS. W.M. Jenkins, M.M. Merzenich and M.T. Ochs*. Coleman Mem. Lab., Depts. Otolaryngology and Physiology, UCSF, San Francisco, CA 94143.

Previous studies have revealed that the map of the hand surfaces within cortical areas 3b and 1 reorganize following restricted peripheral nerve transections and digit amputations in adult owl monkeys (Merzenich, M.M., Kaas, J.H.,Wall, J.T., Sur, M., Nelson, R.J., and Felleman, D.J., Neurosci. 8:33-55, 1983; Neurosci. 10:639-665, 1983; Merzenich, M.M., Nelson, R.J., Stryker, M.P., Cynader, M.S., Schoppmann, A., and Zook, J.M., J. Comp. Neurol. 224:591-605, 1984). On the basis of the details of these results, we have concluded that this cortical map dynamism manifests a normal capacity for alteration of cortical representations by use.

To test this hypothesis, several adult owl monkeys were trained to respond behaviorally for a food reward, to effect a high degree of differential stimulation of restricted skin surfaces of the hand. The apparatus consisted of a 12.5 cm disk with 10, 350μ deep wedge-shaped grooves. The disk was static, or was rotated at about 1 rev./sec. It was mounted so that a monkey could only contact it with the distal phalanges of one or two digits. Monkey's were trained to maintain continuous contact with the static or moving disk for about 15 sec. to obtain a food reward. The disk was continuously available in the home cage and provided the sole source of food. In experiments in which the disk was moving, this procedure resulted in about 2.5 hrs. of skin stimulation, about 180,000 edges moving across the contacted skin surface/day. After several weeks of heavy, repetitive hand use, detailed electrophysiological maps of the hand representation in area 3b were derived. These maps were compared: a) with maps derived in the same monkey before stimulation was initiated; b) with maps derived in the same monkey several months after the stimulation was terminated; and c) with maps derived in a series of normal, untrained monkeys. Highly significant alteration of cortical representational areas resulted from heavy, differential, behaviorally-controlled hand use. Concommitant with apparent cortical representational expansions recorded in these adult monkeys, receptive fields were significantly reduced in size.

These studies reveal that cortical maps within the somatosensory koniocortical field, area 3b, can be altered substantially by use in adult monkeys. Supported by NS10414 and HRI.

195.5 NEUROPHYSIOLOGICAL PLASTICITY IN THE CUNEATE NUCLEUS FOLLOWING DORSAL COLUMN LESION IN THE RAT. M. J. Rowinski and E. L. McGough*. Dept. Physical Therapy, Univ. of Wisconsin-La Crosse, La Crosse, WI 54601.

Specific neuroanatomical changes indicating synaptic rearrangements occur in the dorsal column nuclei of the rat within 30 days following spinal cord damage involving the dorsal columns (Bernstein and Ganchrow, Exp. Neurol. 71: 452, 1981). Behavioral deficits have been found to correlate with these anatomical changes, though the physiological mechanisms underlying this plasticity have not been established. The present study elucidates the neurophysiological nature of neuroplasticity in the cuneate nucleus subsequent to ipsilateral cuneate fasciculus transection at the C_1-C_3 level in the adult rat.

Ten to thirty days following lesion of the cuneate fasciculus, monopolar microstimulation within the ventral forepaw focus of the cuneate nucleus was used to evoke antidromic and orthodromic activity in the forepaw focus of the contralateral sensorimotor cortex of lesioned and non-lesioned sides. Early components of cortical evoked potentials were analyzed for differences in amplitude between lesioned and nonlesioned sides. In most cases, stimulus strength-response amplitude relations were obtained to determine that the electrode was localized within a zone of the nucleus in which response recruitment was demonstrable.

The results indicate that at low stimulus strengths ($\leq$ 100 μA) cortical response amplitudes recorded contralateral to the lesioned side were greater than those responses recorded contralateral to the nonlesioned sides. Response differences between the lesioned and non-lesioned sides were reversed at stimulus strengths exceeding 100-200 μA in the same animals, and stimulus-response relations were found to plateau at lower stimulus strengths for the lesion related side than those for the alternate side. It is like ly that only the lower stimulus range pertained to activation within the boundaries of the cuneate nucleus. Control animals did not exhibit such consistent differences between sides tested.

It is proposed that the cuneate nucleus deafferented by cuneate fasciculus lesion exhibits synaptic rearrangements which are more easily activated by focal direct electrical stimulation than are normally present. Physiological analysis of such changes suggests the possibility of increased density of corticofugal fiber terminals resulting from post-lesion sprouting phenomenon and/or the increased excitability of rostrally projecting cuneate neurons.

195.6 **SURVIVAL OF CATECHOLAMINERGIC CELLS IN ADRENAL MEDULLA AUTOGRAFTS TO THE PRIMATE BRAIN.** J.M. Morihisa, R.K. Nakamura, W.J. Freed, M. Mishkin and R.J. Wyatt. National Institute of Mental Health, Washington, D.C. 20032.

It has been demonstrated that both fetal substantia nigra and adrenal medulla tissue survive transplantation into the rat central nervous system. Furthermore, these tissue grafts act as biological sources of dopamine and improve behavioral abnormalities produced by experimental deprivation of the striatum of its dopaminergic innervation. This work has raised the possibility that catecholamine-containing tissue grafts might eventually be used as replacements for destroyed or damaged dopaminergic neurons in patients with Parkinson's disease. Before the method can be applied clinically, however, important technical and theoretical questions must be addressed. One essential requirement is the successful application of an analogous grafting procedure to a nonhuman primate. To investigate this potential therapeutic approach, fetal substantia nigra or host adrenal medulla were implanted into the denervated caudate nucleus of the rhesus monkey (Macaca mulatta). No fetal substantia nigra grafts survived in either of two animals tested. Some adrenal medulla tissue, however, survived for up to eight months. The grafted tissue contained catecholamines as demonstrated by the presence of cells with specific glyoxylic acid-induced catecholamine fluorescence. Two of the five animals with adrenal medulla grafts had fewer than 10 surviving cells. The other three animals had 190, 300 and 600 catecholamine fluorescent cells. Despite the limited cell survival, it is concluded that peripheral tissue autografts can survive implantation into the nonhuman primate central nervous system and retain the potential to provide a biological source of catecholamines to a denervated brain structure.

195.7 **INFLUENCE OF DONOR AGE ON THE BEHAVIORAL EFFECTS OF ADRENAL MEDULLA GRAFTS.** W.J. Freed and H.E. Cannon-Spoor*. NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032

Adrenal medulla can be removed from young adult rats and transplanted to the lateral ventricle. These grafts decrease apomorphine-induced rotation consequent to unilateral substantia nigra lesions. Intraventricular adrenal medulla grafts from one- or two-year-old donors were, however, behaviorally ineffective even though they survived and contained catecholamine-specific histochemical fluorescence (Freed, Biol. Psychiatry, 18:1205, 1983). The possibility of employing intraparenchymal adrenal medulla grafts has also been considered, and attempted in rhesus monkeys (cf. Morihisa et al., this volume) and human patients with Parkinson's disease. This procedure was therefore further investigated in rats.

Rats (n=46) with unilateral right substantia nigra lesions received grafts into six distributed sites in the striatum through a 20-ga. deflected-point needle. Adrenal medulla grafts were obtained from young (4-5 week old) or aging (22-24 month old) donors. Small sciatic nerve grafts were equivalent in volume to young adrenal medulla, and large sciatic nerve grafts were similar to aging adrenal medulla.

For nine animals that received small sciatic nerve grafts, rotation 5-9 weeks after transplantation was decreased by 10. ± 15% (means ± SEM), and for the young adrenal medulla graft group, rotation was decreased by 36 ± 7% (n=15). For the animals that received large sciatic nerve grafts the decrease was 30. ± 12% (n=7), while for the aging adrenal medulla graft group, rotation was increased by a mean of 11 ± 21% (n=15). These differences were not statistically significant ($F(3,42)=2.16$, $p=0.11$).

On the sensorimotor neglect test, the sciatic nerve group showed a slight deterioration in performance from baseline to two months after transplantation (median ± S.I.Q.R. decrease in total left score of 4.0 ± 2.8 units; baseline= 14, n=12). The young adrenal medulla graft group showed an decrease of only 0.5 ± 3.0 units (n=14) and the old adrenal medulla graft group showed an decrease of 1.0 ± 4.5 units (n=10). This difference was statistically significant ($p=0.044$, Kruskal-Wallis test). After five days, 800-2400 surviving catecholaminergic cells per animal were found.

Thus, whether grafted intraventricularly or intraparenchymally, only adrenal medulla grafts from young donors tended to decrease apomorphine-induced rotational behavior. Deficits in sensorimotor performance were, however, positively influenced by intraparenchymal adrenal medulla grafts obtained from both young and aging donors. Although the behavioral deficits were certainly not eliminated, these data are generally encouraging for the use of autologous adrenal medulla grafts to alleviate the behavioral consequences of destruction of the nigrostriatal dopamine system.

195.8 PLASTICITY OF TRANSMITTER EXPRESSION IN MATURE AND AGED SYMPATHETIC NEURONS IN CULTURE. I.B. Black and J.E. Adler. Division of Developmental Neurology, Cornell Univ. Med. Coll., N.Y., NY 10021.

Abundant evidence suggests that developing neurons are remarkably plastic, altering transmitter metabolism and even phenotypic expression in response to extracellular stimuli. It is presently unclear, however, whether transmitter plasticity is restricted to the developing neuron, or whether it persists during maturity. To examine the effect of age on transmitter mutability, the putative peptide neurotransmitter substance P (SP) was examined in the rat superior cervical ganglion. Explantation of ganglia from mature 6 month old rats resulted in a 10-fold rise in SP, reproducing results previously described in the neonatal ganglion. Veratridine prevented the increase in adult ganglia, while tetrodotoxin blocked the veratridine effect, suggesting that membrane depolarization and sodium influx prevent the rise in SP as in neonates. However, the time courses of the increase in the peptide differed in neonatal and mature ganglia, suggesting that some aspects of regulation may differ in ganglia of these two ages.

The effects of aging on neural plasticity was further analyzed by explanting ganglia from very old rats, aged 2 years. While basal levels of SP did not differ in mature and aged ganglia, the peptide failed to increase significantly in the aged ganglia. Our observations suggest that remarkable plasticity persists in mature neurons, but may be deficient in aged sympathetic neurons.

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195.9 ELECTROPHYSIOLOGICAL AND STATISTICAL STUDY OF THE MECHANISM OF LONG TERM FACILITATION AT THE CRAYFISH NEUROMUSCULAR JUNCTION. J.M. Wojtowicz and H.L. Atwood, Department of Physiology, University of Toronto, Toronto, Ontario Canada M5S 1A8

Repetitive stimulation of the crayfish opener motor axon leads to long term facilitation (LTF) of the excitatory post synaptic potential (EPSP). The facilitation can last for hours (Atwood et al. Brain Res. 100, p. 95, 1975) and it resembles long term potentiation reported for other preparations. We investigated this long lasting effect, using techniques of quantal analysis.

The EPSP amplitude was measured using test stimuli delivered at 5 Hz. LTF was produced by 20 Hz stimulation of a single excitatory axon over a 10 min period. At 60 min following the train, the amplitude of EPSPs was elevated from the initial 270 μV (S.E. = 40) to 470 μV (S.E. = 60) (n = 10) and remained at that level for as long as recordings could be maintained. (i.e. 4 hrs). The presynaptic membrane potential and action potential were also measured in axon terminals at 60 min after the train, but showed no significant changes.

Quantal analysis was performed on amplitudes of evoked EPSPs. This approach was justified since the muscle fibers from which recordings were obtained are nearly isopotential. The results showed that no significant change in the average amplitude of the miniature EPSPs occurred during LTF, while the quantal content of evoked EPSPs increased. In 3 out of 10 cases, the amplitude-frequency histograms had distinct peaks corresponding to multiples of the average miniature amplitudes. Further analysis of these favorable cases showed that the distribution of amplitudes was adequately described by a binominal model, using chi-square test as a measure of goodness of fit. According to this model, parameter n represents the number of release sites while p corresponds to the average probability of release of quantal units at a release site. LTF produced a change in the distributions of evoked EPSPs which was best described by an increased n rather than p. This suggests the possibility that new active synapses are appearing in the terminal boutons. Such a mechanism constitutes a novel form of neuronal plasticity which can lead to strengthening of synaptic pathways following periods of intense activity

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195.10 HOMOSYNAPTIC DEPRESSION (HD), LONG-LASTING POTENTIATION (LLP) AND ^{3}H-GLUTAMATE (^{3}H-GLU) ACCUMULATION IN RAT HIPPOCAMPAL SLICES. J. W. Goh* and B. R. Sastry, Neuroscience Research Laboratory, Department of Pharmacology & Therapeutics, The University of British Columbia, Vancouver, Canada, V6T 1W5.

Baudry and Lynch (Expt. Neurol. 68: 202, 1980) postulated that LLP of the hippocampal CA_1 population spike is due to an increase in the number of glu receptors. The Na^+-independent (presumed to be receptors) rather than Na^+-dependent (presumed to be related to the uptake system) amino acid binding is reportedly increased following tetanic stimulations and by Ca^{++} (Baudry and Lynch, Nature 248: 748, 1979). Recent studies in our laboratory, however, indicate that LLP is presynaptic (Sastry, Life Sci. 30: 2003, 1982; Sastry et al., Life Sci. 34: 1075, 1984). In the present investigation, we examined the accumulation of ^{3}H-glu into hippocampal slices in the presence of normal extracellular medium; we also determined the Na^+-independent ^{3}H-glu binding to hippocampal membranes 10 min after LLP-inducing (400 Hz, 200 pulses) and HD-inducing (20 Hz, 600 pulses) stimulations of Schaffer collaterals. The low frequency tetanus increased (107-280% of control; 7 of 8 groups of slices, each group has 5 slices), decreased (18-86% of control, 4 of 12) or unchanged (1 of 12) ^{3}H-glu accumulation into the slices. If verapamil (1 μM) was applied during the 20 Hz tetanus, ^{3}H-glu accumulation was decreased by 22.7-84.4% (compared with 20 Hz in absence of verapamil, 7 of 9). The LLP-inducing high Hz tetanus, however, consistently reduced ^{3}H-glu accumulation (24.5-88.6% of control, 7 of 8) and if the tetanus was given in the absence of Ca^{++} (0.5 mM Mn^{++}, 3.5 mM Mg^{++} added) the accumulation was further decreased (8 of 8). Na^+-independent ^{3}H-glu (100 nM) binding was increased (by 5-25% , 6 of 9) or unchanged (3 of 9) after the 20 Hz tetanus. In contrast the high frequency tetanus produced no increase in Na^+-independent ^{3}H-glu (100 nM, 4 of 4) binding.

Since the induction of LLP is generally thought to be Ca^{++}-dependent, it is unlikely that a reduction in glu uptake is responsible for this process. HD, however, appears to be associated with a verapamil sensitive increase in the amino acid uptake. The high affinity saturable Na^+-independent glu binding sites, which probably cannot contribute to synaptic transmission (concentrations of 100s of μM glu are needed to produce a response), do not seem to correlate with LLP.

195.11 FREQUENCY-DEPENDENT NORADRENERGIC MODULATION OF LONG-TERM SYNAPTIC POTENTIATION. W.F. Hopkins* and D. Johnston. Neurosci. Prog., Baylor Col. of Med., Houston, TX 77030.

Long-term synaptic potentiation (LTP) in the hippocampus is generally assumed to be a homosynaptic phenomenon, although this possibility has not been adequately tested. We have obtained evidence that bath applied norepinephrine (NE,1-10 μM) or the β-adrenergic agonist isoproterenol (1 μM) reversibly increase the duration, magnitude and probability of induction of LTP of evoked mossy fiber population EPSP's in the CA3 subfield of the in vitro rat hippocampal slice. In addition, these effects, as well as LTP itself, are blocked by the β-adrenergic antagonists propranolol or timolol.

Rat hippocampal slices were prepared using standard techniques. Field potentials were recorded from stratum lucidum and pyramidale of the CA3 subfield. The amplitude of the population EPSP relative to the pre-tetanus response was measured as a function of time after the high-frequency train.

NE, bath applied during high-frequency stimulation only, reversibly increased LTP duration, measured as response amplitude half-decay time, by a mean of 225%. The magnitude of LTP was reversibly increased by a mean of 92%. Isoproterenol had similar effects on LTP duration and magnitude. Neither drug had consistent nor marked effects on control input-output curves of the response amplitude vs. stimulus intensity. Also, response augmentation was never observed when either drug was bath applied during low-frequency (0.2 Hz) stimulation of the mossy fibers for 20-30 minute periods. Propranolol (10-100 nM) blocked these effects, and propranolol or timolol (100 nM) alone reversibly blocked LTP when either drug was bath applied.

NE or isoproterenol, when bath applied during a low-intensity conditioning train, reversibly enabled the induction of LTP under conditons in which the same low-intensity high-frequency stimulation alone did not.

These results suggest that NE, acting on β-adrenergic receptors, exerts long-term, frequency-dependent neuromodulation of synaptic transmission in the CA3 subfield. Furthermore, the possibility exists that tetanic stimulation results in the release of NE from surviving noradrenergic fibers, and that LTP is therefore a heterosynaptic phenomenon. This concept has implications both for the mechanism of LTP, as well as its intriguing associative properties. (Supported by NIH grants NS11535, 15772 and 18295 and a McKnight Development Award.)

195.12 THE ROLE OF EXTRACELLULAR GLYCOPROTEINS IN CNS PLASTICITY: CALCIUM EFFECTS ON POLYMERIZATION. V.E. Shashoua. Mailman Research Center, McLean Hospital, Harvard Medical School, Belmont, MA 02178

The brain extracellular fluid (ECF) contains a class of glycoproteins (ependymins) whose turnover rates are enhanced when goldfish or mice acquire a new pattern of behavior (Shashoua, V.E., Advances in Cellular Neurobiology, 3:97, 1982). This observation suggested that ECF proteins might be involved in some aspect of the CNS plasticity associated with the learning and led to an investigation of properties of goldfish ECF proteins and ependymins that might help define their functional role. Ependymins and other highly soluble ECF proteins were found to polymerize into insoluble fibrous aggregates when ECF calcium was decreased to below physiological levels (2.5 mM). Dialysis of solutions of ependymins at 4°C or the addition of the calcium chelating agent [ethylene glycol-bis-(β-aminoethyl ether)N,N'-tetra-acetate (EGTA)] resulted in the formation of fibrous aggregates. These were insoluble in boiling 2% SDS, 6 M urea and even in trifluoroacetic acid. This result suggests that the capacity to aggregate may be built into the molecular structure of the proteins. A study of the kinetics of the polymerization process indicated that critical concentrations of EGTA and glycoproteins were required. Such a process, if it occurs in vivo, may define a role for some of the proteins present in the brain extracellular space and assign a signaling function for calcium ions in the ECF. It suggests the hypothesis that at specific extracellular sites, where a transient depletion of Ca^{2+} occurs in the ECF, the soluble ependymins and probably other proteins become rapidly converted to an insoluble fibrous matrix.

Transient local decreases of extracellular Ca^{2+} levels have been observed in mammalian brain during stimulation (K. Krnjevic et al., Can. J. Physiol. Pharmacol., 60:1643, 1982). The fibrous matrix formed at such loci may then define where altered neural connections occur. (Supported by NIH Grant NS 09407.)

DEVELOPMENT AND PLASTICITY: VISUAL PATHWAYS

196.1 UNILATERAL SUPERIOR COLLICULAR LESIONS PRODUCE EXTENSIVE RECROSSING OF RETINOTECTAL AXONS. R.W. Rhoades, R.D. Mooney and B.G. Klein. Dept. of Anatomy, Univ. of Medicine and Dentistry of New Jersey, School of Osteopathic Medicine and Rutgers Medical School, Piscataway, NJ 08854.

Several previous studies in hamsters (eg. Schneider, G.E. Brain Behav. Evol. 8:73-109, 1973) have shown that ablation of one superior colliculus (SC) at birth results in a marked reorganization of the projection of the ipsilateral eye to the remaining SC. Tracing the retinocollicular pathway from this eye, primarily with degeneration techniques, revealed a recrossing of retinal axons which was limited to the medial part of the intact tectum. Additional studies showed that axons from the two eyes exhibited little or no overlap in the remaining SC.

Reexamination of this problem with more sensitive tracing techniques (anterograde transport of horseradish peroxidase-HRP and wheatgerm agglutinin-horseradish peroxidase-WGA-HRP) have provided somewhat different results. Hamsters received unilateral (right) SC ablations within 12 hr of birth by application of a heated insect pin to the skull overlying SC. Two-6 mon later the animals were used in one of 4 experiments: 1. Injection of HRP or WGA-HRP into the left eye. 2. Injection of HRP or WGA-HRP into the left eye and [^{3}H]-leucine into the right eye. 3. Injection of HRP or WGA-HRP into the left eye and enucleation of the right eye. 4. Transection of the left optic tract and injection of HRP or WGA-HRP into the left eye.

Experiments 1, 2 and 3 all demonstrated recrossing of retinal axons to innervate the intact SC. The innervation of the left SC by the left eye was, however, much more extensive than previously reported. In most cases labelling was visible throughout the rostrocaudal and mediolateral extents of the superficial laminae. Experiments 2 and 3 showed that axons from the two eyes were extensively intermingled in the left SC, but that fibers from the right eye tended to be excluded from regions where the left eye projection was heaviest. Experiment 4 demonstrated that both recrossing fibers and uncrossed axons which reach the left SC via the left optic tract contribute to the expanded ipsilateral projection observed in these animals.

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196.2 MORPHOLOGY OF REGENERATED OPTIC ARBORS IN GOLDFISH TECTUM. J.T. Schmidt, M.J. Buzzard* and J. Turcotte*. Dept. Biol. Sci., State Univ. of New York at Albany, N.Y., 12222.

The regenerated retinotectal projection is initially diffuse but sharpens via a selective stabilization of synaptic connections. The proposed mechanism utilizes the locally correlated activity of neighboring ganglion cells (Schmidt and Eisele, Neurosci. Abstr., 9:858, 1983), and depends upon the overlap of regenerated arbors to produce summation of EPSP's. Therefore we decided to examine the morphology of individual arbors to see if they are enlarged early in regeneration, providing a high degree of overlap as has been reported in the newt (Fujisawa et al., Dev. Biol. 90:43-57, 1982).

Micropipettes tipped with dried HRP were inserted into the optic tracts of 10-12 cm fish. Two to three days later the fish were perfused. The tecta were fixed and removed, reacted with o-dianisidine, cleared in methyl salicylate and mounted in Canada Balsam for light micrscopic examination. More than 50 normal and 50 regenerated arbors were completely drawn from tectal whole mounts using a drawing tube.

Optic arbors in normal goldfish tectum have been previously described as falling into 3 sizes (Stuermer, Neurosci. Abstr., 9:59, 1983): small (80x100 μm), large (150x 200 μm), and giant (200x300 μm). We find similar sizes but within a continuous range from 30x60 μm to 250x350 μm. Regenerated arbors could be filled as early as three weeks after nerve crush, and were much larger than normal in their extent although more sparsely branched. The largest were approximately 3 to 5 times larger than the largest normal arbors, being well over 1 mm across, and covering more than 1/3 of the tectal extent. The smallest regenerated fibers were 2 1/2 to 4 times larger than the smallest normal arbors. The largest arbors predominate at 3 weeks and may be the first to regenerate. Smaller arbors often had growth cone-like swellings at the tips of their branches. Arbors were still larger than normal at 8 weeks. At six months or longer, we traced 16 mature, regenerated arbors, and these were well within the normal range of sizes(from 50x90 um to 140x230 um). The large early arbors eventually shrink to the normal size. The morphology of individual arbors therefore reflects the sharpening of the retinotopic map recorded electrophysiologically, although the arbors take somewhat longer than expectd to reach their normal size. (Supported by NIH grant 03736 and a Sloan Foundation Fellowship to JTS).

196.3 NEW CELL LABELING TECHNIQUES APPLIED TO STUDIES OF REGULATION IN THE DEVELOPING EYEBUD. N. A. O'Rourke and S. E. Fraser. Depts of Developmental & Cell Biology and Physiology & Biophysics, University of California, Irvine, CA 92717.

Much of our knowledge of the regulative behavior affecting the positional information that guides optic nerve fibers comes from studies of the interactions between "mismatched" eyebud fragments in Xenopus laevis. In one such study, the temporal half of a right eyebud of a St 32 embryo is replaced with the temporal half of a left eyebud. These compound N_rT_l eyes possess a nasal half with normal dorso-ventral orientation and a temporal half with inverted dorso-ventral orientation. In several laboratories, including our own, visuotectal mapping of the projections of these compound eyes at adult stages has yielded both normal and double-nasal maps. In both cases, the dorso-ventral polarity of the projection from the temporal half-eye was normal instead of having the expected inverted pattern. The double-nasal map indicates an additional inversion of the naso-temporal axis of the temporal half-eye. These results have been taken as evidence of an early respecification of the positional information in the temporal eyebud. In a more recent study of eyebud fragments (Ide et al, Soc Neurosci Abs 9:761), cell movement and cell sorting have been suggested as mechanisms for regulative behavior in the eyebud. Applying this argument to the compound N_rT_l eyes, the apparent regulation could be explained by cell movements in which cells of the temporo-dorsal and temporo-ventral quadrants of the eyebud exchange positions.

We have used vital staining with the fluorescent marker, Texas Red to address the possibility of cell movement. Texas Red binds covalently to the cell surface, is later internalized, and remains visible for several days. N_rT_l compound eyes were constructed from a donor eyebud in which the dorsal half of the left eye had been labeled at St 24-25, producing a compound eye with a labeled temporo-ventral quadrant. Observation both in situ and in section indicates that the labeled cells maintain their original position in the graft up to St 45. Therefore, early cell movement and cell sorting would not explain this particular regulatory interaction in the eyebud, although cell movements occuring later in development may contribute to the final pattern observed.

The possibility of respecification of positional information in the eyebud remains. We have investigated whether the original cells in the N_rT_l compound eyes are respecified by marking the cell bodies and axons of the graft with a modification of the Holt & Harris (Nature 301:150) labeling technique. Preliminary results indicate that the cells project to their original target region in the tectum instead of the region expected from the adult pattern of the projection. It appears that regulatory behavior does not become manifest until later stages of development. (NSF 80-23638).

196.4 ALTERATIONS IN THE RETINOTECTAL MAP OF XENOPUS BY ANTIBODIES TO NEURAL CELL ADHESION MOLECULES. S. E. Fraser, B. A. Murray*, C.-M. Chuong and G. M. Edelman. Dept of Physiology & Biophysics, University of California, Irvine, CA 92717, and The Rockefeller University, New York, NY 10021.

The neural cell adhesion molecule (N-CAM) mediates neuron-neuron adhesion, is ubiquitous in the nervous system of developing and mature vertebrates, and undergoes major alterations in both amount and distribution during development. Perturbation of homophilic (N-CAM to N-CAM) binding by univalent fragments of specific anti-N-CAM antibodies has previously been found to alter neural tissue patterns in vitro. Because of the parallels between the known chemistry of N-CAM and the qualities of cell-surface markers proposed in a model of nerve patterning in the retinotectal system (Fraser, Dev. Biol. 79:453), we have investigated the effects of blocking N-CAM mediated adhesion in the Xenopus retinotectal system in vivo.

Antibodies to Xenopus N-CAM were embedded into agarose microcylinders and implanted into the tectal neuropil of juvenile Xenopus laevis frogs. Both normal animals and animals in the midst of regenerating their retinotectal projection (3 weeks after optic nerve crush) were used as hosts for the implants. One week after implantation, the effects of the antibodies on the retinotectal projection pattern were determined by extracellular electrophysiological techniques. Frogs implanted with polyclonal or monoclonal antibodies to Xenopus N-CAM showed both a distortion in the pattern of the projection and an enlargement of the multi-unit receptive fields recorded in the tectum. The receptive fields were nearly doubled in size in comparison with control animals ($p<0.01$-0.001; control animals implanted with agarose alone, pre-immune serum or antibodies directed against chicken L-CAM). Since there was no increase in the single-unit receptive field size in these animals, the increase in the multi-unit receptive field size indicates a decreased precision in the ordering of the retinotectal projection. The results for normal animals and animals regenerating the retinotectal projections were very similar, with the normal animals showing a slightly less pronounced enlargement of the multi-unit receptive field sizes. Time course studies indicate that the effects of the implanted antibodies reach their maximum at seven to nine days after implantation; the pattern and the precision of the projection return to normal over the next few weeks. The results suggest that neuronal adhesion mediated by N-CAM is important in establishing and maintaining the precision and topography of neural patterns. Supported by NSF (BNS-8023638) and NIH (HD-09635, AM-04256, AI-11378).

196.5 PHYSIOLOGICAL RESPONSES IN RETINAL TRANSPLANTS AND HOST TECTA EVOKED BY ELECTRICAL OR PHOTIC STIMULATION OF TRANSPLANTED EMBRYONIC RETINAE. D.J. Simons and R.D. Lund. Dept. of Physiology and Dept. of Anatomy and Cell Biology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

Retinae taken from embryonic rats and transplanted over the tectum of neonates mature and form connections with the host superior colliculus and other subcortical visual centers. Electrophysioloigcal recordings were used to determine if these transplants respond physiologically to light stimuli and if they can effect functional activity of neurons in the host tectum.

Retinae from E14 embryos were transplanted over the left tectum of newborn albino rats. The host right eye was removed at the same time. After 1-2 months survival the transplant and superior colliculus were exposed, and the remaining host eye was covered or removed one day before the recording experiments. Microelectrodes were used to examine field potentials and/or unit responses elicited by electrical or photic stimulation of the transplants.

Slow-wave evoked potentials were recorded from the transplants in response to brief flashes of light. Response amplitudes (to a maximum of 150 uv) varied directly as a function of light intensity, and the time course of the potentials depended on the duration of the light flash (from 50-100 msec). These field potentials were similar in form to ERG's recorded from normal, intact eyes. Units recorded from innervated host tecta were activated by low, but not high, frequency electrical stimulation of the transplants (5-10/s). Discharge patterns of tectal units were altered by illumination of the transplants with ambient light and with light flashes. Both "on" and "off" responses were observed; the precise nature of the responses depended on stimulus duration. Units also responded to dark edges moving across the light field illuminating the transplant.

These findings demonstrate that transplants can mediate functional activity in a host brain in response to natural stimuli.

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196.6 THE EFFECTS OF VISUAL DEPRIVATION ON THE RESPONSE PROPERTIES OF SINGLE UNITS IN THE CAT ACCESSORY OPTIC SYSTEM. Keith L. Grasse and Max S. Cynader. Dept of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

Abnormal visual experience during the first few months of life produces pronounced alterations in cellular responsivity in many different parts of the visual pathway. Neurons in the visual cortex are especially susceptible to the effects of selective rearing conditions such as monocular deprivation and dark rearing. The concommitant abnormalities in cortical output which result from these manipulations may also have profound consequences for subcortical visual structures which receive a substantial cortical afference. For example, Hoffmann and co-workers have shown that cells in the nucleus of the optic tract (NOT) of the pretectum also show a decrease in binocular responsivity and in addition, NOT cells in decorticate cats prefer much slower stimulus velocities than normal animals.

Previous work from this laboratory has demonstrated that lesions of the visual cortex lead to changes in the responses of cells in the lateral (LTN) and dorsal (DTN) terminal nuclei of the cat accessory optic system (AOS) which are similar to those observed in the NOT. Data obtained from both normal and decorticate animals strongly suggests that the visual cortex provides the LTN and DTN with ipsilateral eye input, high velocity responses, and upward direction selectivity.

We have undertaken a series of experiments designed to examine the function of LTN and DTN cells in dark-reared and monocularly deprived cats. The data indicate that 95% of all LTN and DTN cells in the dark reared and monocularly deprived cat respond only to stimulation via the contralateral eye. In addition, there is a shift in the distribution of preferred velocities toward slow stimulus speeds (approximately 1.0 deg/sec) and, in the LTN, there is a substantial decrease in the incidence of upward direction selective units. Similar results are observed in the responses of AOS neurons in monocularly deprived cats with an increase in the number of monocularly driven cells and a preference for low stimulus velocities. These data support the notion that early visual deprivation reduces or completely abolishes cortical influence upon AOS cells.

196.7 IMMUNOCHEMICAL HETEROGENEITY AND FASCICULAR ORGANIZATION OF EMBRYONIC OPTIC NERVE FIBERS.
S. C. Fujita* and K. Obata. Dept. of Pharmacol., Univ. of Gunma Sch. of Med., Maebashi 371, Japan

To explore the chemical basis of precise retinotectal projection, a library of 60 monoclonal antibodies (MAbs) was generated using mice immunized against homogenates of optic nerves from 8 day chick embryos. Screening for MAbs was achieved by indirect fluorescence immunohistochemistry on frozen sections of embryonic nervous system fixed in 3.5% formaldehyde/PBS and cryoprotected in 30% sucrose. A majority of the MAbs obtained exhibited restricted or preferential staining of specific regions in the embryonic spinal funiculi (Fujita & Obata, Neurosci. Res. in press) or of layers in the cerebellum (Obata & Fujita, ibid).

When tested on cross sections of the developing optic nerves, some stained the optic fibers more or less uniformly, e.g. MAb 95H2. But a number of MAbs revealed differential distribution of respective immunoreactivities across the nerve. This was most apparent in the 8 day embryo, in whose optic nerve the number of optic fibers is increasing most rapidly. MAb 82E10 stained the fibers in the central and ventral regions of the nerve. Immunoblot analysis indicated that this MAb is specific to the medium and large subunits of neurofilament. On the other hand, MAb 87C7 stained the dorsal region most intensely. MAb 87D10 immunoreactivity was in the central region with the ventral region and the dorsal margin negative. The boundary of the stained regions were graded rather than sharp.

MAb 84F9, which stained epithelial basement membrane, pia mater and inner limiting membrane of retina, stained the outer limiting membrane of the 8 day optic nerve. After 10th day 84F9-positive membraneous structure apparently invaginated into the nerve, partitioning the fascicles into sheet-like structures. In the mature adult optic nerve, MAb 82E10 stained presumably all fibers, while 87D10 immunoreactivity had disappeared. MAb 87C7-positive fibers tended to be locally clustered within the "sheets" delineated by MAb 84F9.

These results show the occurance of immunochemically heterogeneous nerve fibers in the developing chick optic nerve.

196.8 INITIAL DEVELOPMENT OF THE RETINOTECTAL PROJECTION IN XENOPUS: AN EXAMINATION OF RETINAL GANGLION CELL TERMINAL ARBORIZATIONS. D.S. Sakaguchi and R.K. Murphey. Neurobiol. Res. Center, Dept. of Biol. Sci., SUNYA, Albany, N.Y.

A description of the time course and pattern of development of the retinotectal projection was carried out with special reference to the examination of the morphology of the young retinal afferents in Xenopus frogs. A technique was developed for staining a small number of ganglion cells, thus permitting the elucidation of their terminal arbors in the brain. The experimental preparation consisted of an animal restrained on its side and the neural retina exposed following removal of the sclera, lens and vitreous. The dye, hexamminocobaltic chloride, was injected into the retinae of late stage embryos and young tadpoles (St 35-50; Nieuwkoop and Faber, 1956). The cobalt was then precipitated and intensified according to the Timm's method to reveal the pattern of the developing retinal projection in the brain. In 18% of the successful injections, single retinal ganglion cells were stained using this technique. Analysis of wholemounts, and the subsequent sectioning of selected brains, revealed details of the retinal projection.

The earliest retinal ganglion cell axons to reach the tectum generally took direct routes to their appropriate regions of termination. Injections into dorsal retina labelled axons terminating ventrolaterally and injections into ventral retina stained axons ending dorsomedially. The terminal portions of these axons were usually simple in appearance and often ended in growth cones.

By stage 39, retinal axons began taking on the appearance of more mature terminal arbors. The arborizations of most ganglion cells tended to be elongated along the rostral-to-caudal dimension of the tectum and were restricted along the dorsal-to-ventral axis. Terminal arbors covered approximately 75% of the available rostral-to-caudal tectal neuropil during early larval life (St 40-45). During mid-larval stages (46-50), the terminal arbors covered a smaller region (40-50%) of the rostral-to-caudal extent of the neuropil. Thus, the early projection was characterized by a considerable degree of coverage by terminal arbors with respect to the tectal neuropil. During subsequent larval stages, the terminal arbors were seen to cover a relatively smaller region of the neuropil. Comparative measurements of the terminal arbors with respect to the tectal neuropil suggest that the change in coverage was due primarily to tectal growth and not to arbor shrinkage or pruning.
Supported by NSF Grant BNS 8317929.

196.9 NEURON COUNTS AND SYNAPSES/NEURON RATIOS DURING POSTNATAL DEVELOPMENT OF MONKEY LATERAL GENICULATE NUCLEUS. Pedro Pasik, Tauba Pasik and Gay R. Holstein. Depts. Neurol. and Anat., Mount Sinai Sch. Med., CUNY, New York, N.Y. 10029.

Neuron densities in magnocellular (L1) and parvocellular (L6) laminae were determined using randomized and coded sections of resin-embedded material from newborn, 1, 4, 8 and 17 week old monkeys (M. mulatta). The number of neuronal nucleoli was counted, and the area of the sections measured at 400X magnification. Exact thickness was determined by the Jamin-Lebedeff interference microscopy method which utilized the difference between the paths of monochromatic light through the specimen and air, divided by the refractive index of the resin minus one. The latter index was obtained by the Becke line method. The sum of section thickness and maximum nucleolar diameter gave the effective thickness, which was used to calculate tissue volume. The lengths of synapses made by retinal elements in L1 and L6 had previously been measured in electron micrographs of material from the same animals, and used to calculate synaptic densities by a stereologic method. The ratio of synapse density to neuron density provided an index of relative changes in the presynaptic and postsynaptic populations during development.

The ratio decreases substantially during the first postnatal week from similar neonatal peaks in both laminar regions. Subsequently, the number of synapses/neuron in L1 increases somewhat, and stabilizes by eight weeks, while the ratio in L6 continues to decline throughout the first two months. Estimates of neuronal populations, derived from the density values and previously determined aggregate volumes of corresponding laminae, show a 39% decrease in the magnocellular component, from 0.1 $x10^6$ at birth to 0.06 $x10^6$ at 17 weeks. In contrast, the number in the parvocellular division is estimated to be 0.9 $x10^6$ at birth, increasing 49% during the first postnatal month, and reaching 1.2 $x10^6$ by 17 weeks.

The declining synapses/neuron ratios indicate that synapse elimination occurs primarily at the expense of presynaptic elements, which concurs with a previous finding of marked losses in retinal boutons during the same period. The earlier stabilization in L1 may be due to simultaneous decreases in both presynaptic and postsynaptic components, and indeed, a reduction in neuron number is seen in these laminae. The significance of the higher neuron counts obtained in L6 over the first month is uncertain, since no mitotic figures are visible. This lack of evidence for postnatal neurogenesis forces us to conclude that some nucleoli, and consequently their neurons, are not fully differentiated in younger animals, and, therefore, remain uncounted.

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196.10 REARING WITH EXOTROPIA OR ESOTROPIA: EFFECTS ON CELL SIZE IN THE LATERAL GENICULATE NUCLEUS (LGN) OF THE CAT. R.E. Kalil. Dept. of Ophthalmology, Univ. of Wisconsin, Madison, WI 53706.

Recent electrophysiological studies in the cat (Kalil, Spear and Langsetmo, J. Neurophysiol. 1984) show that the effects of rearing with exotropia or esotropia are not equivalent when the ability of the deviated eye to drive cortical cells in the ipsilateral hemisphere is examined. In exotropes the deviated eye drives the same percentage of cells in the ipsilateral hemisphere as at other cortical locations, but in esotropes the deviated eye is markedly deficient in driving neurons that represent the peripheral part of the nasal visual field.

In an effort to clarify this specific cortical deficit, LGN cells in strabismic cats were measured to determine if neurons that receive input from the temporal retina of the deviated eye show changes in cell body size related to the differential effects of exotropia and esotropia on cortical physiology. Cell sizes were measured in lamina A1 ipsilateral and contralateral to the strabismic eye in cats reared to adulthood with an esotropia or exotropia that was produced surgically at the time of natural eye opening. The cross-sectional areas of LGN cells were sampled at two locations in lamina A1: (a) near the medial edge of the lamina where the central visual field is represented and (b) near the lateral border where the representation of the peripheral nasal visual field is mapped.

In exotropes, cells in the lateral part of A1 were, on average, smaller in size (about 9%) than cells in the medial part of A1. This difference in cell size was found in lamina A1 ipsilateral or contralateral to the strabismic eye. Similar medio-lateral differences in cell size are seen sometimes in normal cats. In esotropes, medial and lateral A1 cells showed no difference in size in the LGN contralateral to the converged eye. However, in lamina A1 ipsilateral to the esotropic eye, lateral A1 cells were, on average, 26% smaller than medial A1 cells.

These anatomical results agree with the cortical physiology mentioned above insofar as a specific reduction in LGN cell size can be demonstrated in the pathway from the temporal retina of the deviated eye in esotropes but not in exotropes. However, this change in LGN cell size is not accompanied by a corresponding loss in cell responsiveness (Jones, Kalil and Spear, J. Neurophysiol. 1984). This suggests that the reduction in LGN cell size in esotropes may contribute to the cortical deficit via a corresponding change in geniculocortical connectivity.

196.11 OBSERVATIONS ON THE DEVELOPMENT OF THE CAT'S RETINOGENICULATE PATHWAY IN THE ABSENCE OF BINOCULAR INTERACTIONS.
D.W.Sretavan,M.Kliot and C.J.Shatz.Dept.of Neurobiology,Stanford Univ.Sch. of Med.,Stanford,CA 94305.

We have studied developmental interactions between axons from the two eyes at the optic chiasm (OC) and within the lateral geniculate nucleus (LGN) by examining the retinogeniculate projection from the remaining eye after early monocular enucleation. Cat fetuses (6 normally pigmented,2 Siamese) were monocularly enucleated at embryonic day 23 (E23;gestation=65),a time when retinal axons have yet to reach the OC (Silver et al.,in prep.),and examined at E59 when the axons would normally have formed well segregated layers within the LGN.

In all fetuses,autoradiography after an eye injection of ^{3}H-leucine showed an ipsilateral projection to the LGN,indicating that formation of an uncrossed pathway need not involve the presence of axons from both eyes at the OC. Although the ipsilateral projection zone in the LGN definitely filled area normally occupied by the contralateral eye,it did not completely fill the nucleus. The contralateral projection also extended into areas normally occupied by the ipsilateral eye;however the density of labelling was not uniform,with the region of future layer A1 frequently more lightly labelled. LGN arborizations of axons were filled at E59 using an *in vitro* HRP method. So far,reconstruction and measurements of arbors from the remaining eye in one enucleated (non-Siamese) fetus show that at least some arbors are remarkably similar in shape and in total linear length to those of normal fetuses (enucleates 2550±1220um, n=5;controls 2520±810um, n=10) although trajectories taken by these axons in the LGN are unusual. We examined in the same fetus the retinal whole mount and the optic nerve. The appearance of the retinal ganglion cells,their distribution and peak density were very similar to normal fetuses indicating that differentiation and maturation of the retina is qualitatively normal. An electron microscope count of axons in the optic nerve gave approximately 2.3×10^{5} axons. This is very comparable to a control nerve count of 2.6×10^{5} and to estimates of Ng and Stone (Dev.Brain Res.,5:263,1982).

These preliminary observations suggest that features in the development of the retinofugal pathway such as formation of an ipsilateral pathway,the massive prenatal reduction in axon number in the optic nerve and the formation of normally restricted arbors within the LGN can occur in the absence of binocular interactions. It remains to be seen whether other aspects such as topography are normal and whether postnatal influences may further modify the pattern seen in monocularly enucleated animals.

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RECEPTOR MODULATION I

197.1 ELIMINATION OF GUANINE NUCLEOTIDE SENSITIVITY OF MUSCARINIC ACETYLCHOLINE RECEPTORS FROM RAT BRAINSTEM BY ENDOGENOUS PROTEOLYTIC ACTIVITY.
R.S. Aronstam and L.M. Greenbaum*. Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912.

Muscarinic acetylcholine receptors in the central nervous system display multiple affinities for receptor agonists. At least part of this heterogeneity reflects interactions between the binding subunit and different regulatory or effector structures in synaptic membranes. Guanine nucleotides lower the affinity of muscarinic receptors in brainstem for agonists, presumably by affecting the coupling of the binding site subunit with a guanine nucleotide-dependent regulatory protein. Such proteins mediate receptor activation or inhibition of metabolic processes in postsynaptic cells in a variety of systems.

Carbamylcholine binding to membranes prepared from rat brainstem (medulla-pons-midbrain) was determined in competition studies with 0.1 nM [^{3}H]methylscopolamine ([^{3}H]MS). In the presence of 10 uM 5'-guanylylimidodiphosphate (a stable GTP analog; Gpp(NH)p), carbamylcholine binding affinity was reduced 12-16 fold. Incubating brainstem membranes at 37°C for 30 min did not alter carbamylcholine binding affinity; however, the ability of Gpp(NH)p to depress carbamylcholine binding was abolished. This loss of sensitivity could be prevented by pretreating the membranes with any of a number of proteinase inhibitors, including the serine proteinase inhibitors phenylmethylsulfonyl fluoride, diisopropylfluorophosphate and aprotinin, and a carboxyl proteinase inhibitor, pepstatin. Bestatin (an aminoproteinase inhibitor) and leupeptin (a thiol proteinase inhibitor) were inactive in this regard.

A loss of the guanine nucleotide sensitivity of muscarinic receptors has been reported by others after partial tryptic digestion, heat treatment, urea treatment, and exposure to media of high pH. In each of these cases, loss of guanine nucleotide sensitivity was accompanied by a lowering of agonist affinity. In the present work, there was no alteration in carbamylcholine affinity, suggesting a different mode of inactivation. With respect to currently proposed modes of muscarinic receptor organization and function, we suggest that an endogenous proteolytic activity in brainstem membranes inactivates the nucleotide regulatory protein without engendering its dissociation from the receptor binding subunit.

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197.2 ATYPICAL CONTROL OF BETA-RECEPTORS.
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Rats exposed to forty minutes of intermittent inescapable shock will develop a transient learning deficit which can be reversed specifically by a number of antidepressant treatments (Sherman and Petty, 1980). We have found that hippocampal beta-adrenoreceptors are elevated in rats with the learning deficit as compared to animals exposed to identical shock conditions with no response deficit. This biochemical change induced by environmental manipulations was reversed upon treatment with the antidepressant imipramine, showing a correlation between a behavioral effect and hippocampal beta-receptors. Thus, we were interested in the generality of the correlation as it applies to novel antidepressants.

Rats were subjected to a forty minute training session and tested in an escape response task twenty-four hours later. Thirty percent of these animals were response deficient, and thirty percent operated the task at control levels. Mianserin (10 mg/kg) and an analog, 6-azamianserin (2.5 mg/kg), were administered IP for four days and the rats were retested. Mianserin, a proven tetracyclic antidepressant was effective in reversing the escape reponse deficit ($p<.01$), while 6-azamianserin (which lacks NE-uptake inhibition) was ineffective. Subsequently, hippocampal beta-receptor assays were performed on those animals receiving mianserin. Preliminary work indicates that mianserin lowers the upregulation of the beta-receptor in response deficient animals, while causing no change in the hippocampal beta-receptor of nondeficient animals.

Mianserin has previously been shown not to downregulate the beta-adrenoreceptor in behaviorally naive rats (Mishra, 1980). The present study indicates that the upregulated receptor seen in our animal model may be either; 1) more sensitive to presynaptic influences or 2) under exquisite control of a postsynaptic regulatory process. Additionally, atypical antidepressants may have an exclusive effect on a pathologic transmitter system and exhibit no control over receptors in a normal, naive animal.

197.3 **REGULATION OF SUBTYPES OF RENAL BETA-ADRENERGIC RECEPTORS.** David M. Robinson* and Barry B. Wolfe (SPON: N. Krieger). Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

The densities of both beta-adrenergic receptor subtypes have been shown to decrease after chronic in vivo administration of agonists. β-Adrenergic receptors were visualized and quantified by incubating 10 μm thick sections of rat kidney with (^{125}I)-iodopindolol for 40 minutes in the absence and presence of various adrenergic agents. The resulting labelled sections were opposed to LKB Ultrofilm for 72 hours and then developed. Using quantitative autoradiography with computer assisted densitometry the concentration of (^{125}I)-iodopindolol binding sites was analyzed on sections of kidney from control rats and rats chronically treated with either epinephrine (EPI) or norepinephrine (NE). The binding was localized, saturable, reversible, and stereospecific. After subcutaneously implanting osmotic minipumps to deliver EPI (452 nmoles/kg/h) or NE (444 nmoles/kg/h) for 7 days, the pumps were removed and after 12 hours the animals were sacrificed and kidneys sectioned. The density of β-adrenergic receptors was determined from Scatchard analysis of (^{125}I)-iodopindolol binding to slide mounted kidney sections that contained both medulla and cortex and revealed a K_d value of 79 pM in each group. There was a decrease in βmax from 8.30 ± .92 fmoles/mg protein for controls to 5.16 ± .40 fmoles/mg for EPI and 3.72 ± .66 fmoles/mg for NE ($p < .01$). In addition, using autoradiography the density of β-adrenergic receptors associated with glomeruli and the juxtaglomerular apparatus was found to be decreased (EPI: 42 ± 1.8%; NE: 46 ± 3.0%; EPI or NE vs control; $p < .01$). β-Adrenergic receptor subtypes were visualized by using the selective antagonists ICI 118,551 (β_2 selective) and ICI 89,406 (β_1 selective). Analysis of β-receptor subtypes in the glomeruli from these kidneys showed striking differences. Treatment with EPI caused a 42 ± 1.8% decrease in β_1-receptors and a 66 ± 4.8% decrease in β_2-receptors while NE caused a 61 ± 3.3% decrease in β_1-receptors and a 25 ± 8.8% decrease in β_2-receptors (EPI vs NE $p < .01$). In areas not associated with glomeruli and the juxtaglomerular apparatus, regulation of β_2-receptors was similar to that seen in the glomerular region (EPI 70% ± 2.6, NE 22% ± 6.1 loss of receptors) but the regulation of β_1-receptors was less than that of glomeruli (EPI 30% ± 5, NE 48% ± 8.0). Thus, administration of pathologically relevant doses of catecholamines results in selective alterations in the density of renal β-adrenergic receptor subtypes. These differences in receptor regulation by catecholamines may produce differences in the expression of agonist stimulation of adenylate cyclase and of renin release.

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197.4 ^{3}H-MIANSERIN AND ^{3}H-KETANSERIN BIND TO DISTINCT RECOGNITION SITES. O. Gandolfi, M.L. Barbaccia and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

In minces prepared from the frontal cortex of rats receiving ketanserin (10 mg/kg i.p.) or mianserin (5 mg/kg i.p.) twice daily for 21 days the amplification of the cAMP (cyclic AMP) generating system stimulated by NE is reduced suggesting that repeated injections of ketanserin and mianserin, similarly to other antidepressants, down regulate the β-adrenoreceptor signal amplification caused by specific agonists.

In crude synaptic membranes prepared from rat brain homogenates the sites occupied by ^{3}H-spiroperidol that are displaced by μM concentrations of serotonin (5HT) have been defined operationally as $5HT_2$ receptors. Since the ^{3}H-spiroperidol displaced by 5HT is also displaced in nM range by ketanserin and mianserin it was suggested that spiroperidol, mianserin and ketanserin are labeling the same recognition site.

In the present study we found that in crude synaptic membranes from the frontal cortex of rats receiving ketanserin twice daily for one week or longer the density (Bmax) of $5HT_2$ recognition sites labelled by ^{3}H-ketanserin is decreased. Moreover this drug given twice daily for three weeks fails to change the characteristics of ^{3}H-mianserin specific binding when the radioligand assay is run in the presence of a H_1 receptor blocker. In contrast, a significant decrease in the Bmax of ^{3}H-ketanserin binding is elicited by a single injection of mianserin which fails to regulate its own binding following unless two daily injections are given for three weeks.

It is concluded that ^{3}H-ketanserin and ^{3}H-mianserin bind to two different recognition sites. The possibility that $5HT_2$ and $5HT_1$ recognition sites are functionally related and that the serotonergic synapses are modulated by multiple chemical signals is considered.

197.5 RAPID DOWN-REGULATION OF S_2-SEROTONIN RECEPTORS BY ANTIDEPRESSANTS : NORADRENERGIC - SEROTONERGIC INTERACTIONS. D.M. Helmeste and S.W. Tang, Psychopharmacology Dept., Clarke Institute of Psychiatry, Toronto, Ontario, Canada M5T-1R8.

Recent studies show that some antidepressants (mianserin) and neuroleptics acutely decrease cortical S_2-serotonin receptor densities. The mechanism of this unusual action is unclear but may represent an indirect interaction through another neurotransmitter site. A good candidate is a noradrenergic(NE) site since yohimbine(α_2 receptor antagonist) accelerates S_2 down-regulation by the antidepressant desipramine (DMI). Accordingly, mianserin may acutely decrease S_2 binding because it also possesses potent α_2 antagonistic action. The stereoselectivity of mianserin's action would help determine the role of α_2 sites since (+) and (-) mianserin have very different potencies on these sites. (+)Mianserin is potent on α_2 receptors mediating inhibition of NE release; the (-)-enantiomer is virtually inactive on NE release, but equipotent on the α_2 site mediating inhibition of serotonin (5-HT) release. In contrast, (+)mianserin is only ten times more potent than the (-)enantiomer on S_2 receptors. Administration of (+), (-) or racemic (±) mianserin (10 mg/kg i.p., 48h before, n=5-6) decreased S_2 binding (0.5 nM ^{3}H-ketanserin, 1 μM methysergide baseline) as follows: Control = 204 ± 10 fmol/mg prot.; (±) = 124 ± 8 (60% of control); (-) = 117 ± 17 (57%); (+) = 67 ± 17 (33%). (+)Mianserin caused a further reduction in binding of 45% compared to the (±) and (-)mianserin groups, which were equipotent. These results are not consistent with an α_2 adrenergic site of action, but rather parallel the stereoselectivity of mianserin for the S_2 site.

In a second set of experiments, the role of 5-HT release on yohimbine's acceleration of DMI-induced S_2 down-regulation was examined. Treatment (days 3-6 of expt.) with yohimbine(Y) (5 mg/kg b.i.d.) plus DMI (10 mg/kg once daily) decreased S_2 binding (0.5 nM ^{3}H-ketanserin, 1 μM methysergide baseline) by 38% compared to DMI alone [DMI + Y = 83 ± 4 fmol/mg prot.; DMI = 134 ± 7]. PCPA (400 mg/kg once daily, days 1-6) which depleted cortical 5-HT content by more than 94%, did not inhibit the yohimbine effect [PCPA + DMI + Y = 88 ± 6; PCPA + DMI = 128 ± 8; n = 6-10; 32% decrease].

In summary, yohimbine's acceleration of DMI-induced S_2 down-regulation is not dependent on 5-HT release since PCPA did not inhibit this action. This suggests that yohimbine may act indirectly through NE neurons. However, mianserin's ability to acutely decrease S_2 binding may not be dependent on α_2 receptor action since its stereoselectivity did not parallel α_2 adrenergic affinities.

197.6 EFFECT OF APOMORPHINE UPON SPONTANEOUS AND POTASSIUM STIMULATED RELEASE OF ENDOGENOUS DOPAMINE FROM SUPERFUSED MALE RAT CORPUS STRIATUM M.R. Carter* and V.D. Ramirez, Dept. of Physiology and Biophysics, Univ. of Illinois, Urbana, IL 61801

The effect of in vitro long-term (LT, 2 h pre-K^+) infusion using three doses of apomorphine (APO, 0.01, 0.1, and 1 uM) and a short-term (ST, 20 min pre-K^+) infusion of 1 uM APO upon spontaneous and K^+-stimulated release of dopamine (DA) from corpus striatum (CS) from male rats was investigated. CS fragments of one animal were placed in a superfusion chamber and perfused with modified Krebs-Ringer phosphate (KRP) medium, pH 7.4. Following a 60 min equilibration period and 6 collection intervals (10 min/interval), the KRP medium was changed to one containing 30 mM KCl for a 20 min period. Infusion of medium containing APO in the LT conditions started immediately following CS dissection and continued until the end of collection (170 min total). In the ST condition, APO infusion started 20 min prior to K^+ and continued for a total of 70 min. At the conclusion of the experiment, DA content in the CS fragments and perfusates were assayed using a radioenzymatic method. Basal DA release levels for all conditions were not significantly different. As expected, control superfused CS fragments responded to K^+-stimulation with a marked release of DA (219 ± 59 pg/mg, n=5, peak - basal release). In the LT infusions, 0.01 uM APO did not affect K^+-evoked DA release (246 ± 45 pg/mg, n=4). The 0.1 uM APO, however, effectively inhibited K^+-stimulated release of DA (99 ± 10 pg/mg, n=5). Interestingly, the 1 uM APO did not significantly inhibit K^+-evoked DA release (206 ± 48 pg/mg, n=5). In contrast, infusion of the same dose for only a ST period was very effective in inhibiting K^+-evoked DA release (61 ± 30 pg/mg, n=4). In addition to these effects, all doses of APO significantly decreased the latency between K^+ infusion and maximal K^+-evoked DA release. Thus, depending on the dose and time of infusion, in vitro APO can either be effective or ineffective in inhibiting K^+-evoked DA release. These data suggest that a relatively rapid down regulation of DA autoreceptors can occur in response to LT infusion of a high dose of APO.

197.7 ESTROGEN-INDUCED CHANGES IN D2 RECEPTOR AGONIST AFFINITY STATES. J.K. Clopton* and J.H. Gordon. Dept. of Pharmacology, Univ. Hlth. Scis./The Chicago Med. Sch., N. Chicago, IL 60064.

Administration of estrogen to ovariectomized rats results in a biphasic change in striatal dopamine receptor sensitivity, characterized by a striatal dopamin receptor hyposensitivity 24 hours after the last dose of estrogen, followed by a striatal dopamine receptor hypersensitivity 48-72 hours after the last dose of hormone. We have recently demonstrated that the hypersensitivity phase is mediated, at least in part, by the catecholestrogens. As the catecholestrogen 2-hydroxyestradiol has been shown to elicit a striatal dopamine receptor hypersensitivity at both 24 and 72 hours after the last dose of 2-hydroxyestradiol and the inhibition of the conversion of estrogen to its catecholestrogens attenuates this hypersensitive response. In an attempt to further characterize this biphasic response neurochemically, we have studied the effect of estradiol benzoate and 2-hydroxyestradiol on D2 receptor agonist affinity states. Ovariectomized rats were treated with estradiol benzoate 10 or 100 µg/kg) for 3 days and sacrificed 24 or 72 hours after the last dose. Animals sacrificed at 24 hours displayed a significant decrease in the ratio of high to low affinity agonist conformation sites of the D2 receptor regardless of the dose of estradiol benzoate used. On the other hand, animals treated with 100 µg/kg estradiol benzoate or 2-hydroxyestradiol 100 µg/kg and sacrificed 72 hours later showed no change in the ratio of high to low affinity conformation sites, but did display an increase in the total number of (3)H-spiro binding sites. The increased number of (3)H-spiro binding sites following 2-hydroxyestradiol has also been replicated in vitro, as incubation of striatal membranes with 2-hydroxyestradiol resulted in an increase in the B_{max} for (3)H-spiro with no change in the ratio of high to low affinity agonist sites. Thus, the estradiol benzoate-induced dopamine receptor hyposensitive phase appears to be associated with a decrease in ther ratio of high to low affinity agonist sites; whereas, the hypersensitive phase is associated with an increase in the total number of these sites.

197.8 CENTRAL ADRENERGIC RECEPTORS IN THE INHERITED NORADRENERGIC HYPERINNERVATED MUTANT MOUSE TOTTERING. P. Levitt, C. Lau, A. Pylypiw*, L.L. Ross, Dept. Anatomy, The Med. Coll. of Pennsylvania, Philadelphia, Pa. 19129.

Tottering mice (tg) express spike-wave discharges and focal motor absence seizures. The only CNS anatomical and pharmacological abnormality detected thusfar is a marked overgrowth of a single central noradrenergic system. Most terminal fields innervated by the nucleus locus coeruleus contain 100-200% more norephinephrine with a similar increase in the number of fluorescent axons visualized (Levitt and Noebels, PNAS 78: 4683, 1981). In many instances where hyperinnervation of terminal fields are induced by chemical or mechanical lesions, the presynaptic changes classically lead to a diminution of postsynaptic receptor density. To determine whether the CNS adrenergic receptors respond similarly to an inherited hyperinnervation, alpha and beta receptors in mature homozygous tg/tg and wild-type (+/+) mice were characterized.

Using the radioligand 3H-dihydroalprenolol (DHA; 10nM) specific binding of -adrenoceptors was measured in two terminal fields that receive an increased locus coeruleus innervation. In the +/+ hippocampus and cerebellum, specific binding of 3H-DHA was 13.4±0.7 pmol/g and 9.84±0.95 pmol/g, respectively. In both areas, specific binding of -adrenoceptors between tg/tg and +/+ mice did not differ significantly . Scatchard analysis revealed a remarkable similarity in receptor numbers and binding affinity. Specific binding of hippocampal alpha -receptors, measured with the ligand 3H-prazosin (0.8nM), was 1.76±0.09 pmol/g in the +/+ and 1.78±0.12 pmol/g in the tg/tg.

The abnormal locus coeruleus hyperinnevation may be directly related to the gene-linked pathophysiology, since removal of this axon system during development or in the adult tg/tg either prevents or reverses expression of the spike-wave absense seizures, respectively (Noebels, Neurosci. Abs. 9: 906, 1983). The failure of a classical postsynaptic adrenoceptor down-regulation may be an additional factor in the pathophysiological aberration in the tg/tg mouse.

Supported by NIH grant NS20196, the Commonwealth of Pennsylvania, and the Pharmaceutical Manufacturers Association Foundation.

197.9 MODULATION OF NEUROBLASTOMA ADENYLATE CYCLASE ACTIVITY BY MEMBRANE POLYUNSATURATED FATTY ACIDS: POSSIBLE INVOLVEMENT OF ENDOGENOUS PROSTAGLANDINS.
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During initial studies of the effects of phospholipid fatty-acid composition on neuroreceptor function, we observed that adenylate cyclase activities in N1E-115 neuroblastoma were consistently higher in cells supplemented with linoleic acid ($C_{18:2\omega6}$) than in control cultures. Both basal and prostaglandin (PG)-stimulated activities were affected. Incubation of control cells in culture medium containing 0.7mM Ro 20-1724 at 30°C for 40 min results in the accumulation of 28.7 ± 3.0 pmol cAMP/mg protein (mean ± SEM, N=17); supplemented cells accumulate 84.4 ± 12.9 pmol/mg protein (N=15). Addition of PGE_1 (1µM) 10 min prior to the end of incubation dramatically stimulates cyclase activity: levels in control and supplemented cells increase to 1717.4 ± 220.3 (N=11) and 3283.7 ± 555.5 (N=10) pmol/mg protein, respectively. Prostaglandin D_2 also stimulates cyclase activity in NIE-115; however, maximum stimulation in both cultures is less than four-fold.

Since these neuroblastoma have prostaglandin receptors that are cyclase-linked, we examined the possibility that elevated basal activity in supplemented cultures is related to receptor activation by endogenously-synthesized PGs. Exogenous linoleic acid is readily taken up from the culture medium, modified by chain elongation and desaturation to arachidonic acid (AA), and incorporated into membrane phospholipid. Phospholipid AA levels are > two times higher in these cells. Radioimmunoassay of media clearly indicates that PGE production is increased > four fold in cells with the elevated AA (272.7 vs. 62.9 pg/ml, respectively). There is good correlation (r=0.846) between PG production and basal levels of cAMP. To determine whether blockade of PG synthesis in supplemented cells affects basal activity, acetylsalycylic acid (ASA) was included in the medium throughout the three-day culture period. PG synthesis was inhibited under these conditions; however, basal levels of cAMP were even higher in the presence than in the absence of ASA. This suggests that elevated adenylate cyclase activity in linoleate-supplemented cells is not due to receptor activation by endogenously-synthesized PGs, and that the effects observed may be related directly to membrane lipid environment. (Supported by the Medical Research Council of Canada).

197.10 Theophylline-Induced Up-Regulation of Cerebellar Adenosine A_1 Receptors in Neonatal Rats. R.C. Sanders*, P. Szot* and T.F. Murray. (Spon: R.A. Dodson), Oregon State University, College of Pharmacy and Hatfield Marine Science Center, Corvallis, OR 97331.

Chronic treatment with caffeine (B.B. Fredholm, Acta Physiol. Scand. Suppl., 508, 31, 1982) or theophylline (T.F. Murray, Eur. J. Pharmacol., 82, 113, 1982) elicits an up-regulation of adenosine (ADO) A_1 receptors in rat brain. The ontogeny of ADO A_1 receptors has been shown to be consistent with neuronal differentiation (P.J. Marangos et al., J. Neurochem. 39, 267, 1982). The purpose of the present investigation was to examine the effects of pre- and postnatal administration of theophylline (THEO) on the ontogenesis of ADO A_1 receptors in the rat cerebellum. A_1 receptors were selectively labeled in brain membrane preparations using N^6-cyclohexyl-[^{3}H]adenosine([^{3}H]CHA) (New England Nuclear, spec. act. 25Ci/mmol) as the radioligand. Pregnant Sprague-Dawley rats received twice daily subcutaneous injections of THEO (25 mg/kg) or 0.9% saline on gestation days 5 through 15 to determine the effects of in utero exposure to THEO on postnatal development of [^{3}H]CHA binding sites. This in utero exposure did not produce any significant alterations in the specific binding of [^{3}H]CHA in either the cerebral cortex (CX), hippocampus (HC) or cerebellum (CB) of pups on postnatal day 45 as compared to offspring from pregnant rats receiving saline injections on the same days of gestation. In another series of experiments the effects of neonatal exposure to THEO on the ontogenesis of [^{3}H]CHA binding sites were assessed. Beginning on postnatal day 5, pups were treated daily with THEO (75 mg/kg, s.c.) or isotonic saline (s.c.). Six control and six THEO-treated neonates were sacrificed at postnatal days 5, 15, 30 and 45 twenty-four hours after their last THEO injection. THEO treatment of the neonates elicited a significant increase in the number of [^{3}H]CHA binding sites in the CB and CX, but not in the HC. In all cases examined the CB appeared to be the brain region most susceptible to this effect. After 10 daily THEO injections on postnatal day 15 there was a 32% increase in the specific binding of [^{3}H]CHA in cerebellar membranes as compared to saline injected pups. Similar increases of 28% and 17% were observed on postnatal days 30 and 45, respectively. Considered together, these results indicate that neonatal, but not in utero, exposure to THEO elicits an up-regulation of [^{3}H]-CHA binding sites in the rat CB. (Supported in part by a PMA Foundation Research Starter Grant)

197.11 WISTAR RATS CARRYING THE 'fa' GENE HAVE DECREASED BRAIN INSULIN BINDING. D.P. Figlewicz*, D.M. Dorsa, H. Ikeda*, L.J. Stein*, D. Baskin*, S.C. Woods, and D. Porte, Jr.* Depts. of Psychology, Pharmacology, and Medicine, Univ. of Washington, Seattle, WA 98195.

We have hypothesized that insulin acts in the central nervous system (CNS) as an inhibitor of food intake. If so, hyperphagia and concomitant obesity may be due to either diminished exposure of the CNS to insulin, or to resistance to central insulin action. To test these hypotheses, we measured both cerebrospinal fluid immunoreactive insulin levels (CSF IRI) and brain insulin binding in an obese, hyperphagic rat, the Wistar "fatty", a Wistar Kyoto rat into which the Zucker 'fa' gene has been introduced. 18-hr fasted, 4 mo. male fatties (n=10) were hyperinsulinemic (IRI=120±11 μU/ml) as compared to Wistar Kyoto lean controls (n=12; IRI=23±3 μU/ml). Further, CSF IRI was elevated in the fatties (3.6 ± 0.6 μU/ml) as compared to the leans (0.2±0.1 μU/ml). This observation--that the fatties' CSF IRI was increased, and not decreased, compared to the leans--suggests that insulin access to the CNS per se may not account for the hyperphagia of the obese animals. Alternatively, the hyperphagia may be due to central insulin resistance, as manifested by either an alteration of brain insulin receptors, or a post-receptor defect. Therefore, we measured insulin binding in several brain regions. Specific insulin binding was decreased in the fatties compared to the leans in olfactory bulb (OB) (8 vs. 29 fmol insulin bound/mg protein), in cerebral cortex (CC) (7 vs. 16 fmol/mg protein), and in lateral hypothalamus (LH) (16 vs. 47 fmol/mg protein). To determine whether this apparent downregulation was due to high ambient insulin levels, or to the presence of the 'fa' gene, we measured brain insulin binding in a group of Wistar "mixed lean" rats (Wistar Kyoto's which are phenotypically non-obese with a genotype of either Fa/fa, or Fa/Fa, in a statistical proportion of 2:1). Although the "mixed leans'" CSF IRI (n=10, 1.1±0.1 μU/ml) was not as elevated as the fatties', brain insulin binding was also reduced in the OB (17 fmol/mg protein), CC (6 fmol/mg protein) and LH (10 fmol/mg) as compared to the leans. Thus, brain insulin binding in the Wistar "fatty" and "mixed lean" rats appears to be regulated by the expression of the fat gene, and not by ambient insulin levels. Decreased brain insulin binding may contribute to, but cannot completely account for, the hyperphagia and obesity of the Wistar fa/fa rat.

197.12 REVERSIBLE IN VITRO IMIPRAMINE RECEPTOR DOWN REGULATION AND DETECTION OF DRUGS IN WASHED MEMBRANES PREPARED FROM FRONTAL CORTEX OF RATS RECEIVING CHRONIC IMIPRAMINE. P.A. Shea, C. Liang-Haskell*, H.S. Schwier,* and E.B. Solow.* Depts. Psychiatry and Biochemistry, Indiana Univ. Sch. Med., Indianapolis, IN 46223.

Male Wistar rats were injected i.p. for 19 days with 10 mg/kg IMP or saline in order to see if residual drug was present in the washed membranes used for the ^{3}H-IMP binding assay based on the procedures of R. Raisman et al., 1980. Some of the membrane samples were preincubated at 37°C, centrifuged and then binding studies performed. It was hypothesized that this preincubation would eliminate residual drugs from the preparations and that the usually observed down regulation would no longer occur.

After washing membranes prepared from frontal cortex three times, a portion was used for analysis of IMP and DMI using gas chromatography methods. We found amounts of IMP of 94.5 ± 35.2 ng/g original wt. wet and DMI, 135.0 ± 55.8 ng/g, N=4 in the 19 day Imipramine treated rats. Membranes from control rats had non-detectable amounts. If all the drug in the membranes were in solution in the binding assay the concentrations would be 4.67 nM for IMP and 6.86 nM DMI. When using the normal ^{3}H-IMP binding procedures, the expected decrease in B_{max} was observed in drug-treated animals (234 ± 22 fmol/mg protein in IMP treated rats compared to 300 ± 12 in saline treated rats; $P < .01$). When samples of membranes were incubated in buffer for 30 min at 37°C and these washed membranes were used for the binding assay, the resultant B_{max} values were 198 ± 11 fmol/mg protein, chronic treatment vs. 194 ± 8 in saline controls. We assume the disapperance of down regulation was due to the removal of drug at 37°C. Further work is in progress to confirm this hypothesis.

PAIN MODULATION III

198.1 NEURONAL ACTIVITY IN THE MONKEY MEDULLARY DORSAL HORN ASSOCIATED WITH THE DETECTION OF NOXIOUS THERMAL CUES IS MAINLY RELATED TO STIMULUS INTENSITY. R. Dubner, W. Maixner, D.R. Kenshalo, Jr., M.C. Bushnell and J.L. Oliveras. Neurobiology & Anesthesiology Br., NIDR, NIH, Bethesda, MD 20205.

Monkeys can detect small changes in intensity of noxious thermal stimuli applied to the face. Temperature increases of 0.2°C to 0.4°C from a 45°C or 46°C baseline produce reliable increases in neuronal discharge in medullary dorsal horn nociceptive neurons in awake monkeys performing the detection task. In earlier studies we showed that neuronal discharges associated with panel release following a temperature decrease in the innocuous range were independent of stimulus intensity or modality and were related to behavioral performance (Dubner et al., J. Neurophysiol., 46:444, 1981). In the present study, we determined whether such task-related responses were associated with panel release signalling the detection of an increase in temperature in the noxious range. Two monkeys were trained to release a panel button when they detected a second temperature increase of 0.2°C to 1.0°C (T2) following a temperature change from a 39°C baseline to 45°C or 46°C. In addition, they were trained to release the panel button after innocuous thermal stimuli or at the onset of a visual stimulus instead of T2. The increase in neuronal discharge following T2 was related to stimulus intensity and behavioral detection latency (Maixner et al., this volume). Neuronal responses to T2 stimuli presented outside of the behavioral task were also related to stimulus intensity. The administration of Ketamine (1 mg/kg) temporarily suppressed performance of the task and reduced slightly the magnitude of neuronal responses. Under Ketamine, neuronal activity following experimenter-presented T2 stimuli was still monotonically related to stimulus intensity. Most neurons exhibited no change in activity when monkeys detected an innocuous T2 stimulus of 2.0°C to 3.0°C from a 39°C baseline or when the relevant cue for panel release was a visual stimulus. A few neurons exhibited small changes in neuronal activity associated with the detection of innocuous thermal or visual stimuli. These responses were independent of stimulus intensity and only occurred during the task. These findings indicate that thermal nociceptive neurons in the monkey medullary dorsal horn encode stimulus intensity in a detection task when small temperature increases in the noxious range are the relevant cues for panel release. Task-related responses associated with stimulus detection are rare and their magnitude is independent of stimulus intensity or modality.

198.2 DORSAL COLUMN (DC) MODULATION, THROUGH THE BRAINSTEM, OF SPINAL WITHDRAWAL REFLEXES EVOKED BY C FIBER AFFERENT VOLLEYS. S.F. Atweh, N.E. Saadé*§ and S.J. Jabbur. Fac. of Med., Amer. Univ. of Beirut, Beirut and §Fac. of Sci., Lebanese Univ., Hadath-Beirut, Lebanon.

In decerebrate and decerebellate cats, DC stimulation rostral to selective DC cuts has been shown to modulate activities of dorsal horn neurons and spinal reflexes evoked by nociceptive and innocuous stimuli (Saadé et al., '84). Using similar preparations, we now demonstrate that DC stimulation can modulate the withdrawal reflexes evoked by afferent C fiber volleys.

In decerebrate and decerebellate cats, DC fibers were sectioned at both C_1 and C_3 levels to avoid antidromic activation of the spinal cord by the conditioning DC stimuli delivered rostral to the C_1 cuts. Withdrawal flexor reflexes were recorded as either gross potential discharge in the S_1 ventral root or single α-motoneuron discharge from a small bundle of axons isolated from S_1 or L_7 ventral roots (see Chung, J.M. et al., '83).

The S_1 reflex discharge was evoked by nociceptive radiant heat directed to the footpad (at 50-60°C for 3-5 sec) and repeated once every 2 min. Conditioning DC stimulation (10 min train at 100 Hz) resulted in a depression of the reflex discharge (reaching a maximum 50% of control) which started 2-3 min after the beginning of the conditioning and lasted 5-15 min after the end of the conditioning stimulus.

The late discharge of 20 α-motoneurons (latency of 150-200 msec) evoked by electrical stimulation at C-fiber strength applied to the sural or superficial peroneal nerve (single shock 0.5 msec or 100 msec train at 30 Hz) was inhibited by conditioning DC train (100 msec duration at 300 Hz). This inhibition peaked at 50-70 msec and lasted between 30 to 500 msec and was also apparent in neuronal discharge evoked by noxious heat stimulation.

Pain relief following DC stimulation in man has been traditionally ascribed to antidromic activation of large afferent fibers into the dorsal horn. Our findings show that afferent DC fibers can modulate nociceptive input and interact with C fiber input via a brainstem loop.

Supported by two grants from the Lebanese National Research Council.

198.3 **CHANGES IN THE MEMBRANE PROPERTIES OF SPINAL CORD NEURONS PRODUCED BY NUCLEUS RAPHE MAGNUS STIMULATION AND METERGOLINE.** M.M. Behbehani and F.P. Zemlan, Dept. of Physiology and Biophysics and Psychiatry. Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267-0576

Cells in the nucleus raphe magnus (NRM) and its surrounding area, the nucleus magnocellularis, project to the spinal cord through the dorsolateral funiculus and synapse with both the dorsal and the venteral horn (VH) neurons. The interaction between the NRM and dorsal horn neurons have been examined extensively. Although such interaction is believed to be involved in inhibition of pain, the nature of the interaction between NRM and the ventral horn cells is not clearly understood. Biochemical and histochemical investigations have shown that a significant number of NRM neurons that project to the spinal cord are serotonergic. Accordingly, we hypothesized that serotonin may be involved in the interaction between the NRM and the VH cells. Therefore, we studied the effect of NRM stimulation and a serotonin antagonist, metergoline, on intracellularly recorded VH neurons in the rat.

Male Sprague-Dawley rats were anesthetized with choral hydrate (400 mg/kg) or urethan (1.2g/kg). The spinal cord was exposed between L1 and L5. A monopolar stimulated electrode was placed in the NRM (AP 12.0-12.5, L 0.0-0.1, D 8.7-8.9) and a 40 microsecond constant current pulse of 1.5 to 3.0 mA was used to stimulate this region. Intracellular recordings were made from the VH neurons. When a cell with stable resting membrane potential better than 50 mV was isolated, its input resistance and its response to NRM stimulation both before and after application of metergoline was evaluated.

Approximately 20% of the ventral horn cells responded and all these neurons were excited by NRM stimulation. The latency of response was between 20 to 32 msec yielding a conduction velocity of 3 to 5 m/sec. The input resistance of these neurons ranged between 0.5 to 15 mega ohms with a mean value of 8 megaohms. Metergoline had an inhibitory effect on the majority of the cells and produced slight hyperpolarization of the neurons. In the majority of the neurons that were excited by NRM stimulation, metergoline significantly reduced the excitatory effect of NRM stimulation. The input resistance of the cells was not significantly affected by metergoline. The results of this study suggest that excitation of VH cells by stimulation of NRM may be mediated by serotonin. Supported by USPHS grant NS18326.

198.4 RAPHE MAGNUS UNIT RESPONSES TO DORSAL RAPHE NUCLEUS AND SOMATIC STIMULATION. G.J. Prieto, H. Quijano and J.A. Roig. Departamento de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México, México, D.F. 04510. MEXICO.

Electric stimulation of dorsal raphe nucleus (DR) of the rat results in profound analgesia. Physiological studies have suggested that this analgesia depends upon descending pathways ending selectively among dorsal horn neurons with nocieceptive inputs. However, direct spinal projections from DR are not extensive; therefore, it has been proposed that DR establishes synaptic relays with several midbrain nuclei before reaching the spinal cord. The role of raphe magnus nucleus (RM) in production and conservation of analgesia indicates that it might be a synaptic relay in this pathway. Nevertheless, anatomic relationships between DR and RM are not clear. The following experiments were performed to elucidate possible connections between these nuclei.

Male Wistar rats were anesthetized with urethane (1.5 g/kg i.p.). Bipolar stimulating electrodes were placed in DR. Contralateral sciatic nerve stimulation was applied simultaneously or independently of DR stimulation. Raphe magnus single units were recorded with stainless steel extracellular microelectrodes. Thirty two sweeps were made for each post-stimulus histogram.

A total of 28 neurons were studied during DR stimulation. Twenty eight percent showed facilitation, 25% inhibition, 17% both facilitation and inhibition, and 28% no response. The mean latencies to onset ($\bar{x}$=5.77 ms, σ=3.38; $\bar{x}$=16.45 ms, σ=3.05) and peak ($\bar{x}$=9.44 ms, σ=5; $\bar{x}$=28.02 ms, σ= 2.86) of facilitated responses suggest two populations of neurons. The mean latencies to peak of inhibitory responses were ($\bar{x}$=4.0, σ=2.8).

Somatic projections to RM were studied in 32 neurons. Facilitation occurred in 46%. When DR and somatic stimulation were applied simultaneously (N=26), 45% were inhibited, 26% facilitated, and 28% did not modify their response pattern.

Since electrical stimulation of DR can produce facilitation or inhibition of RM neuron discharges with either short or long latencies, this indicates that the projections could involve either direct or indirect pathways. Moreover, these findings suggest that DR inputs to RM neurons can modify somatic responses arriving at this level.

198.5 MIDBRAIN INHIBITION OF RAT LUMBAR NEURONAL RESPONSES TO NOXIOUS SKIN HEATING. E. Carstens & L. R. Watkins. Dept. of Animal Physiol., Univ. of Calif., Davis, CA 95616.

Previous data (J. Neurophysiol. 43:332, 1980) indicate that differential spinal inhibitory effects are produced by electrical stimulation in midbrain periaqueductal gray (PAG) or lateral reticular formation (LRF) in anesthetized cats. We similarly determined whether PAG or LRF stimulation differentially inhibits rat spinal neurons as a prelude to a study of behavioral correlates in awake rats.

In rats anesthetized with sodium pentobarbital, tungsten microelectrodes were used to record responses of single lumbar spinal neurons to noxious radiant or contact heating of glabrous hindfoot skin. Responses to heat stimuli (50°C, 10 sec) repeated at 2 min intervals were stable, providing a baseline against which to test effects of midbrain stimulation (100 msec trains at 100 Hz; 3/s; 25-300 μA) delivered via stereotaxically placed bipolar steel electrodes. Responses of each neuron tested to date (N >30) were reduced during PAG or LRF stimulus trains which began 10 s prior to heating and continued for 25 s.

In 14 experiments we systematically mapped inhibitory sites by testing effects of identical stimulation (100 or 200 μA) through each of 3 electrodes spaced 2 mm apart and lowered in 1 mm depth intervals. At collicular through posterior diencephalic levels, powerful inhibition (to 50% or more of control) was generated from PAG and subjacent tegmentum, and from widespread areas of the ipsi- and contralateral LRF. Stronger inhibition was usually produced by LRF compared to PAG stimulation.

The degree of inhibition increased with graded increases in PAG or LRF stimulation intensity, and the slopes of current-inhibition plots were much steeper for LRF compared to PAG stimulation. The mean current intensity at threshold for inhibition was lower (38.3 ± 25.4 [S.D.] μA) for LRF compared to PAG stimulation (89.4 ± 60 μA).

Neuronal responses generally increased linearly with graded increases in temperature from threshold (39.2-48.9°C) to 54°C. Slopes of these temperature-response lines were reduced (to a mean of 47% of control; N=10) during PAG stimulation with minor changes in threshold (range: -0.2 to +0.7°C). In contrast, slopes were reduced less (to a mean of 72%; N=11) during LRF stimulation, while thresholds were raised (mean: 1.7°C; range 0-3.7°C).

These data provide evidence for differential inhibitory spinal effects of PAG vs. LRF stimulation in the rat.

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198.6 **PERIVENTRICULAR GRAY INHIBITION OF PRIMATE T_2-T_5 SPINOTHALAMIC TRACT NEURONS WITH VISCEROSOMATIC CONVERGENT INPUTS.** M.-N. Girardot*(SP@N: R. W. BLAIR), W. S. Ammons, and R. D. Foreman. Dept. of Physiology & Biophysics, Univ. of Oklahoma HSC, Oklahoma City, OK 73190.

Studies on primates and humans show that the periventricular gray (PVG) region of the thalamus and hypothalamus is a very effective site for stimulus-produced analgesia. Inhibition by PVG stimulation of dorsal horn interneurons activity has been shown. The present study was designed to demonstrate that PVG stimulation also inhibits activity of spinothalamic tract (STT) neurons. The effect of PVG stimulation was studied on the discharge rate of STT cells at the T_2-T_5 level during spontaneous activity, noxious and non-noxious somatic activation and electrical and "natural" activation of cardiopulmonary visceral input. Thirteen monkeys (Macaca fascicularis) were anesthetized with α-chloralose and paralyzed with pancuronium. STT neurons were identified by antidromic activation from medial thalamic nuclei, or the ventral posterior lateral nucleus (VPL). Extracellular unit recordings were obtained from 28 STT cells. All neurons had somatic receptive fields. Electrical stimulation of cardiopulmonary sympathetic afferent fibers excited 27 cells and inhibited 1 cell. PVG stimulation inhibited all 28 neurons with no difference between STT cells projecting to either medial or VPL thalamus. PVG stimulus current thresholds ranged from 30 to 900 μA. The lowest threshold sites were in the nucleus reunions of the thalamus and the dorsomedial hypothalamus. The highest threshold sites were in the lateral hypothalamus. PVG stimulation (360 ± 50 μA) inhibited the responses of 22/22 neurons to noxious pinch of the chest or triceps region by 89 ± 3%. The response of 3/3 cells to hair movement was inhibited by 51 ± 21%. Conditioning stimuli applied to PVG inhibited responses of 6/6 STT neurons to stimulation of cardiopulmonary sympathetic afferents. Effectiveness of PVG was greater with longer conditioning stimulus trains, particularly when tested against responses to C-fiber sympathetic input. Injection of bradykinin into the left atrium of the heart increased the discharge rate of 13/18 STT neurons from 13 ± 3 to 30 ± 5 spikes/s. PVG stimulation (360 ± 30 μA) during the response to bradykinin reduced the discharge rate to 7 ± 4 spikes/s. The results provide evidence for 1) descending inhibition on neurons projecting to the medial thalamus, and 2) inhibition by PVG stimulation of STT neurons responses to noxious somatic and cardiopulmonary visceral stimuli. (Supported by NIH grants HL22732, HL00557, NS07114 and HL07440).

198.7 EVIDENCE THAT VAGINAL SELF-STIMULATION IN WOMEN SUPPRESSES EXPERIMENTALLY-INDUCED FINGER PAIN. Barry R. Komisaruk and Beverly Whipple*. Institute of Animal Behavior, Rutgers University, Newark, N.J. 07102

The purpose of these studies was to extend to humans previous findings from this laboratory that vaginal stimulation in rats strongly suppresses behavioral and neuronal responses to noxious (pinch) but not innocuous (tactile) stimulation. A definitive answer to the question of whether VS blocks pain per se requires a verbal report.

METHOD. Informed consent and all other procedures received prior approval by the Rutgers University Institutional Review Board. Women (28-56 yr) were asked to apply a controlled amount of pressure to the vagina or control areas of their body (e.g. knee) with a specially-designed device containing a calibrated force transducer with digital and chart recorder readout. We used a Ugo-Basile Analgesia Meter to apply a compressive force to the fingers in an experimental design using a) the method of limits to determine pain detection and pain tolerance thresholds and b) the signal detection method. In addition, we determined the tactile threshold on the dorsal surface of the hand using graduated monofilaments (von Frey fibers).

RESULTS. In the first study, with 10 women, vaginal self-stimulation significantly increased pain tolerance and pain detection thresholds over control (unstimulated) levels by 31.9% and 41.5% (group means) respectively, whereas tactile thresholds were unaffected. In a second study, based on preliminary results with 6 women, the pain tolerance and pain detection thresholds increased by 55.3% and 71.3% (group means) respectively, when the women were requested to maximize the pleasurable quality of the vaginal self-stimulation. One of these subjects experienced an orgasm at which time these pain thresholds increased 98.8% and 135.0% respectively, whereas the tactile thresholds remained unchanged. Distraction controls (film segment or tactile self-stimulation) were comparatively ineffective on pain and tactile thresholds. We conclude that vaginal stimulation produces analgesia rather than anesthesia and that its effect does not involve painful or non-painful distraction. Thus, a vaginally-activated mechanism exists which may normally attenuate pain during coitus and perhaps parturition; this mechanism may be utilizable clinically for controlling pain of pathological origin.

This study was supported by the Rutgers University Graduate Dean's Fund and other Rutgers University sources.

198.8 DIFFERENTIAL SENSORY AND MOTOR CORRELATES OF ANALGESIA INDUCED BY COLD-SWIM STRESS AND BY MORPHINE. D. S. Leitner and D. D. Kelly. New York State Psychiatric Inst. and Dept. of Psychiatry, Columbia Univ., New York, NY 10032

It is not normally clear whether the sensory changes induced by a given stressor are specific to the modality of pain, or whether some forms of stress analgesia might occur as part of a broader sensory deficit, or as part of a deficit in motor responsivity. We have approached this question by comparing the equivalent effects of cold-swim stress and of morphine upon a reflex analgesimetric test with their differential effects upon a psychophysical procedure based upon the inhibition of the unlearned startle response.

The whole-body startle response can be modulated in either amplitude or latency by weak stimuli that slightly precede the startle-eliciting stimulus. Whether amplitude or latency is affected depends upon the interstimulus interval which in this experiment was set at 70 msec for inhibition of startle amplitude. Startle sessions lasted 15 mins and included in mixed order: 10 trials with a 5K-Hz acoustic prestimulus, 10 trials with a light-flash prestimulus, and 10 trials when the 25-msec white-noise startle-eliciting stimulus was presented alone. Nociceptive tail-flick thresholds were assessed at three radient heat intensities adjusted to produce withdrawal latencies of 3, 6 and 10 secs.

Thirty rats were divided into groups equated for startle amplitude and exposed in counterbalanced order to five experimental conditions: (CWS) a forced, 3.5-min swim in cold water (2°) followed 30 mins later by six tail-flick trials and at 40 mins by startle; (WWS) a forced swim in warm water (28°) followed by the same tests; (MOR) morphine sulfate (5 mpk i.p.) 30 mins pretest; an equal volume injection of acetate buffer vehicle; and a baseline condition in which only the behavioral tests were administered.

Each active experimental condition produced a unique profile of sensory and motor effects. WWS did not significantly affect tail-flick, startle or startle inhibition. MOR elevated tail-flick thresholds, but it increased sensitivity to both tone and light prestimuli in the startle paradigm. Thus morphine reduced sensitivity only to noxious stimuli. In contrast, CWS lengthened tail-flick latencies, but also signficantly decreased the startle response to white noise without affecting sensitivity to either visual or auditory prestimuli. Hence the apparent antinociceptive properties of cold-water swims might result from a more general arreflexia that is principally motor in nature.

(Supported by PHS Grant 2 R01 NS 18822)

198.9 OPIOID AND NONOPIOID FORMS OF FOOTSHOCK STRESS ANALGESIA CAN BE DISTINGUISHED BY STARTLE RESPONSIVITY. D.D. Kelly and D.S. Leitner. New York State Psychiatric Inst. and Dept. of Psychiatry, Columbia Univ., New York, NY 10032

Lewis et al (Science, 208:623, 1980) demonstrated that inescapable footshock stress (IFS) could elicit either an opioid or nonopioid form of analgesia depending upon the temporal parameters of shock. With Maier, the same group found that only the opioid form of stress analgesia was correlated with a two-way shuttlebox escape deficit interpreted as "learned helplessness" (JEP:Anim Beh Proc, 9:80, 1983). We now report that the same stressors also differ in their effects upon an unlearned startle response and that these effects of IFS upon reflex responsivity to an acoustic stimulus can be differentially manipulated by naloxone (NAL), independent of the latter's effects upon analgesia.

Fifteen rats were exposed over weeks in counterbalanced order to six conditions: Brief-Continuous IFS (3-min at 3.5-ma); Prolonged-Pulsed IFS (1-sec at 3.5-ma every 5 secs for 20 mins); No Stress; and to the same conditions preceded by 10-mpk of NAL. Each condition was repeated on successive days, followed immediately on the first by startle testing and on the second by tail-flick. Startle sessions consisted of 30 trials spaced 30 secs apart. On 10 trials the white-noise startle-eliciting stimulus was presented alone. On 10 a 5K-Hz, 40-msec pure-tone acoustic stimulus preceded the white noise by 70 msec (onset to onset). On 10 there was a light-flash prestimulus. Nociceptive tailflick thresholds were assessed in 6-trial sessions at three heat intensities that produced withdrawal latencies of 3, 6 and 10 secs.

Brief IFS and Long IFS produced an identical analgesia on the tail-flick test; however, the two differed widely in their effects upon startle and in their sensitivity to NAL. Long IFS produced a 54% increase in the startle response to white noise, whereas Brief IFS had no effect upon startle. In both unstressed rats and those exposed to Brief IFS, NAL increased the startle response by a moderate, yet significant, degree (17-29 %), but had no effect upon tail-flick latencies. Following Long IFS, NAL resulted in differential changes in analgesia and startle. NAL completely blocked tail-flick analgesia, confirming Lewis et al. At the same time NAL interacted synergistically with Long IFS to produce an exceptional state of startle hyperresponsivity (+109.3 %). These and prior data suggest that stressors that induce an opioid form of analgesia may also induce a concurrent state of hyperresponsivity to select non-noxious sensory stimuli. (Supported by PHS Grant 2 R01 NS 18822)

198.10 MODULATION OF MORPHINE ANALGESIA BY PERIPHERAL CHOLECYSTOKININ (CCK) AND B-ENDORPHIN (End). P.L. Faris*, C.L. McLaughlin, C.A. Baile and J.W. Olney (SPON: T.J. Cicero). Dept Psychiatry, Washington Univ Schl Med, St. Louis, MO.

Exogenous CCK attenuates analgesia produced by morphine (Faris et al, Science 219,310,1983). Thus we assessed the function of peripherally circulating CCK in the modulation of morphine analgesia (Fed Proc 43, 935, 1984). Endogenous CCK was sequestered by antibodies produced by an active immunization procedure. Rats were immunized against bovine serum albumin (BSA-AB) or CCK conjugated to BSA (CCK-AB). The capacity of serum from the CCK-AB group to bind ^{125}I-CCK was 50 pg/ml. Baseline levels of pain responsivity, assessed by the tailflick (TF) test, did not differ between groups. The analgesic response to 10 mg/kg morphine sulfate (M) was significantly potentiated and prolonged in the CCK-AB group ($p<0.03$). Rats then received M at 12 hr intervals through the next 60 hr. TF latencies (TFL) were measured at 60 & 90 min after M. While the CCK-AB group displayed a greater analgesic response at all times compared to BSA-AB ($p<0.02$) the rate of decline in the efficacy of M to produce analgesia did not differ between groups.

It is known that M increases plasma End (Rossier et al, Nature, 1982). To determine if this released End contributes a component to the overall analgesic response we examined the effect of autoimmunization against End on M analgesia. Serum from the End immunized (End-AB) rats specifically bound 658 pg/ml ^{125}I-End and free End was decreased by 87% (4 vs 30 pg/ml in the End-AB and BSA groups respectively). Baseline TFL did not differ between groups. M analgesia was significantly reduced in the End-AB group (eg, 25% lower 90 min post-M; $p<0.004$). This suggests that M-induced release of End may contribute to M analgesia. However, when M was injected repeatedly at 12 hr intervals, this difference was not demonstrable after the third and subsequent injections. Thus, by an undetermined mechanism, the effect of End autoimmunization is apparently reversed or overcome by repeated M exposure.

Since CCK inhibits End analgesia (Itoh et al, Eur J Pharm 80,421,1982), possible mechanisms underlying CCK attenuation of morphine analgesia include inhibition of the component contributed by End. Further studies are needed to directly examine this possibility. [Supported in part by RSA MH38894 (JWO), a grant from Monsanto Co (CAB & CLM) and ES07066.]

198.11 EVIDENCE FOR A SUPRASPINAL ANALGESIC EFFECT WITH THE KAPPA RECEPTOR AGONIST ETHYLKETOCYCLAZOCINE. S. Sasson and C. Kornetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Agonists of the kappa receptor have recently been shown to produce analgesia supraspinally in addition to their accepted spinal mechanism of action (Carr,K.D., et al.,Brain Res.,245:389-393, 1982; Llewelyn,M.B., et al.,Pain,16:313-331, 1983). However, it had been suggested previously that the presumed antinociceptive effect with this class of opioids is due to sedation and/or motor incapacitation (Tyers,M.B.,Brit. J. Pharmacol., 69:503-512, 1980). The present study examines the effect of the pure kappa agonist ethylketocyclazocine (EKC) on escape thresholds maintained by electrical stimulation to the midbrain reticular formation (MRF) in the rat. Additional nonspecific measures allow us to determine if an increase in the escape threshold is due to antinociception or to sedation and/or motoric effects.

Animals were trained to escape from the aversive stimulation by turning a cylinderical manipulandum. The stimulation intensity was varied according to a modification of the psychophysical method of limits. The escape threshold was determined pre- and post-injection and drug test days were expressed as Z-scores based on the standard deviation of the mean threshold difference for all saline test days. Results indicate that EKC (0.06 - 0.25 mg/kg) raises the escape threshold in a dose-dependent manner. The latency to respond at threshold as well as the strength of response as measured by number of wheel turns were calculated and found to be unaffected by these doses of EKC. This suggests that the threshold elavating effect of EKC is not due to sedation and/or motor incapacitation but to a specific antinociceptive effect. (Supported in part by NIDA grant DA02326 and by NIDA Research Scientist Award [CK] K05 DA00099).

198.12 ELIMINATION OF VASOPRESSIN ANALGESIA FOLLOWING LESIONS PLACED IN THE RAT HYPOTHALAMIC PARAVENTRICULAR NUCLEUS. R.J. Bodnar, L. Treusdell*, J. Haldar, J.H. Kordower and G. Nilaver. Dept. of Psychology, Queens Col., C.U.N.Y., Flushing, NY 11367, Dept. of Biology, St. John's Univ., Jamaica, NY 11439 and Dept. of Neurology, Columbia Univ. Col. of Physicians and Surgeons, New York, NY 10032.

Vasopressin (VP) elicits analgesic responses in rats following central or peripheral administration that is unaffected by pretreatment with either opiate antagonists or chronic morphine injections. The VP projection to the posterior lobe of the pituitary does not appear to mediate the analgesic response since hypophysectomy fails to attenuate VP analgesia. Therefore, the extrahypothalamic VP projections, particularly those emanating from the hypothalamic paraventricular nucleus (PVN) and terminating in either the midbrain, pontine or medullary tegmentum as well as the substantia gelatinosa of the spinal cord, were examined for their role in VP analgesia. Matched for preoperative tail-flick latencies, rats were cannulated in the lateral ventricle and received either no lesion or lesions placed bilaterally in the PVN. Seven days following surgery, control and lesioned rats received arginine VP (500 ng, ICV) with tail-flick latencies determined 5, 15, 30, 45 and 60 min thereafter. Control animals displayed VP analgesia at 5, 15 30 and 45 min following injection. Animals with lesions placed in the PVN exhibited marginal analgesia only at 5 min following injection, the magnitude of which was 36% of control values. In a separate experiment, animals with control or PVN lesions were tested for VP analgesia six weeks after surgery. Preliminary data indicate that VP analgesia returned to within normal levels. Following treatment, all rats were sacrificed and samples of pons-medulla and lumbosacral spinal cord were saved for VP radioimmunoassay. Histological analysis of the PVN lesions confirmed placements. These data indicate that extrahypothalamic neural VP projections mediate VP analgesia and will be discussed in terms of alterations of ponto-medullary and spinal concentrations of VP. (Supported by PSC/CUNY Grant 6-63210).

REGULATION OF PITUITARY FUNCTION III

199.1 LHRH NEURONS AND THEIR SYNAPTIC PATTERNS IN MALE RAT MEDIAL PREOPTIC AREA (MPOA). J. W. Witkin and A-J. Silverman. Dept. Anatomy & Cell Biology., Columbia U. Coll. P.& S., New York, N.Y., 10032

Ultrastructural immunocytochemical procedures have been used to study the morphology and organization of the synaptic input to LHRH neurons in the MPOA of adult male Fischer 344 rats. Tissue was prepared by perfusing animals with 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1M phosphate buffer (pH7.3). Vibratome sections were cut through the MPOA and were treated for the immunohistochemical demonstration of LHRH using LR-1 (gift of R. Benoit; see abst. 343.5, Soc. Neurosci. 8, '83). LHRH neurons were recognized by the granular reaction product which was unevenly distributed in perikarya and processes. Some LHRH neurons contained a single cilium which projected into the neuropil. Reaction product was often in aggregates along the cytoplasmic side of the nuclear membrane and appeared to be associated with the rough endoplasmic reticulum (RER). The Golgi apparatus was generally free of reaction product. Dendrites, recognized by the presence of RER, contained diffuse reaction product though there were occasional filled vesicles. Axons, which were of narrow diameter, contained densely packed reaction product mainly in vesicles (80-120nm diameter). Synapses were present on both LHRH dendrites and perikarya. Both the axo-dendritic and axo-somatic synapses were generally asymmetric and presynaptic terminals contained either pleomorphic or round, clear vesicles. A quantitative comparison of the amount of synaptic input to LHRH dendrites vs. non-immunoreactive dendrites in the same field (using an Apple II and a Bioquant image analysis program) showed that LHRH dendrites were sparsely innervated. Only 0.44% of their membrane was in synaptic apposition compared to 5.32% of the membrane of non-reactive dendrites. For axo-somatic input, 38 LHRH perikaryal profiles were examined and only 6 axo-somatic synapses were found. Axons could be seen traversing MPOA tissue but in addition, some of these LHRH processes formed axo-dendritic synapses on non-immunoreactive elements. In these cases there was a well defined synaptic cleft and a post-synaptic density of a typical asymmetrical terminal. It is clear the LHRH neurons in these animals are complexly related to other elements of the MPOA. Supported by USPHS HD 10665 (AJS), F32 AG05290 (JWW) and Whitehall Fdn (AJS).

199.2 COMPLEX SYNAPTIC CIRCUITS OF LHRH NEURONS IN THE GUINEA PIG BRAIN A.J. Silverman AND J. WITKIN. Dept. Anatomy and Cell Biology, Columbia Univ., P&S, NY 10032.

We previously reported that LHRH axons in the diagonal band medial preoptic area of the guinea pig form axo-dendritic synapses with non-immunoreactive dendrites (Abst 343.5, '83). We now report on electron microscopic (EM) analyses of the organization of synaptic input to LHRH neurons in this region. Immunocytochemical procedures are identical to those described previously with a minor change in fixative to 4% paraformaldehyde/ 0.1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3. The LHRH neurons of the guinea pig are fusiform, with an indented nucleus and cytoplasm filled with rough endoplasmic recticulum (RER), numerous Golgi stacks, large irregular lysosomes and secretory vesicles. The distribution of reaction product varies from cell to cell and from region to region within a cell, even in the same thin section. It is associated with some but not all of the stacks of RER and secretory granules. Reaction product is rarely associated with the Golgi stacks and is absent from the nucleus. Asymmetrical axo-somatic synapses onto LHRH neurons are present. The presynaptic elements contain either clear round synaptic vesicles or a mixture of round and flattened vesicles; both types of clear vesicles are accompanied by dense cored ones measuring 50 nm diameter. Synapses are sometimes associated with protuberances of the somal surface. In addition to the synaptic input to LHRH cell bodies we also have evidence for somatodendritic syanpses with the LHRH neuron being the presynaptic element. In these cases a synaptic cleft and postsynaptic density are observed but no synaptic vesicles have been found to date. LHRH dendrites, defined as such by their large size, irregular shape, presence of RER and the longitudinal orientation of microtubules, are innervated by presynaptic elements similar to those described for the soma; the majority contained clear round vesicles. By following LHRH dendrites through several serial sections it is possible to show that, in addition to receiving a varied axo-dendritic innervation, the same structure can form (1) a reciprocal dendro-dendritic synapse and (2) a dendro-somatic synapse. Several other examples of LHRH dendrites forming dendro-dendritic relationships occur but there is no evidence for LHRH-LHRH synapses. LHRH neurons clearly participate in complex synaptic relationships which must underly their integrative functions in control of gonadotropin secretion. Supported by HD 10665 (AJS), F32 AG05290 (JW) and The Whitehall Fnd.

199.3 THE EFFECT OF PINEALECTOMY ON PROLACTIN AND LH DURING SUCCESSIVE PREOVULATORY SURGES OF INDIVIDUAL FEMALE RATS. A. Purcell* and O.K. Rønnekleiv. Oregon Health Sciences University, Portland, Or., 97201, and Oregon Regional Primate Research Center, Beaverton, Or. 97006.

The pineal organ of the female rat is known to influence the estrus cycle. There has been reports of increased estrus-type smears, increased frequence of 5-day cycles and altered timing of the preovulatory luteinizing hormone (LH) surge following pinealectomy (PX). This study was initiated to further evaluate the effects of PX on LH and prolactin (PRL) plasma levels throughout the estrous cycle and on successive proestrus afternoons.

Pinealectomized (n=18) and sham-operated (n=12) adult female rats were kept on a 12L:12D lighting schedule. Vaginal smears were monitored before PX and for 2 months afterwards. The first series of blood samples (0.4 ml) were obtained every 20 min. for 4-5 hrs on the afternoon of the first proestrus 3-5 days following jugular cannulation and one month after PX. The plasma was retained for hormone determination, the blood cells were resuspended in plasmanate (5% plasma protein fraction: Cutter Lab.) and reinjected. This sampling procedure was repeated in 11 PX and 8 controls during the afternoon of estrus and diestrus.

The remaining animals were sampled on successive proestrus afternoons. The PX animals continued to cycle normally, although some of them did show a shift from 4- to 5-day estrous cycles.

Plasma levels of LH and PRL were low during diestrus and PRL elevated during estrus in both groups of animals. On the afternoon of proestrus we observed substantial variation between all animals in the timing of LH and PRL surges. However, each control animal had very consistent timing (0.2-1 hr), whereas, individual PX animals showed a greater variation (0.5-4 hrs) in timing of LH and PRL on successive proestrus afternoons ($p < 0.005$, PX vs sham).

The PRL surge consistently occurred 0.5-2 hrs prior to the LH surge and preliminary evaluation indicates that this timing was not altered by PX. These data suggest that the pineal organ of the female rat plays an important role in the timing of the individual preovulatory surge of LH and PRL.

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199.4 REVERSAL BY EXERCISE OF PHOTOPERIODIC ANESTRUS IN SYRIAN HAMSTERS: CHANGES IN THE PATTERN OF LH RELEASE. Katarina T. Borer, Reproductive Endocrinology Program and Dept. Kinesiology, University of Michigan, Ann Arbor, MI.

Exercise reinstates estrus in hamsters rendered anestrous by 8L:16D photoperiod and normalizes serum PRL, LH, and FSH concentrations to levels seen in cycling hamsters in long photoperiods (Biol. Reprod., 1983, 29:38). We examined the effect of exercise on the pattern of LH secretion by addressing 4 questions: How does exercise affect: (1) the sensitivity of the LH secretory mechanism to estradiol benzoate (EB); (2) the pulsatile pattern of LH release; (3) the incidence of diurnal LH surge; and (4) is the presence of estradiol during exercise necessary for the changes in LH secretion to take place? Of 82 hamsters, 62 became anestrous after 7 to 15 weeks of 8L:16D exposure (lights on at 7 am). In Exp. 1, 16 cycling and 15 anestrous hamsters remained sedentary (SED), while 26 anestrous hamsters were exposed to exercise (EX). Cycles were reinstated in 15, after 3 to 26 days of running. All were ovariectomized (OVX) and 11 days later, injected with 500 μg/kg of EB. Blood was collected by c.p. 24 (8:30 am), 27, and 51 (11:30 am) hrs later Twenty-four hours post-injection, EB suppressed serum LH in EX and in cycling SED hamsters to 15% of preinjection level, but 27 and 51 hrs after EB, it failed to do so in EX hamsters and continued to do so in SED hamsters. In Exp. 2, 6 hamsters were exposed to 38 days of exercise, and 5 remained sedentary. All were OVX, implanted with jugular-vein catheters 28 days later, and bled at 20-min intervals (9 am to 5 pm) 24 hrs later. Between 11 am and 2 pm, mean plasma concentrations (130.5 vs 31.2 ng/ml), pulse frequency (3.6 vs 0.8 pulses/6 hrs), pulse amplitude (136.2 vs 20.2 ng/ml) and baseline values (74.4 vs 24 ng/ml) of LH were all higher ($p < 0.001$) in EX than in SED hamsters. Between 2 and 5 pm, LH surge was present in all SED, but not in any EX hamsters. In Exp. 3, 14 anestrous hamsters were OVX, and assigned to EX (n=7) and SED (n=7) treatments 7 days later. They were bled by c.p. at 3:30 pm after 1,3,5,7 and 9 days of exercise. LH surges (5-10 μg/ml) occurred in SED hamsters from day 10 post-OVX on, but not in EX hamsters (serum LH less than 0.8 μg/ml). We conclude that exercise: increases mean and baseline concentrations, pulse frequency, and amplitude of LH; suppresses diurnal LH surge in OVX hamsters (for which the presence of estradiol during exercise is not necessary), and appears to reduce the sensitivity of LH secretory mechanism to estradiol negative feedback.

(Supported by NSF grant PCM 81-04375)

199.5 EFFECTS OF PROGESTERONE ON THE LH SURGE AND ON SINGLE UNIT ACTIVITY OF THE MEDIAL PREOPTIC AREA-ANTERIOR HYPOTHALAMUS IN OVARIECTOMIZED AND ESTROGEN-PRIMED RHESUS MONKEYS. R.R. Yeoman and E. Terasawa. Wis. Reg. Primate Res. Ctr., Univ. of Wis., Madison, WI 53715-1299.

Despite anatomical studies reporting that considerable numbers of LHRH-containing cells as well as estrogen and progesterone accumulated neurons were present in the medial preoptic-anterior hypothalamus (MPO-AH) of nonhuman primates, physiological studies with hypothalamic deafferentation in primates failed to support the positive role of the MPO-AH in controlling reproductive function. In the present experiment we have attempted to clarify the role of the MPO-AH of the rhesus monkey in LH release using electrophysiological techniques. Since previously we have found that progesterone (P) injection after a small dose of estrogen induces a neural excitation of the medial basal hypothalamus (MBH) in association with an LH surge (Neuroscience Abst. 9: 96.7, 1983), we have used similar methods to approach the question. Single unit activity (SUA) was recorded from the MPO-AH of 5 ovariectomized and estrogen-primed female rhesus monkeys 2 h before to 12 h after P injection under light ketamine sedation. Blood samples were also obtained from the animals every 3 h in order to determine the P-induced LH release. SUA was recorded from the area 2 mm lateral of the 3rd ventricle, 4 to 6 mm rostral to the infundibular recess and 0 to 5 mm above the base of the brain with stainless steel electrodes (2-5 μm tip dia.). In all monkeys P induced an LH surge with mean peak latency at 9 h after P and mean peak amplitude of 88.2 ng/ml. The firing rate of SUA of MPO-AH neurons before P injection was 6.4 ± 0.8 Hz (n=105). Injection decreased the firing rate of SUA to 2.0 ± 0.2 Hz (n=178) with a nadir occurring at 6-10 h after P (1.1 ± 0.2 Hz). The interspike interval before P (120.2 ± 10.8 msec) increased after P (264.4 ± 32.5 msec) and the pattern of frequency distribution was also changed to predominantly slow firing components after P. The number of units encountered per mm with advancement of electrodes was not significantly affected with P. Therefore, P injection to ovariectomized and estrogen-primed monkeys induced a decrease in unit activity of the MPO-AH with a concomitant LH surge. Although the physiological significance of the P-induced reduction in SUA of the MPO-AH is not clear, our data suggest that neurons of the MPO-AH respond to P differently from the neurons of the MBH, when the LH surge, presumably LHRH release, occurs.

(Supported by NIH grants RR-00167, HD-15433 and HD-11355.)

199.6 EPISODIC LH RELEASE IN THE OVARIECTOMIZED GUINEA PIG: RAPID INHIBITION BY ESTROGEN M.A. Dykshoorn*, T.P. Condon* and M.J. Kelly (SPON: J. Hammerstad), Dept. of Physiology, Oregon Health Sci. Univ., Portland, OR 97201

Similar to its actions in the female rhesus monkey, estrogen induces an LH surge (positive feedback) in the ovariectomized female guinea pig with a mean latency of approximately 36 hrs. (Terasawa et al., Endo. 104:680 (1979). We have recently corroborated these findings and presently have investigated the time course of the negative feedback actions of estrogen in the ovariectomized female guinea pig.

Adult female guinea pigs of Topeka strain (500-550g) raised in our colony were examined daily for vaginal opening to determine estrous cyclicity. Animals displaying regular estrous cycles were bilaterally ovariectomized two weeks prior to experimentation. Two to three days before bleeding sessions guinea pigs were implanted with intra-atrial cannulas to allow frequent blood sampling from freely moving undisturbed animals. Blood samples (0.6-0.7ml) were taken at 10 min intervals for 3-5 hours. RBC's were resuspended in a plasma protein fraction and were reinfused after each subsequent sample to maintain hematocrit. Plasma LH was determined by RIA (anti-oLH antiserum, oLH trace, NIADDK rRP-2 reference). 17β-estradiol (E_2) was dissolved in 10 μl or 25 μl ETOH.

During control bleeding periods, prominent LH secretory episodes were observed in all animals studied. The mean pulse frequency was 1.6 pulses/hour with a mean pulse amplitude of 140.1 ± 13.3 ng/ml. After a 2-3 hour control period two groups of animals were infused with either 10 μg or 25 μg E_2 and bled for an additional 1-2 hours. E_2 infusion resulted in a rapid inhibition of episodic LH release. Within 30-50 min following E_2 infusion, overall mean plasma LH levels had declined to 60% of control. In addition, both pulse amplitude as well as the number of detectable pulses (0.8 pulses/hour) decreased with both doses of steroid.

These data demonstrate that in the guinea pig E_2 can inhibit LH secretion with a short latency. Whether this rapid inhibition results from estrogen action at the hypothalamic and/or pituitary level remains to be determined.

(Supported by PHS Grants NS 18989 and Fellowship HD06332)

199.7 FORSKOLIN-ASSOCIATED AUGMENTATION OF LH RELEASE IN VITRO: EFFECTS OF CYCLOHEXIMIDE. W.S. Evans*, E.R. Limber*, M.J. Cronin and M.O. Thorner* (Spon: C. Desjardins), University of Virginia Medical Center, Charlottesville, Virginia 22908

The role of cyclic AMP (cAMP) in luteinizing hormone (LH) secretion remains controversial. Using rat anterior pituitary cells in primary culture we have previously reported that cholera toxin, forskolin (F) and pertussis toxin, agents known to increase intracellular levels of cAMP, enhance both basal and gonadotropin releasing hormone (GnRH)-stimulated LH release but that such augmentation occurs only after a time lag (Am J Physiol 9:E44, 1984). To further define the temporal aspects of these effects, we exposed dispersed and continuously perifused anterior pituitary cells from rats at random stages of the estrous cycle to medium alone for 4 h followed by 10 nM GnRH, 0.3 uM F, or GnRH + F for 5 h. In addition, to determine the protein synthesis dependency of enhanced basal and GnRH-stimulated LH release by F, cells in parallel chambers were exposed to 80 uM cycloheximide (C) for 1 h prior to and during exposure to GnRH, F, or GnRH + F. Unless noted, all experiments were repeated at least 3 times. F alone had no discernable effect on basal LH release for 15-30 min after which there was a progressive increase in LH release culminating in 4-fold higher levels by the end of the fifth h. LH release in response to GnRH was biphasic with the interphase nadir occurring between 30 and 45 minutes. Simultaneous addition of GnRH + F resulted in a two-fold increase in LH release during both the first (initial 30 min) and second (final 270 min) phases of LH secretion as compared to GnRH alone. Preincubation of cells with C had no apparent effect on first phase GnRH-stimulated LH release but diminished second phase release by approximately 75%. C completely blocked the F-associated increase in basal LH release. In a single experiment C enhanced the F-associated increase in first phase GnRH-stimulated LH release by 40% and diminished second phase release by 60%. In summary, these data demonstrate that exposure of anterior pituitary cells to F results in delayed LH release and in augmentation of GnRH-stimulated LH release. We hypothesize that cAMP may promote a protein synthesis dependent process by which LH secretion is facilitated. However, the mechanisms involved in and the physiologic significance of such facilitation remain to be defined. Supported by HD-00439 (WSE), NS-00601 (MJC), and HD-13197 (MOT).

199.8 MODULATION OF LHRH SECRETION IN VITRO BY HYPERPROLACTINEMIA: ROLE OF OPIATES. Pushpa S. Kalra* (SPON: B.Y. Cooper) Dept. OB-Gyn, Univ. Fla. Col. Med., Gainesville, FL 32610

Although hyperprolactinemia (hyperprl) has been reported to suppress LH release, the mechanism of this effect is not understood. We have examined the effects of chronic hyperprolactinemia (hyperprl) and castration on LHRH content of the medial basal hypothalamus (MBH) and on the basal and naloxone-induced in vitro release of LHRH from the MBH-preoptic area (POA). Adult Wistar-Furth male rats were inoculated with MtTW15 tumor fragments and three weeks later one-half of the rats were castrated. Hyperprolactinemic (H) and normoprolactinemic (non-H) rats were decapitated two weeks later and the MBH LHRH and serum PRL and LH levels were measured. Elevated PRL levels (>2 µg/ml) resulted in increased MBH LHRH stores (3819±200 vs 2989±181pg in non-H rats). Castration caused massive depletion of MBH LHRH (1289±97 pg) in non-H rats. In castrated H rats the MBH LHRH content was also reduced (2275±158 pg, P<.01) but not to the extent seen in non-H castrated rats. Serum LH levels of intact non-H and H rats (0.95±0.18 and 0.87±0.28 ng/ml, respectively) were not different; however, the post-castration LH hypersecretion was attenuated in H rats (non-H: 9.82±0.72 and H: 5.22±0.86ng/ml, P<.01).

In a parallel study, the LHRH release rate was assessed by in vitro perifusion of the MBH-POA of similarly treated rats (6 MBH-POA/chamber; 4-5 perifusions/group). Basal LHRH release rate of intact non-H and H rats was similar (22.8±2.0 and 22.2±0.2 pg/10 mins). Castrated, non-H rats released LHRH at a reduced rate (11.6±0.6 pg/10 mins; P<.01), while castrated H rats released LHRH at an intermediate rate (17.6±1.4 pg/10 mins; P<.01). To examine the possibility of opiate involvement in the post-castration attenuation of LH release in H rats, naloxone (1mg/ml) was added to the perifusion media. Naloxone infusion (30 mins) resulted in significant increments of LHRH release from the MBH-POA of intact and castrated, H and non-H rats with similar magnitude of increase (150-170% of basal rate). In summary, results show that LHRH release rate in vitro after hyperprl and castration (1) parallels the changes in the MBH LHRH content and (2) is inversely related to changes in serum LH levels. Further, our studies do not support the view that attenuation of LH release in castrated H rats may be mediated by increased hypothalamic opioid tone. Instead, other factors such as altered pituitary responsiveness may have a major causal role in hyperprl associated suppression of LH release. (Supported by NIH HD 11362).

199.9 INHIBITION OF SECRETOGOGUE INDUCED LUTEINIZING HORMONE RELEASE BY CALMIDAZOLIUM, BUT NOT TRIFLUOPERAZINE IN CALF PITUICYTES. J.P. Kile and M.S. Amoss, Jr. Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, Texas, 77843-4466.

Calcium-calmodulin (CA^{2+}•CM) has been proposed as a mediator of both gonadotropin releasing hormone (GnRH) and potassium-induced luteinizing hormone (LH) release in rats. The intracellular mechanism of LH release in large domestic animals has not received similar attention. It was of interest, therefore, to determine if CM mediates LH release induced by GnRH and other secretogogues in cattle by the use of the CM inhibitors, calmidazolium and trifluoperazine. Prepubertal, calf pituitaries were dispersed with 0.2% collagenase, and plated at $3x10^5$ cells/ml/well in multiwell dishes. On day 5, cells were treated with secretogogues (GnRH $8.3x10^{-8}$M; Theophylline $5-40x10^{-3}$M; KCl ($10-50x10^{-3}$M); Prostaglandin E_2 (PGE_2), $10^{-7}-10^{-4}$M; Estradiol (E_2), $1.8x10^{-8}-3.6x10^{-7}$M) or secretogogue plus CM inhibitors (Trifluoperazine $10^{-7}-10^{-4}$M; or calmidazolium $10^{-8}-10^{-5}$M) in HEPES-buffered HBSS (20 mM) in 1 ml total volume for 6 hours at 37°C, 5% CO_2. Agent effects were determined in 2 or 3 separate experiments. GnRH and maximal effective doses of theophylline (2 mM) and E_2 ($1.8x10^{-7}$) induced 2-4 fold increases in LH ($p<0.05$) compared to controls, while KCl ($50x10^{-3}$M) and PGE_2 (10^{-5}M) induced 3-6 fold ($p<0.01$) increases in LH release compared to controls. Calmidazolium in concentrations as low as 10^{-8}M completely abolished LH release induced by submaximal doses of GnRH ($8.5x10^{-8}$M), E_2 ($9x10^{-8}$M), KCl ($25x10^{-3}$M), PGE_2 (10^{-6}M) in 2/3 experiments, and theophylline ($1x10^{-3}$M) in 3/3 experiments. Trifluoperazine at 10^{-6}M was able to significantly inhibit PGE_2-induced LH. However, trifluoperazine had no effect on LH release induced by any other agent. Neither CM inhibitor alone affected basal LH levels. These results demonstrate that there is a significant difference between the antipsychotic trifluoperazine and calmidazolium in their ability to inhibit LH release from calf gonadotrophs. The dichotomy of the responses to these inhibitors indicates the possibility of two different mechanisms, either specific or non-specific, involved in inhibition of CM. Therefore, the role of Ca^{2+}•CM in secretogogue induced LH release in cattle remains to be clarified.

199.10 THE POSSIBLE ROLE OF THE TRH AND ITS METABOLITE HISTIDYL-PROLINE DIKETOPIPERAZINE (CYCLO-HIS-PRO) IN THE REINFORCEMENT OF DOPAMINERGIC INHIBITION OF PROLACTIN (PRL) TRANSFORMATION FOLLOWING SUCKLING. S.W. Shyr* and C.E. Grosvenor. Dept. Physiol., Univ. Tenn. Ctr. Hlth. Sci., Memphis, TN 38163

Suckling in the rat causes a rapid reduction in anterior pituitary (AP) concentrations of PRL, i.e., transformation; AP PRL then becomes refractory to further reduction by suckling for 1 1/2-3 h. We have reported (Soc. Neuroendo. 9:460, 1983) that this refractoriness is due at least in part to a reinforcement of dopaminergic inhibition at the level of the AP but is not due to an increase in the hypothalamic secretion of dopamine, judging from turnover rate studies or to an increase in the affinity or number of dopamine receptors in the AP.

In the present studies we have examined whether TRH and/or its metabolite cyclo-His-Pro might be involved. Cyclo-His-Pro previously has been reported to inhibit PRL secretion. Primparous lactating rats (Holtzman strain) each with 6 pups were implanted with intra-atrial catheters 1 1/2 days before experiment. In the first experiment, the normal suckling-induced fall in concentration of PRL in the AP was prevented and the subsequent normal rise in plasma PRL was reduced by more than 60% in dams injected intravenously at the time of pup replacement either with TRH (500 ng, single injection) or with cyclo-His-Pro (400 ng/min x 5). The reductions were comparable to those following injection of 200 ng/min x 5 dopamine; 400 ng/min x 5 dopamine totally suppressed PRL secretion in response to suckling.

In the second experiment, the dopamine antagonist, haloperidol (0.2 mg) and domperidone (0.001, 0.01, 0.1 mg) were used to reduce AP PRL and release PRL to ascertain if the reduction in the suckling response by cyclo-His-Pro is via the dopamine inhibitory mechanism. Cyclo-His-Pro (200 or 400 ng/min x 5) reduced the plasma elevation in PRL to each dose of domperidone tested by 60-70% and to the one dose of haloperidol tested by 90%. Injection of 500 ng TRH also effectively reduced by 60% the plasma concentration resulting from a single test dose (0.01 mg) domperidone.

We hypothesize from these data that cyclo-His-Pro may act as a co-factor with dopamine in restraining PRL transformation in response to suckling in the rat. TRH may contribute to this inhibition by increasing the rate of entry of the dopamine-receptor complex into the cells through its reported facilitation of endocytosis as well as providing the source of cyclo-His-Pro. (Supported by grant HD-04358)

199.11 LUTEINIZING HORMONE AND THYROID-STIMULATING HORMONE IN THE CNS; PEPTIDERGIC AND AMINERGIC SECRETORY DYNAMICS.
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Immunoreactive and bioactive luteinizing hormone (LH) and thyroid-stimulating hormone (TSH) are widely distributed in the rodent central nervous system. Studies from this laboratory have described subcellular localization of LH and TSH in hypothalamic synaptosomes, and in vitro release by potassium-induced depolarization suggests these peptides may act in trans-synaptic neuromodulatory roles. In studies reported here, characterization of secretory control of brain-based LH and TSH was compared with pituitary LH and TSH release. Adult male rats were sacrificed by decapitation, hypothalami removed and quartered, and four quartered hypothalami incubated in KRB. The depolarizing concentration of potassium used was 60 mM. All other test agents were used at concentrations of 10^{-5} M. Potassium induced the release of LH and TSH from both the pituitary and hypothalamus. Gonadotropin-releasing hormone (GnRH) caused release of both pituitary and hypothalamic LH. However, beta-endorphin, norepinephrine, dopamine, serotonin, and acetylcholine, agents which modulate pituitary LH release, had no effect upon the secretion of LH from hypothalami in vitro. Furthermore, beta-endorphin did not alter potassium-induced release of LH from the hypothalamus. Hypothalamic TSH release was not altered by thyrotropin-releasing hormone (TRH), somatostatin, beta-endorphin, norepinephrine, dopamine, serotonin, or acetylcholine, agents known to alter pituitary TSH release. Further, potassium-provoked hypothalamic TSH release was not altered by the addition of TRH, somatostatin, or beta-endorphin in vitro. Conclusion: While there are some similarities between LH and TSH release from the pituitary and from the CNS, the substantial differences reported here suggest that hypothalamic LH and TSH do not simply serve as supplemental sources of these pituitary peptides and probably subserve entirely different role(s) from that of their pituitary counterparts.

199.12 NEUROENDOCRINE AND BEHAVIORAL EFFECTS OF INTRAHYPOTHALAMIC INJECTIONS OF QUINOLINIC ACID.
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Intrahypothalamic injections of the convulsant and excitotoxic brain constituent quinolinic acid (QUIN) were made in an attempt to examine its effects on the secretion of luteinizing hormone (LH), prolactin (PRL), growth hormone (GH) and thyrotropin (TSH). Compounds with close structural similarities to QUIN, e.g. glutamate, kainate and N-methylaspartate (NMA), can stimulate hormone release, probably by directly affecting receptors on the dendrosomal surface of neurons in the arcuate nucleus of the hypothalamus (ARH). In the present study, the specificity of QUIN-effects was assessed by co-administration of QUIN and (-) 2-amino-7-phosphonoheptanoic acid (-APH), a selective NMA- (and QUIN-) antagonist.

Acute injections of QUIN, nicotinic acid (the non-excitatory decarboxylation product of QUIN), -APH and QUIN + -APH were made bilaterally into the ARH of unanesthetized male rats chronically implanted with subdural guide cannulae. The rats were decapitated 7.5 min after injection and trunk blood collected for measurement of LH,PRL,GH and TSH using NIAMDD antisera and standards. Histological verification of cannula placements was performed on thionin-stained coronal cryostat sections. In a dose-dependent fashion, QUIN caused increases in serum LH, PRL and GH but not TSH concentrations (316%, 607% and 1134% of nicotinic acid-treated controls, respectively, after 50 µg QUIN; N=6). At low doses, a preferential effect of QUIN on PRL release was observed (12.1±5.7 ng PRL/ml after 1.5 µg QUIN vs. 3.9±0.7 ng PRL/ml after 100 µg nicotinic acid; $p<0.05$; N=6). All hormonal changes were blocked by co-injection of equimolar amounts of -APH, consistent with the presence of QUIN-sensitive receptors on neurons intimately associated with endocrine regulation.

Doses of 5 µg and higher produced the immediate precipitation of an array of seizure phenomena. No abnormal behaviors were observed in rats co-treated with QUIN and -APH.

The data indicate that QUIN may be a modulator of endocrine function. Moreover, the present findings offer a conceptual link between mechanisms underlying seizures and endocrine phenomena.

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NEUROTRANSMITTERS, MODULATORS: METABOLISM OF TRANSMITTERS AND MODULATORS

200.1 METABOLIC ACTIVITY CLOSELY PARALLELS ELECTRICAL ACTIVITY IN THE RAT LOCUS COERULEUS.
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The possibility to reliably monitor the activity of central noradrenaline (NA) neurons within the locus coeruleus (LC) through estimation of extracellular 3,4-dihydroxyphenylacetic acid (DOPAC), using in vivo differential pulse voltammetry (DPV)(1,2) has raised 2 questions a) Is there a parallelism between dopamine (DA) synthesis in the NA-LC neurons and single unit activity (SUA) as revealed by combined HPLC and DPV measurements (1,2) ? b) Is extracellular DOPAC a reliable index of NA-LC neurons activity when compared to SUA ?

Rats were placed in a stereotaxic apparatus after chloral hydrate (350 $mg.kg^{-1}$ ip). Temperature was kept constant. SUA was monitored within one LC while catechol peak was recorded in the contralateral nucleus after standard identifications (1,3). The SUA was continuously recorded except during DPV scans (ie 20s every 2 min). After 20 min of stabilization of both signals, alpha 2 selective drugs were injected iv over 2 min.

Under these experimental conditions no delay was noticeable between changes in SUA and catechol peak. Clonidine (20 $ug.kg^{-1}$ injected over 45 min in 5 $ug.kg^{-1}$ increments every 15 min) brings the NA-LC neurons to electrical silence (5 Hz to 0) and decreases the catechol peak height by 30%. Piperoxane (20 $mg.kg^{-1}$ iv) given 15 min after the last bolus of clonidine brings the SUA above control levels (0 to 6.5 Hz) and at the same time restores the catechol peak back to control. Similar parallelism was found under other conditions of stimulation (treatment by RU 24722 and RU 24969).

This first attempt to combine in vivo measurements of SUA and metabolic activity of the 2 LC shows a parallelism between variations in the SUA and in extracellular DOPAC levels. Within the present limits of detection the results suggest that DA synthesis in NA neurons is closely linked to the electrical activity of these neurons. These results are in agreement with previous electrophysiological (3,4) or biochemical (1,2) studies of central NA-LC activity and validate in vivo DPV as a reliable monitoring technique when compared to conventional methods.

1) Buda M et al. Brain Res,1983,273:197-206. 2) Gonon F et al. Brain Res,1983,273:207-216. 3) Svensson TH et al. Brain Res.,1975,92:291-306 4) Marwarha J et al. J. Pharmacol Exp. Ther.,1982,222:287-292.

200.2 CLONIDINE ABATES CENTRAL NORADRENERGIC HYPERACTIVITY INDUCED BY IMMOBILIZATION STRESS.
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Clonidine 18 $ug.kg^{-1}$ iv decreases the spontaneous firing of noradrenergic (NA) neurons in the rat locus coeruleus (LC) and blocks the NA-LC neuron response to peripheral stimuli (1). Furthermore prolonged hyperpolarization in these neurons is induced by clonidine 50 to 200 $ug.kg^{-1}$ ip (2). In vivo electrochemical detection appears a reliable technique to follow central NA-LC activity in freely moving rats through the estimation of extracellular 3,4-dihydroxyphenylacetic acid (DOPAC) (3) and is sensitive enough to detect NA-LC hyperactivity induced by mild immobilization stress (4). This experiment was undertaken to see if clonidine in the 50-200 $ug.kg^{-1}$ ip range could modulate the NA-LC hyperactivity in such a model.

A guiding cannula was implanted in the LC under anesthesia in naïve rats 48 h before the experiments. A treated carbon fiber electrode was threaded through this cannula to allow DOPAC monitoring (3,4).Control value (100%) was taken as the mean of the 5 catechol peaks recorded during the last 10 min before ip injection. Results are expressed as variations of catechol peak heights (%). After at least 50 min of stabilization of the catechol peak, rats were injected with clonidine (50,100 $ug.kg^{-1}$ ip) or vehicle 30 min before immobilization stress (10 min). Experiments were also done with the same doses of clonidine without stress.

Clonidine 100 $ug.kg^{-1}$ ip given 30 min before stress (n=5) diminished by 25%+3 (mean+sem) the catechol peak height and totally abated the increase induced by stress. Clonidine 50 $ug.kg^{-1}$ blocked that increase only partially. Saline treated rats (n=6) exhibited 32%+5 increase in the catechol peak height.

This first attempt to evaluate, under strictly chronic conditions,the pharmacological modulation of behavioral induced NA-LC hyperactivity shows also an original way to suppress it, which is in agreement with previous studies in anesthetized rats (1,2).

1) Marwarha J et al. J. Pharmacol. Exp. Ther.,1982,222:287-93.2) Aghajanian GK et al. Science,1982,215:1394-6.3) Buda M et al. Brain Res.,1983,273:197-206. 4) Gonon F et al. Brain Res.,1983,273:207-16.

200.3 Δ^1-PYRROLINE-5-CARBOXYLATE DEHYDROGENASE ACTIVITY IN RAT BRAIN: EFFECTS OF VARIOUS L-AMINO ACIDS

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The enzymes ornithine aminotransferase (EC 2.6.1.13, Orn-T) and Δ^1-pyrroline-5-carboxylate dehydrogenase (EC 1.5.1.12; P5CDH) sequentially converts ornithine to glutamic acid. Previous studies on Orn-T and on the metabolism of ornithine suggest that ornithine may act as a precursor for the neurotransmitter pool of glutamic acid and/or for the GABA precursor pool of glutamic acid in glutamatergic and GABAergic neurones, respectively. Little is documented on the enzyme P5CDH and its presence has yet to be demonstrated directly in the mammalian brain. We report here the presence of P5CDH in rat cerebellum and the effects of some amino acids on the activity of this enzyme.

Cerebella, from which high P5CDH activity has been observed with respect to other brain regions, were dissected and homogenised. The homogenate was treated with 0.1%(v/v) Triton X-100 and centrifuged (20,000g, 20 min). The supernatant was used for all measurements. P5CDH activity was monitored spectrophotometrically at 340 nm due to the formation of NADH from the second substrate NAD^+. A variety of L-amino acids were tested for their effects on the activity of P5CDH. Results revealed that GABA (5mM) produced marked inhibition (84%) on P5CDH activity. At 10mM, glutamate (44%), glutamine (50%), proline (56%), hydroxyproline (76%) and glycine (85%) also strongly inhibited the enzyme. In contrast, ornithine and aspartate were found to produce no significant effect on P5CDH activity.

It appeared that GABA and glutamate can control their own formation from ornithine by negative feedback inhibition on P5CDH as well as Orn-T in the case with GABA. Since glutamine is known to be the major source of neuronal glutamate, its inhibitory action on P5CDH may indicate a supportive role for ornithine as an alternative source. P5CDH inhibition by proline, however, is more difficult to understand in terms of negative feedback control.

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200.4 REGULATION OF SULFOCONJUGATION OF DOPAMINE BY MONOAMINE OXIDASE IN THE RAT BRAIN. N.T. Buu, Clinical Research Institute of Montreal, Montreal, Quebec H2W 1R7, Canada.

Sulfoconjugation plays a major role in the metabolism of dopamine (DA) as evidenced by the presence of large concentrations of DA sulfate in cerebrospinal fluid (CSF) and plasma of man and animal. However although activity of phenolsulfotransferase (PST), the enzyme catalyzing sulfoconjugation, is present in most areas of the brain, large increase of free DA in the brain following L-dopa administration was not accompanied by any change in the level of DA sulfate in brain tissues. The reasons for this absence of PST reactivity amid large concentrations of substrate were not known. We studied the possibility that this lack of activity was due to a lack of access of DA to the sulfoconjugating enzyme. We now report that following MAO inhibition DA sulfate can readily be formed in brain from newly generated free DA.

Sprague Dawley rats (200 g) were injected with saline or with various doses (20-80 mg/kg, i.p.) of pargyline. After one hour, control and treated rats were administered intraperitoneally with L-dopa (100 mg/kg). All rats were killed by decapitation 60 minutes following L-dopa. Free DA, DA sulfate, homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) in the hypothalamus and striatum were analyzed by high performance liquid chromatography (HPLC) with electrochemical detection.

We found that after pargyline treatment DA sulfate increased proportionally with free DA in both the hypothalamus ($r = 0.85$, $p < 0.01$) and the striatum ($r = 0.54$, $p < 0.001$) suggesting a direct interaction between free DA and PST. The DA sulfate increase was markedly more pronounced in the hypothalamus (from 56 ± 36 to 1327 ± 142 ng/g tissue) than in the striatum (from non detectable values to 559 ± 111 ng/g tissue) indicating that DA sulfoconjugation occurred primarily in the hypothalamus. The influence of MAO inhibition on PST was further supported by the significant correlation ($r = 0.85$, $p < 0.001$) between decrease of HVA, indicating increased MAO inhibition, and increased formation of DA sulfate.

The results demonstrated that MAO inhibition by pargyline stimulated DA sulfate formation in the rat brain. The effect of MAO inhibition on PST activity is likely due to a regulation, controlled by MAO, of the entry of newly formed DA into the brain compartment containing PST. (Supported by Medical Research Council of Canada).

200.5 4-[2,6-^{3}H] FLUORO-3-NITROPHENYLAZIDE BINDING SITE(S) ON THE PURIFIED BOVINE LIVER MONOAMINE OXIDASE. S. Chen, J.C. Shih, Q-P. Xu*, and M-C. Hsu.* Institute for Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA 90033.

We have previously shown that 4-fluoro-3-nitrophenylazide (FNPA) is a selective photoaffinity label of type B monoamine oxidase (MAO-B) in rat cortex. The FNPA binding site on MAO-B has been further investigated using purified bovine liver MAO-B. A single radioactive band, associated with MAO, was observed when [^{3}H] FNPA photolabeled bovine liver MAO was subjected to sodium dodeceyl sulfate polyacrylamide gel electrophoresis. Furthermore, this labeling could be inhibited by the presence of phenylethylamine, the substrate for MAO-B, during photoirradiation. In order to further characterize the [^{3}H] FNPA binding site(s), the labeled enzyme was completely digested with trypsin and chymotrypsin. Two radioactive peaks were separated by Sephadex G-25 gel filtration. One is eluted in the void volume (I) and the other represented a lower molecular weight peptide(s) (II). The photodependent labeling of both peaks could be inhibited by phenylethylamine, trans-phenylcyclopropylamine, pargyline, or phenylhydrazine in a concentration dependent manner. However, the radioactive labeling of (II) was consistently reduced by a greater extent than that of (I). Interestingly, under the same experimental conditions only one radioactive peak was observed for [^{3}H]-pargyline labeled MAO and its elution volume was different from that of the two [^{3}H] FNPA labeled peptides. This result suggests that the sites labeled by FNPA may be different from the site labeled by pargyline--the flavin binding site. (Supported by NIMH Grant No. MH 37020.)

200.6 PURIFICATION AND SOME PROPERTIES OF HUMAN BRAIN ARYL SULFOTRANSFERASE. P.H. Yu*, B. Rozdilsky* and A.A. Boulton, Psychiatric Research Division, Saskatchewan Health, CMR Building, and Dept. of Pathology, University of Saskatchewan, Saskatoon, Saskatchewan, S7N OWO, Canada.

An alternative major catabolic pathway for monoamines is known to be sulfate conjugation. In this process aryl sulfotransferase (PST, EC 2.8.2.1) catalyzes the transfer of the sulfate group from 3'-phosphoadenosine-5'-phosphosulfate to the hydroxyl group of the aromatic ring of the substrates. In the human brain at least two types of PST have been described; PST-M with amine as preferential substrates and PST-P with non-amine phenolics as preferential substrates. The human brain PST has not yet been isolated so its properties are only partially known. This report describes the purification of PST enzymes from the frontal cortex of post mortem human brain utilising ammonium sulfate precipitation, Sephadex gel filtration, DEAE cellulose ion exchange chromatography, hydroxyapatite chromatography and chromatofocusing. PST-P has been separated from PST-M. PST-M has been found to contain at least two physically separable subtypes. The pH profile, stability to heat treatment, effect of salt sensitivity towards the PST inhibitor dichloronitrophenol, molecular weight, isoelectric point as well as the kinetic properties with respect to different amine and phenolic substrates have been investigated. The purified PST-M exhibits its highest affinity to dopamine and m-tyramine, followed by p-tyramine, noradrenaline and serotonin; only negligible activity towards phenol and nitrophenol was found. PST-P, on the contrary, possesses very high affinity towards phenol and nitrophenol but much lower affinity towards the amines. The high affinity of PST-M to m-tyramine ($Km = 5\times10^{-6}$M) rather than p-tyramine ($Km = 4\times10^{-5}$M) is in accord with the observation that the formation of 3-O sulfate ester with dopamine predominates over that of the 4-O sulfate conjugate. PST activity is unevenly distributed in different brain regions with the highest activity being found in the cortex. The frontal cortex exhibits higher activity than that of the occipital cortex. Application of Percoll density centrifugation technique indicates that PST activity is located in the synaptosomal fraction of human brain cortex. Results to date support the view that sulfate conjugation of monoamines, especially dopamine and m-tyramine, may significantly contribute to the catabolism of these amines in the CNS.

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200.7 THE EFFECTS OF MINAPRINE ON DOPAMINE METABOLISM IN THE RAT AND MOUSE CAUDATE NUCLEUS AND ON GLUTAMATE, GABA AND ASPARTATE CONCENTRATIONS IN VARIOUS REGIONS OF THE RAT BRAIN.
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Minaprine (morphinoethylamine-3-methyl-4-phenyl-6-pyridazine) is a novel psychotropic drug which has been suggested to inhibit monoamine oxidase. We investigated this aspect of minaprine's action by measuring dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) using a sensitive gas chromatography-mass spectrometric (GC-MS) method. This technique employs negative chemical ionization to detect the hexafluoroisopropyl-pentafluorpropionyl esters of the DA metabolites. In the rat caudate, minaprine (10 mg/kg; intraperitoneally) produced decreases in the DA metabolites in a manner similar to pargyline. 3-MT concentrations increase as well. In the mouse, however, minaprine (25 mg/kg; i.p.) produced a decrease in DOPAC concentrations at 30 min. 3-MT increased at 20 min and 30 min. Both DOPAC and 3-MT concentrations returned to normal by 60 min. Pargyline (25 mg/kg; i.p.) produced a decrease in DOPAC by 10 min with 3-MT increasing by 10 min. These effects were still in evidence at 4 hours. Minaprine, in contrast, did not affect DA or HVA concentrations thus suggesting a different mode of action for minaprine. Minaprine, at high doses, produces convulsions. We, therefore, investigated minaprine's effects on glutamate, GABA and aspartate concentrations in the caudate nucleus, nucleus accumbens, cerebellum, globus pallidus and substantia nigra of the rat brain. These amino acids were measured by electron impact GC-MS as their heptafluoroisopropyl-pentafluorpropionyl esters. Minaprine (25 mg/kg; i.p.) produced a decrease in the glutamate concentration in the substantia nigra and in aspartate concentrations in the cerebellum. Glutamate concentrations declined (to 78% of control) by 30 min in the nucleus accumbens while GABA concentrations increased (to 125% of control). At 60 min these concentration changes had normalized. No changes were observed in the amino acid concentrations in the caudate nucleus or globus pallidus. These results suggest that minaprine has yet another mechanism of action - possibly acting as an inhibitor of GABA degradation in the nucleus accumbens. (Supported by the MRC of Canada and the Douglas Hospital Research Centre).

200.8 INTERACTIONS OF RECENTLY DEVELOPED α_2-ADRENERGIC ANTAGONISTS WITH BRAIN MONOAMINE SYNTHESIS: MODULATION BY PRAZOSIN.
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The effects of two recently-developed α_2-adrenergic (α_2) antagonists, RX781094 and WY-26703, on monoamine synthesis in rat brain were compared to those of yohimbine (YOH), its diastereomer rauwolscine (RAU), and mianserin. Intraperitoneal injection of these compounds increased cortical norepinephrine (NE) synthesis (DOPA formation after NSD-1015), an indication of α_2 antagonism, with the potency order (ED + 60%, μmoles/kg): YOH (5.3), RX781094 (6.6), WY-26703 (7.0) > RAU (33.8) > mianserin (>100). Neither haloperidol nor prazosin influenced cortical NE synthesis. Within a similar dose range YOH, RAU and WY-26703 also stimulated striatal dopamine (DA) synthesis (DOPA accumulation) but were considerably less potent than haloperidol. YOH and the structurally-related WY-26703 were also active as DA antagonists in the γ-butyrolactone model for DA autoreceptor function. In addition, YOH, RAU and WY-26703 reduced hypothalamic serotonin (5-HT) synthesis (5-HTP accumulation) while RX781094 and mianserin were very weak or inactive.

The six-fold lower potency of RAU to enhance cortical NE synthesis (α_2 antagonism) compared to YOH was surprising in view of the fact that they are diastereomers and that RAU is actually a slightly more potent competitor of α_2 radioligand binding (Perry and U'Prichard, Eur. J. Pharm. 76: 461, 1981). RAU, however, is ~ 5-fold less potent than YOH at α_1 binding sites (Tanaka and Starke, Eur. J. Pharm. 63: 191, 1980). Pretreatment of rats with the specific α_1-antagonist prazosin (1-10 μmoles/kg) significantly enhanced the activity of RAU, WY-26703 and RX781094 to increase cortical DOPA formation but was without effects by itself.

Collectively, these data suggest that newly developed α_2-antagonists which contain the benzoquinolizine structure of YOH (e.g. WY-26703) will also interact with with DA- and 5-HT systems of the brain. Furthermore, α_1 mechanisms may influence α_2 control of cortical NE synthesis.

200.9 TOLERANCE TO HALOPERIDOL: MORE RAPID DEVELOPMENT IN FEMALE THAN MALE RATS.
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The anti-psychotic drug haloperidol (HALDOL®) is a potent dopamine receptor blocking agent. The major biochemical manifestation of this receptor blockade is a dramatic increase in the concentration of the dopamine (DA) metabolites DOPAC and HVA relative to DA. After repeated administration of haloperidol, there is evidence of tolerance to the biochemical effects of the drug (Asper, H. et al, Europ. J. Pharm., 22:287, 1973) which may involve development of receptor supersensitivity. We report evidence of a more rapid development of tolerance to repeated injections of haloperidol in the female than in the male adult rat. Furthermore, ovariectomy decreases this difference.

Twelve week old male and female rats received daily intra-peritoneal injections of haloperidol (0.5 or 1.0 mg/kg) for 1,2,3,4 or 7 days and were killed by decapitation 2 h. after the last injection. The brains were rapidly removed, the striatum dissected free, weighed and homogenized 1:20 in 0.2N perchloric acid containing 100mg/L EGTA. Concentrations of DA, DOPAC and HVA were analyzed by high-performance liquid chromatography with electrochemical detection.

A single haloperidol injection led to a large increase in DOPAC/DA ratios in striatal tissue of both males and females. However, following repeated daily injections, there was a significantly more rapid drop in DOPAC/DA ratios in females than in males. This phenomenon was slowed in females ovariectomized two weeks prior to daily injections of 1 mg/kg haloperidol. Similar, although less dramatic results were noted when HVA/DA ratios were examined.

These results demonstrate a sexual dimorphism in the development of tolerance to the biochemical effects of haloperidol. Thus, ovarian hormones not only affect the density of striatal DA receptors (Hruska, R.E. et al., Neuropharm. 19:923, 1980) but may also play a role in determining the rate of development of supersensitivity following receptor blockade. These results may aid in explaining clinical observations of greater movement disorders in female than male patients receiving neuroleptic therapy.

HALDOL® was a gift from McNeil Laboratories.

200.10 MINIMAL INHIBITION OF BRAIN CATION PUMP ENZYME BY ACETYL-L-CARNITINE.
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Acetyl-L-carnitine, a naturally occurring biologically active derivative of carnitine, has many putative cholinergic like effects in the mammalian central nervous system. Among the effects of acetylcarnitine are hyperexcitability, clonic contractions, and motor hyperactivity after intrathecal administration. This study was undertaken to determine whether acetyl-L-carnitine has actions on the biochemical expression of the cation pump Na,K-ATPase, as measured by the partial reaction K-paranitrophenylphosphatase (K-pNPPase). Acetyl-L-carnitine was preincubated with rat brain homogenate protein, 0.1 mg/ml protein, for 20 minutes on ice. Other experiments have shown that inhibition reaches a maximum by about 10 minutes. Final concentrations were 75 mM imidazole-HCl (pH 7.4), 5 mM nitrophenylphosphate, 5 mM $MgCl_2$, in the presence and absence of 20 mM KCl in a final volume of 80 ul. K-pNPPase activity was taken as the difference between the values obtained in the presence and absence of KCl. The remaining activity of K-pNPPase was 100.0 ± 2.4%, 98.2 ± 8.1%, 97.1 ± 6.6%, 95.8 ± 4.6%, 75.5 ± 6.0%, and 22.8 ± 2.6% at 0, 0.001, 0.01, 0.1, 1, and 10 mM acetyl-L-carnitine, respectively. Mg-pNPPase was only inhibited by about 10% at the highest acetyl-L-carnitine concentration tested. We conclude that only at very high levels does acetyl-L-carnitine significantly inhibit the cation pump, and its physiologic effects are most likely due to other mechanisms.

200.11 MICROCHEMICAL DETERMINATION OF CHOLINE ACETYLTRANSFERASE ACTIVITY IN RETROGRADELY-LABELED MOTONEURONS OF SEDENTARY AND EXERCISED RATS. D.D. Dunning, D.A. Godfrey, C.D. Ross, and R.B. Armstrong*. Dept. of Physiology, Oral Roberts Univ. Sch. of Med., Tulsa, OK 74171.

The purpose of this study was to determine the effect of chronic treadmill exercise, a functional form of increased neuronal activity, on the choline acetyltransferase (ChAT) activity of spinal motoneurons. The ChAT activities of three regions of the motoneuron were measured: its terminals in the muscle, its axon in the ventral root, and its cell body. To measure activities of the latter, a method was developed for measuring the ChAT activity of individual cell bodies specifically identified by retrograde-labeling with horseradish peroxidase (HRP).

Samples of individual motoneuron cell bodies were microdissected from 10 µm-thick freeze-dried transverse sections of rat spinal cord and were chosen for dissection by comparison with adjacent sections stained for HRP by a modification of the method of Mesulam et al. (J. Histochem. Cytochem. 28:1255, 1980). Cell bodies were labeled following injection of 100 µl 25% HRP (Sigma Type VI) in normal saline into the left gastrocnemius muscle.

High-intensity treadmill training (10 wks, 60 m/min protocol - Dudley et al., JAP 53:844, 1982) produced significant changes in muscle oxidative capacity (23% increase in succinate dehydrogenase activity in mixed gastrocnemius, p=0.05) and muscle weight (11% increase for quadriceps femoris, p=0.01). There were, however, no significant differences in ChAT activities of muscle, ventral root, or cell bodies between sedentary control and exercise trained groups.

Data below are averages of ChAT activity (µmol/kg dry wt/min at 38°C) ± S.E.M. for motoneuron cell body samples microdissected from 6 rats (n=number of samples) of each group.

CONTROL	n	TRAINED	n
5167 ± 323	9	6058 ± 595	12
5902 ± 738	9	4341 ± 438	11
3779 ± 353	10	3461 ± 452	5
3845 ± 260	10	5858 ± 690	10
3768 ± 267	7	4361 ± 283	7
3509 ± 337	11	4102 ± 680	7
4329 ± 396	6[a]	4696 ± 421	6[a]
4301 ± 200	56[b]	4915 ± 263	52[b] (p=0.07)

[a]Average for group (n=number of rats)
[b]Average for group (n=number of soma samples)

(Supported by ORU intramural research funds)

200.12 EFFECTS OF 60 HZ ELECTRIC FIELDS ON BRAIN BIOGENIC AMINES IN THE RAT. B.J. Vasquez, P. McNeeley*, and W.R. Adey. Loma Linda University and VA Medical Center, Loma Linda, CA 92357.

Several clinical as well as experimental reports indicate that man-made electromagnetic fields might significantly affect the nervous system in central and/or peripheral aspects. However, neurohumoral correlates of these changes have not been systematically studied. The present study mapped brain regional biogenic amines in non-grounded animals exposed in air to vertical 60 Hz electric fields generated between two parallel metallic plates. Male albino rats were continuously exposed to a 14 kV/m, 60 Hz sinusoidal electric field, for either 1 day or 1 week. Sham groups included rats similarly housed but not exposed. Animals were sacrificed and brains removed immediately after treatment. Striatum, hippocampus, hypothalamus and cerebral cortex were dissected and kept frozen until HPLC determination of catecholamines, serotonin and acid metabolites. Adrenal glands were also assayed to test for possible generalized stress effects. Levels of norepinephrine (NE) were found significantly decreased in striatum and hippocampus after 1 week but not after 1 day exposure. The effects reported here appear independent of field-induced stress, since the adrenal concentration of catecholamines was unchanged. These experiments are being repeated on animals exposed to the same electric field for prolonged periods. Future research will focus on the impact on the whole animal of decreased neurotransmitter in specific brain regions as well as the chemical nature of this effect.

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200.13 CATECHOLAMINE AND INDOLEAMINE LEVELS IN VARIOUS BRAIN REGIONS IN RATS EXPOSED TO 60-Hz ELECTRIC FIELDS. D. I. Hilton* and L. E. Anderson, Biology and Chemistry Department, Pacific Northwest Laboratory, Richland, Washington 99352.

A number of recent studies have been conducted to determine if exposure to extremely low frequency (ELF) electric fields can cause significant biological effects. These investigations provide several indications of a possible interaction between ELF exposure and the nervous system. High-strength electric fields have been shown to affect the excitability of synapses, change behavior patterns, alter biological rhythms, and influence neuroendocrine function. In the study reported here, we have assessed changes in specific neurotransmitter levels by measuring norepinephrine, epinephrine, dopamine, 5-hydroxyindoleacetic acid, homovanillic acid, and serotonin. Rats were exposed or sham-exposed to 65 kilovolts/meter for 20 hours/day for 1 month. Subsequently, the neurotransmitter and major metabolites listed were measured in tissue from the frontal cortex, hypothalamus, hippocampus, pons, cerebellum, striatum, and midbrain. Neurotransmitter levels in the frontal cortex and hypothalamus were comparable in exposed and control groups, however hippocampal levels of norepinephrine were significantly ($p < 0.02$) lower in exposed animals when compared to controls. We are currently exposing animals to various levels of electric field exposure in order to determine dose response relationships.

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200.14 EFFECT OF CHEMICAL HYPOXIA ON MONOAMINE NEUROTRANSMITTER METABOLISM. G.B. Freeman, P. Nielsen*, and G.E. Gibson. Cornell Medical College, Burke Rehabilitation Center, White Plains, NY 10506.

Hypoxia (low oxygen) alters mental function by an unknown mechanism. Mild hypoxia decreases acetylcholine (ACh) synthesis and release (Gibson and Peterson, 1982) and diminishes amino acid neurotransmitter formation. The effect of hypoxia on the catecholamines and indoleamines has been difficult to evaluate because both their synthesis and degradation are oxygen dependent. Both decreases and no change in whole brain dopamine (DA) levels have been reported at similar degrees of hypoxia (Saliguat et al., 1981; Cymerman et al., 1971; Davis and Carlsson, 1973; Brown et al., 1974; Prioux-Guyonneau et al., 1979). In the present studies, graded, chemical (anemic) hypoxia was induced in male CD-1 mice (30-40 days old) by an I.P. injection of sodium nitrite (0, 37.5, 75, or 150 mg/kg) in saline 30 minutes before sacrifice. Sodium nitrite converts hemoglobin to methemoglobin and thus reduces the oxygen carrying capacity of the blood. Animals were sacrificed by focussed microwave irradiation and the concentration of serotonin (5-HT), DA, tryptophan (TRP), tyrosine (TYR), dihydroxyphenylacetic acid (DOPAC), and 5-hydroxyindoleacetic acid (5-HIAA) were determined in the striatum by electrochemical detection after separation by HPLC. The striatum is particularly suited for investigation of relative sensitivity of neurotransmitters to hypoxia because all of the neurotransmitters are present in high concentrations. Hypoxia did not alter DA, 5-HT, 5-HIAA, TRP or TYR levels in the striatum. However, hypoxia (37.5, 75, or 150 mg/kg of $NaNO_2$; n=8) caused a dose-dependent decrease in DOPAC levels (as percent of control): 88.11 ± 3.93%, 75.85 ± 10.3%, 69.49 ± 2.83%, respectively. Hypoxia also reduced the DOPAC/DA ratio: 87.56 ± 4.27%, 76.52 ± 9.89%, and 67.86 ± 6.09%, respectively. By comparison, ACh synthesis in striatum declined to 18.5% of control at 150 mg/kg (Peterson and Gibson, 1982). These results suggest that hypoxia decreases DA utilization. Current experiments that utilize incorporation of radioactivity may be an even more sensitive measure of changes in monoamine metabolism. Treatments directed toward a single neurotransmitter (ACh) do not totally reverse aging- or hypoxic-induced deficits. Multiple neurotransmitter involvement in neurological and metabolic dysfunctions may explain the limitation of these previous approaches.

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200.15 **REGIONAL HISTAMINE LEVELS IN THE CENTRAL NERVOUS SYSTEM.** P.J. Chudomelka and L.C. Murrin. Dept. of Pharmacology, Univ. of Nebraska Med. Center, Omaha, NE. 68105.

Histamine is a putative neurotransmitter in the central nervous system. Histamine formation is a simple biochemical process consisting of the decarboxylation of the amino acid L-histidine to form histamine. We examined the regional levels of histamine in the rat brain using a modification of Orr and Pace, J. Neurochem. 42:727, 1984. Rats were sacrificed by decapitation and their brains removed and dissected into the following regions: hypothalamus, hippocampus, striatum and cortex. Tissues were homogenized in ice-cold distilled H_2O. Homogenates were sonicated for 15 sec and then heated for 10 min in a boiling H_2O bath, cooled on ice, and centrifuged at 30,000 x g for 10 min at 4°C. The supernatant was assayed radioenzymatically using a partially purified preparation of rat kidney histamine-N-methyltransferase. Aliquots (10 µl) of supernatant were added to 250-µl microcentrifuge tubes on ice, followed by 10µl 0.1M sodium phosphate buffer (pH 7.9), alone or containing standard histamine levels. Finally, 10 µl of a reaction mixture containing 2µl histamine-N-methyltransferase, 1µl (0.5µCi) [methyl-^{3}H]-S-adenosylmethionine, and 7µl sodium phosphate buffer was added to each tube for a total reaction volume of 30 µl. (Blanks substituted distilled H_2O for tissue.) Tubes were incubated at room temperature for 60 min. The reaction was stopped by the addition of 10 µl of 1 N perchloric acid containing 0.5mg methyl-histamine/ml and then placed on ice. After 30 min the tubes were microcentrifuged for 5 min and an aliquot (25 µl) of each supernatant was spotted on an LK5D TLC plate (Whatman Chemical Separation, Inc.). The perchloric acid -methyl-histamine stop solution was also spotted. Plates were air dried for approximately 45 min and run in a chloroform/methanol/ammonium hydroxide (12:7:1 by volume) solvent system. The TLC plates were then air-dried and visualized with a 0.2% ninhydrin solution. The methyl-histamine spots were scraped into scintillation vials to which 0.5ml 100% ethanol and 5 ml scintillation cocktail were added. After subtraction of blanks, tissue histamine levels were determined by plots of dpm versus internal histamine standards. The hypothalamus contained the highest regional levels of 185 ng/g with the striatum and hippocampus having similar values of 45 and 40 ng/g, respectively. The cerebral cortex contained the least amount of histamine with 35 ng/g. These values are in agreement with current literature values.

200.16 LOCALIZATION AND CHARACTERIZATION OF PSEUDOCHOLINESTERASE IN RAT BRAIN. M.C. Bundman, J.L. Bruce*, J.M. Frigo*, R.T. Robertson and C. Gorenstein. Departments of Pharmacology and Anatomy, University of California, Irvine, CA 92717.

We have localized pseudocholinesterase (pseudo-ChE) in the rat brain using the copper-thiocholine method of Koelle. Unlike previous histochemical studies, we chose conditions of fixation and tissue sectioning which minimized the loss of enzyme activity while maintaining tissue integrity. Rats were perfused with 4% formaldehyde for 30 minutes and the brain sectioned on a Vibratome.

Pseudo-ChE was visualized using propionylthiocholine as the substrate and BW284c51 as a specific inhibitor of acetylcholinesterase. Under these conditions three types of staining could be observed: 1) All the endothelial cells of blood vessels in the brain were positively stained for pseudo-ChE. 2) A small number of brain nuclei displayed intense staining in cell soma and dendrites. These populations included the islands of Calleja and the anterior dorsal, anterior ventral and centromedial nuclei of the thalamus. Additonal staining was also observed in scattered neurons of the cerebral cortex, hippocampus, reticular formation and Purkinje cells of the nodulus and uvula in the cerebellum. 3) A single population of astrocytes, the Bergmann glia and the Bergmann glial fibers of the cerebellum, were also positive for pseudo-ChE. The histochemical localization of pseudo-ChE was clearly distinct from that of acetylcholinesterase.

The presence of pseudo-ChE in several cell types suggested the possibility that multiple forms of the enzyme could exist. We submitted brain extracts to electrophoresis under isoelectric focusing conditions and detected isozymes of pseudo-ChE. The evidence indicates that blood vessels contain a different isozyme than that found in neurons.

We have also localized pseudo-ChE at the electron microscopic level. In neurons, the enzyme is localized to the rough endoplasmic reticulum, and displays an intracellular distribution similar to that seen for acetylcholinesterase.

The data suggest that pseudo-ChE may play a more integral role in neurotransmission than previously proposed.

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200.17 THE MULTIPLE FORMS OF GLUTAMATE DECARBOXYLASE DIFFER IN REGULATORY PROPERTIES. T.G. Porter*, D.C. Spink*, and D.L. Martin, Ctr. for Labs and Research, NYS Dept. of Health, Albany, NY 12201.

Among the properties of glutamate decarboxylase (GAD), the regulation of holoenzyme levels is thought to be a major mechanism controlling GABA synthesis (holoGAD contains bound cofactor, pyridoxal-P, and is active). Previous studies have shown that conversion of holoGAD to apoGAD (inactive enzyme without bound cofactor) does not occur by simple dissociation of pyridoxal-P, as prolonged dialysis does not inactivate the enzyme. GAD does require exogenous cofactor in the presence of saturating concentrations of either its substrate, glutamate, or its product, GABA. This requirement is due to removal of the cofactor from holoGAD by an abortive transamination reaction catalyzed by GAD that produces succinic semialdehyde (SSA) and pyridoxamine-P (which cannot serve as a cofactor for decarboxylation). Pyridoxamine-P readily dissociates from the enzyme, producing apoGAD. Thus transamination leads to inactivation. ApoGAD can be reconstituted to holoGAD by reaction with free pyridoxal-P.

The present study compared the abortive transamination reactions and the reactivation reactions of the three forms of porcine brain GAD, termed α-, ß-, and Y-GAD. In the absence of pyridoxal-P the three forms were inactivated at substantially different rates by glutamate and GABA; α-GAD< ß-GAD<Y-GAD. During incubation with glutamate and pyridoxal-P each form produced SSA and pyridoxamine-P in a 1:1 ratio, indicating that each form undergoes abortive transamination. For α-GAD, ß-GAD, and γ-GAD the frequencies of transamination (as % of decarboxylations) were 0.0083, 0.012, and 0.028. Thus the frequency of abortive transamination accounts for the different rates of inactivation among the forms. The catalytic rate constants (kcat) were calculated from the inactivation rate constants and the frequencies of transamination. The values, (4.6, 5.7, and 5.3 s^{-1} for α-, ß-, and γ-GAD) were similar. The rate constants for reactivation of apoGAD by pyridoxal-P were different for α-, ß-, and γ-GAD (0.041, 0.21 and 0.47, $S^{-1}mole^{-1}$ respectively). The differences among the forms in the rates of inactivation by abortive transamination and the rates of reactivation by pyridoxal-P suggest that the three forms of GAD may be regulated differently and catalyze quite different rates of GABA synthesis, depending on physiological conditions. Supported by Grant MH35664 from NIMH/DHHS/USPHS.

200.18 THE EFFECTS OF PEPTIDASE INHIBITORS ON THE BINDING OF ANGIOTENSIN PEPTIDES TO RAT AND GERBIL BRAIN MEMBRANES. R.H. Abhold, C.G. Cámara, J.B. Erickson and J.W. Harding. Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164-6520.

Our laboratory has been using radioligand binding techniques in attempts at characterizing angiotensin receptors in the brains of several species. In previous studies, HPLC analysis of both bound and free ligand, following incubation of ^{125}I-labeled AII, AIII, or Sar^1,Ile^8-AII with rat or gerbil membranes, indicated that the majority of "bound" ligand was ^{125}I-tyrosine. Not only is tyrosine produced by enzymatic degradation of the parent compound by membrane bound peptidases, it is readily sequestered into synaptosomes by means of a high-affinity amino acid uptake system. In order to more accurately assess tissue binding, new methodologies were employed which involved post-incubation release of sequestered ^{125}I-tyrosine from tissue incubates by sonication, inhibition of ^{125}I-tyrosine reuptake by the addition of ^{127}I-tyrosine prior to sonication, and separation of bound and free radioligand by rapid filtration through BSA-coated glass fiber filters. Using this technique, the effects of various specific and nonspecific peptidase inhibitors on the binding of angiotensins to rat and gerbil brain membranes were examined. The binding of each angiotensin peptide, as well as the inhibitory capability of several peptidase inhibitors, exhibited sensitivity to EDTA, some of which appeared to be species specific. While all inhibitors tested blocked the binding of angiotensins to some degree, only those which inhibited aminopeptidase activities appeared to block binding by more than 50%. Indeed, a combination of specific inhibitors of aminopeptidase-A (amastatin) and aminopeptidase-B (bestatin), in the absence of EDTA, completely blocked radiolabeled AII, AIII, and, to a lesser extent, Sar^1,Ile^8-AII binding. Our results indicate that peptidases (particularly aminopeptidases A and B) may be closely associated with the angiotensin receptor and that occupation of these sites influence the binding of angiotensins. Furthermore, the previously reported noncompetitive characteristics of Sar^1,Ile^8-AII inhibition of AII and AIII activity may well be explained by a greater affinity for, and/or reduced degradation by, receptor associated peptidases.

201.1 NORADRENERGIC STIMULATION OF TRH, RANATENSIN AND CAERULEIN mRNA LEVELS IN FROG SKIN. E.R. Spindel, W.W. Chin*, and J.F. Habener*. Laboratory of Molecular Endocrinology, Massachusetts General Hospital and Howard Hughes Medical Institute Laboratories, Boston, Massachusetts 02114.

Many peptides homologous with mammalian neuropeptides are located in high concentrations in the myoepithelial granular glands of frog skin. The concentrations of these peptides are sensitive to monoaminergic neurotransmitters. Hence, frog skin is a valuable model for studies of neuropeptide biosynthesis. To study the regulation of neuropeptide gene expression, we have performed cell-free translations and nucleic acid hybridization analyses of RNAs isolated from frog skin. Synthetic oligodeoxynucleotide probes were prepared complementary to the mRNAs encoding TRH (Xenopus laevis) and caerulein (Xenopus laevis). In addition, cDNA complementary to the mRNA encoding ranatensin (Rana pipiens) was cloned and characterized.

L-norepinephrine (NE), (0.5 μM) was injected into the dorsal lymph sac of adult, male Rana pipiens or Xenopus laevis. Two days after injection, the TRH content of the dorsal skin had decreased by greater than 95%. After 4 weeks, TRH levels had returned to 25% of control levels. Poly A+ RNA was prepared from dorsal skin of untreated frogs and from frogs 4 weeks post NE injection. Comparing the NE treated frogs to the control, poly A+ RNA translated in a cell free system directed increased protein synthesis and hybridization analyses showed 3-5 fold increases in the levels of mRNA encoding TRH, ranatensin and caerulein. The multiple mRNA species which encode caerulein were all increased by NE treatment as were the multiple TRH-encoding mRNAs.

Thus in frog skin, neuropeptide synthesis can be stimulated by prior treatment with norepinephrine. The high levels of mRNA encoding these neuropeptides make this tissue ideally suited for the study of molecular regulation of neuropeptide gene expression.

201.2 CYCLIC AMP AND CALCIUM REGULATE SYNTHESIS OF EGG-LAYING HORMONE BY THE NEUROSECRETORY BAG CELLS OF APLYSIA. C.L. Brueh1 and R.W. Berry. Dept. Cell Biology and Anatomy, Northwestern Univ. Sch. Med., Chicago, IL 60611.

In peptidergic neurons, protein synthesis is needed to replace material lost through secretion: thus it is possible that the synthesis of neurosecretory peptides is under some form of regulatory control which is proportional to the rate of secretion. We have chosen to study this possibility using the neurosecretory bag cells of Aplysia, which produce and secrete a peptide egg-laying hormone, ELH. Because cyclic nucleotides and calcium are common intracellular messengers, and because both have a role in normal bag cell discharge, we have assessed their possible roles in regulation bag cell ELH synthesis. Previously, we have shown that exposure of bag cells to cAMP derivatives, to dopamine, which increases bag cell cAMP levels, or to a phosphodiesterase inhibitor, all cause significant increases in ELH synthesis. Further, 100mM K+, which elevates ELH synthesis, increases bag cell cAMP levels by over 200% when assayed in the presence of a phosphodiesterase inhibitor. Moreover, the specific adenylate cyclase activator, forskolin (1 μM) increases both cAMP levels (208%) and ELH synthesis (35%). High K+ does not elevate cGMP levels and 8-bromo-cGMP (0.5mM) actually decreases ELH synthesis by 26%. Application of the Ca^{++} ionophore A23187 (50 μM) to increase intracellular Ca^{++} leads to a 22% decrease in ELH production. Conversely, exposing bag cells to a 0-Ca^{++}/EGTA medium leads to a 38% increase in ELH synthesis. Treating bag cells with the calmodulin inhibitor, calmidazolium (50 μM) increases ELH synthesis by 20%. None of the Ca^{++}-altering treatments has any measurable effect on bag cell cAMP levels. These results suggest that cAMP and Ca^{++} act as antagonistic regulators of ELH synthesis and that the Ca^{++} effect is calmodulin-mediated. They allow us to hypothesize that the rise in cAMP levels that occurs early in a bag cell discharge increases ELH production to replace stores of the peptide lost to secretion, and that Ca^{++}, which enters the cells progressively during the discharge, acts to terminate this effect.

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201.3 MOLECULAR CLONING, PRIMARY STRUCTURE, AND CNS DISTRIBUTION OF RAT PREPROENKEPHALIN MESSENGER RNA. K. Yoshikawa and S. L. Sabol. Lab. of Biochemical Genetics, NHLBI, National Institutes of Health, Bethesda, MD 20205.

The rat brain is an important system for studies on the regulation of preproenkephalin (ppEnk) gene expression. While ppEnks of bovine adrenal medulla and human pheochromocytoma have already been characterized by cDNA cloning and sequencing, rat brain ppEnk may differ significantly from these because of evolutionary distances separating the species and because of possible differences between neuronal and non-neuronal ppEnks. Therefore rat brain ppEnk cDNA was cloned to elucidate its structure and to obtain ideal probes for the detection and quantitation of rat ppEnk mRNA.

A cDNA library constructed from striatal poly(A)+ RNA of Fischer rat was screened for plasmids hybridizing with human ppEnk cDNA (a kind gift of Drs. M. Comb and E. Herbert). The insert of one positive clone, pRPE2, was sequenced and found to contain the coding sequence (810 bases), as well as 316 and 155 bases of the 3' and 5' untranslated regions, respectively, of rat ppEnk mRNA. The primary structure of rat striatal ppEnk (269 amino acids, M_r 30,932) is essentially similar to those of bovine and human ppEnks (78% and 82% identical residues, respectively), and contains four copies of Met-enkephalin (Met-Enk), one of Leu-Enk, one of Met-Enk-Arg^6-Gly^7-Leu^8, and one of Met-Enk-Arg^6-Phe^7. One Met-Enk-containing sequence may give rise to metorphamide.

Southern blot hybridization analysis of rat genomic DNA with ^{32}P-labeled fragments of the pRPE2 insert is consistent with a single rat ppEnk gene.

Cell-free translation of rat striatal mRNA selected by hybridization with pRPE2 DNA resulted in the synthesis of a M_r 31,000 protein, presumably intact ppEnk, that binds to Met-Enk-Arg-Phe antibodies. Minor immunoreactive proteins of M_r 32,000, 21,500, and 20,000 were also noted.

A ^{32}P-labeled 941-base-pair fragment of the pRPE2 insert hybridized specifically with ppEnk mRNA (ca. 1500 bases) on Northern blots of poly(A)+ or total unfractionated RNA of various CNS regions, while no hybridization was detected to rat liver mRNA. The relative abundances of ppEnk mRNA in total RNA of rat CNS regions, determined by a sensitive dot-blot hybridization assay, are as follows: striatum = 100, hypothalamus 11.2, pons + medulla 10.8, spinal cord 10.3, cerebellum 6.1, midbrain 5.9, front cortex 4.6, hippocampus 2.0, and thalamus 1.6.

The pRPE2 probe will serve as a useful tool for studies on the regulation of ppEnk mRNA in the rat CNS.

201.4 AN ISOZYMIC FORM OF ANGIOTENSIN CONVERTING ENZYME IN THE CORPUS STRIATUM AND ITS IMMUNOHISTOCHEMISTRY. S.M. Strittmatter*, M.M.S. Lo and S.H. Snyder. Dept. of Neuroscience, Johns Hopkins University Sch. of Medicine, Baltimore, MD 21205.

The specific labeling of angiotensin converting enzyme (ACE) by (^{3}H)captopril permitted the autoradiographic localization of the enzyme to a striatonigral pathway (S.M. Strittmatter et al., Proc. Natl. Acad. Sci. USA, 81:1599, 1984). The absence of endogenous angiotensin and angiotensin II receptors in this pathway suggests that ACE has a role other than angiotensin II production. Purification of ACE from rat corpus striatum by affinity chromatography yields a protein with M_r of 135,000 by SDS-PAGE as compared to 140,000 for ACE from rat lung. If the two tissues are mixed at the initial step of the procedure then two proteins, one with M_r of 140,000 and one of 135,000, are co-purified. Comparison of peptide maps of radioiodinated ACE produced on SDS-PAGE or reverse phase HPLC reveals only a few proteolytic fragments with different mobilities between the two tissues. No differences can be detected between striatum and lung ACE in substrate specificity, in gel filtration, in ion exchange chromatography, in immunoprecipitation with anti-ACE antibodies or in sucrose density gradient sedimentation velocity. The existence of an isozymic form of ACE in the striatum is consistent with a different role for the enzyme in this location.

More precise localization of ACE in the corpus striatum and substantia nigra has been obtained by immunohistology with a monoclonal antibody raised against rat lung ACE. This antibody attached to a resin purifies only one protein of M_r 135,000 from corpus striatum extracts. The distribution of antibody binding to brain sections detected by the avidin biotin complex method parallels the distribution of ACE revealed by (^{3}H)captopril autoradiography. The ability to detect ACE by immunohistologic methods should allow electron microscopic resolution of ACE to specific subcellular sites, and may allow co-localization of the enzyme with known neuropeptides.

201.5 POTENTIAL ENDOGENOUS SUBSTRATES FOR STRIATONIGRAL ANGIOTENSIN CONVERTING ENZYME. E.A. Thiele*, S.M. Strittmatter* and S.H. Snyder. (SPON: C. Andrew). Dept. of Neuroscience, Johns Hopkins University Sch. of Medicine, Baltimore, MD 21205.

Angiotensin converting enzyme (ACE), a dipeptidyl carboxypeptidase, produces the angiotensin II found in plasma and in some brain regions. (^{3}H)Captopril autoradiography (Strittmatter, et al., P.N.A.S. USA, 81:1599, 1984) revealed the presence of high ACE levels in a striatonigral pathway. However, angiotensin is not found in the corpus striatum or the substantia nigra. Thus, it is possible that ACE hydrolyzes an as yet unidentified peptide. Substance P-like immunoreactivity is found in high concentration in a striatonigral pathway and the peptide is a competitive inhibitor of ACE. A recent report (Yokosawa et al., BBRC, 116:735, 1983) described the cleavage of substance P by ACE. We have monitored the cleavage of this potential substrate of striatonigral ACE by reverse phase HPLC. ACE purified from rat corpus striatum or rat lung initially cleaves substance P at two sites to produce the fragments Gly-Leu-Met-NH_4 (9-11), and Leu-Met-NH_4 (10-11) from the C-terminus. Substance P 1-8 and substance P 1-9 are degraded by the sequential removal of C-terminal dipeptides. Substance P 1-5 is not further degraded; it contains a penultimate Pro residue. There is a 5:1 preference for initial cleavage at the Phe^8-Gly^9 bond over the Gly^9-Leu^{10} bond. The K_M of ACE for substance P is approximately 1 μM, and the k_{cat} is 1100 min^{-1}. Thus, ACE has a higher affinity for substance P than angiotensin I and cleaves the two peptides at equal rates. The K_i of captopril and the effect of chloride is similar for substance P and Hippuryl-His-Leu hydrolysis by ACE. Studies were conducted to detect the ability of ACE to hydrolyze substance K. The ability of ACE to cleave substance P and substance K is consistent with these peptides being endogenous substrates of striatonigral ACE. To determine whether there are other endogenous striatal substances which might be candidate ACE substrates, we examined the ability of acid extracts of rat corpus striatum and rat cerebral cortex to inhibit (^{3}H)captopril binding. We have found an inhibitory substance present in at least a 10 fold higher concentration in the corpus striatum. This substance has a molecular weight of approximately 600 by gel filtration and is inactivated by peptidase treatment.

201.6 SPECIFIC BINDING OF ^{3}H-GEMSA TO ENKEPHALIN CONVERTASE: TISSUE HOMOGENATE AND AUTORADIOGRAPHIC STUDIES. D.R. Lynch*, S.M. Strittmatter* and S.H. Snyder. (SPON: D. ROBINSON). Dept. of Neuroscience, Johns Hopkins University Sch. of Med., Baltimore, MD 21205.

Enkephalin convertase (EC), an enkephalin synthesizing carboxypeptidase, has previously been characterized in both membrane and soluble forms from brain, pituitary, and adrenal medulla. The enzyme is inhibited by guanidinoethylmercaptosuccinic acid (GEMSA) with a K_i of 9 nM. ^{3}H-GEMSA binding to rat brain homogenates is saturable with a K_D of 4 nM for soluble binding sites and 6 nM for membrane bound sites. The highest levels of binding are found in the pituitary, with the second highest levels in the brain. The tissue distribution of ^{3}H-GEMSA binding parallels that of EC, and ^{3}H-GEMSA binding and EC activity have similar pharmacological profiles. Purified EC binds ^{3}H-GEMSA with similar affinity to crude tissue, and the ratio of binding to enzymatic activity is the same for purified EC as for crude tissue. Cumulatively, these results prove that ^{3}H-GEMSA binds to EC and only to EC under the assay conditions.

This finding permits the localization of EC within the brain by in vitro autoradiography with ^{3}H-GEMSA. ^{3}H-GEMSA binds to rat brain sections with a K_D of about 10 nM and a similar pharmacological profile to homogenate binding and EC activity. The highest density of ^{3}H-GEMSA labeling is found in the median eminence. Other densely labeled areas include specific nuclei in the hypothalamus, layer II of the piriform cortex, layer II of the entorhinal cortex, the hippocampal formation, the bed nucleus of the stria terminalis, the lateral septum, the central, lateral, and medial nuclei of the amygdala, the locus coeruleus, and the parabrachial nucleus. Moderate labeling is found in the globus pallidus. In the hippocampus, the most densely labeled area is the pyramidal cell layer, especially CA3. Overall, the regions labeled by ^{3}H-GEMSA correspond closely to previous localizations of enkephalins. Further autoradiographic studies of specific neuronal pathways, of ontogeny and of opiate addiction should determine the exact role of EC in synthesis of enkephalins and other neuropeptides.

201.7 DIFFERENTIAL MODULATION OF NEUROPEPTIDE LEVELS IN CHROMAFFIN CELLS. R.M. Pruss*, L.E. Eiden*, J.R. Moskal*, V.Y.H. Hook*, and M.C. Beinfeld*+. (SPON: T.G. Smith). Laboratory of Cell Biology, NIMH, Bethesda, MD 20205 and +Dept. of Pharmacology, St. Louis Univ. Med. Sch., St. Louis, MO 63104.

Primary cultures of bovine chromaffin cells offer a good model system for studying factors controlling the levels of neuroactive peptides. We have studied changes in two neuropeptides, enkephalin (ENK) and vasoactive intestinal polypeptide (VIP), which occur when the cells are treated with agents which deplete their catecholamines (reserpine), elevate cAMP (forskolin), or increase protein kinase C activity (the phorbol ester, TPA). While both ENK and VIP levels are increased by forskolin and/or TPA, only ENK levels are increased by reserpine. Cellular peptide content can be profoundly affected by cell density. We will present data about three aspects of this differential modulation. 1) At high cell density the forskolin stimulated increase in ENK is diminished at the level of the peptide but less so at the level of mRNA. Conversely, reserpine induced increases in ENK are more dramatic with increasing cell density. These data are consistent with reserpine acting as a facilitator of enkephalin precursor processing. 2) These density dependent phenomena are due to cell-cell contact rather than "conditioned medium" and may be a function of the "non-chromaffin cells" present in the cultures. 3) The effects of forskolin and TPA are synergistic in causing both an increase in the amount of VIP in the cultures and in the number of cells containing immunohistochemically detectable VIP. Furthermore, increases in VIP as a result of forskolin and/or TPA treatment are reflected in an equal increase in the amount of intracellular neuropeptide PHI. This tightly coupled biosynthetic regulation supports the hypothesis that VIP and PHI in chromaffin cells, like VIP and PHM in human neuroendocrine tumors, are contained in the same precursor molecule. These data, taken together, domonstrate that neuropeptide biosynthesis in chromaffin cells can be regulated by diverse intra-, inter-, and extra-cellular stimuli.

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201.8 PATHWAY OF CCK-8 DEGRADATION IN BRAIN: REGIONAL DIFFERENCES AND CHARACTERISTICS OF RATE-LIMITING ENZYME. P. Barone*, L. Steardo*, C.A. Tamminga, M. Knight and T. N. Chase. Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20205

To delineate mechanisms of CCK-8 inactivation, products of CCK-8 proteolysis by rat brain synaptic membranes at 37°C were analyzed by HPLC. CCK-5 has been shown to be formed initially by cleavage of the Met-Gly bond; CCK-4 is then generated by a puromycin-sensitive aminopeptidase. Thereafter, either aminopeptidase cleavage of CCK-4 or removal of Gly-Trp from CCK-5 can yield CCK-3. CCK-3, CCK-2 and Trp accumulate as final products. CCK-5, CCK-4, and CCK-3 interact with central CCK receptors. Since CCK-3 is known to antagonize CCK-8 at pancreatic receptors, the tripeptide might also have antagonist activity in brain. Regional CCK-8 catabolism was analyzed in an attempt to determine whether the rate or pattern of degradation correlate with the distribution of the neuropeptide or its receptors. Synaptic membranes were prepared from brain areas and 100 μg/ml were incubated with 10^{-5}M CCK-8. The rate of the initial cleavage was assayed by the formation of CCK-5 and the overall rate was measured by the production of Trp.

CCK-8 Proteolytic Products (pmol/min/mg)

	Whole Brain	Cortex	Striatum	Olf. Tub.	Cere-bellum	Liver	Pancreas
Trp:	60	46	96	37	45	150	0
CCK-5:	38	38	41	20	35	71	0

The pattern of products was the same in all regions. Proteolysis rates were greater in striatum than cortex, even though the intermediate metabolite, CCK-5, was produced at the same rate in both areas. Although the total rate may be higher in the striatum, the rate of formation of intermediates is similar; thus, the level of intermediates may be regulated. The pattern of proteolysis is not unique to brain as liver displays high reaction rates; pancreatic acinar membranes, which contain CCK receptors, possess little degrading activity. The enzyme responsible for the CCK-5 forming step is inhibited by Zn^{++}, Co^{++}, EDTA and PCMB and stimulated by Mg^{++} and dithiothreitol. Therefore the enzyme appears to require a metal ion and sulphydryl groups for the catalysis, suggesting it is a metallo-endopeptidase or a tripeptidyl aminopeptidase. In view of its importance in the regulation of CCK degradation, pharmacologic manipulation of this enzyme may produce substantial alterations in CCK-8 levels.

201.9 TRH AND LHRH IN PRIMARY CELL DISPERSED CULTURES OF RODENT HYPOTHALAMUS. P. Joseph-Bravo*, J.L. Redondo*, M. Theelen*, P. De la Torre*, C. Guerra* and J.L.Charli*. Centro de Investigación sobre Ingeniería Genética y Biotecnología, U.N.A.M., A.P. 70479, México D.F. 04510, MEXICO

The advantages of cell culture in studying regulatory factors has been previously stressed, however the levels of TRH and LHRH reported are too low for biochemical studies. We have developed a culture system where we can detect these peptides at nanogram levels.

The hypothalamic zone of mice embrios (14-16 days old) was mechanically dispersed in Spinners media and the cells were plated in 2 ml of DMEM suplemented with fetal calf serum, antibiotics, insulin, glucose, glutamin on polylysine coated plates. One ml of media is replaced every 4 days. We found that the density of plating was important for the good development of the culture (10^5cells/dish), being necessary to control growth of non neuronal cells with cytosine arabinoside. TRH and LHRH were detected in the media by specific radioimmunoassays taking care of possible interferences in the assays and their identity confirmed by HPLC methods. Measured within the same culture TRH and LHRH followed different behaviour during the development of the culture, being LHRH the one that appeared at later periods. The cell content of TRH dropped drastically during the first week as did the levels of protein, probably representing death of some cells during the adjustment of the culture conditions. During the second week, the levels were stabilized, lots of processes were observed and the most reproducible results obtained. TRH had a large half life in the medium. We could also detect incorporation of ^{3}H-Pro into TRH (detected by immunoprecipitation followed by two HPLC). In conclusion, the system seems adecuate for a biochemical analysis of TRH and LHRH neurons.

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201.10 PUTATIVE PRECURSOR FORMS OF SUBSTANCE P IN NERVOUS TISSUES DETECTED BY NOVEL ANTIBODIES. R.M.Kream*, R.Mancuso*,W. El-Bermani*,T.A.Schoenfeld, A.N.Clancy*,and F.Macrides, Anesthesia Research, Tufts Univ. Sch.Med.,Boston, MA 02111 and Worcester Foundation for Exptl. Biol.,Shrewsbury, MA01545.

Identification of precursor forms of Substance P (SP) in nervous tissues has proven to be difficult. Antisera previously generated to authentic SP recognize only mature undecapeptide or C-terminal fragments. We have therefore generated antisera to unamidated C-terminal extentions of SP, SP-gly-lys(SP-G-L) and SP-gly(SP-G) that are thought to be internal sequences in larger precursor forms of the peptide. Custom synthesized SP-G-L was purified by cation-exchange high performance liquid chromatography (HPLC) followed by reversed-phase HPLC. SP-G was generated from SP-G-L by mild carboxypeptidase B (CB) digestion and purified by HPLC. SP-G-L (2mg) and SP-G (2mg) were conjugated to bovine serum albumin (4mg) in separate incubations using 0.4% glutaraldehyde. Incorporation(over 80%) was monitored by HPLC of unconjugated peptide. Female New Zealand rabbits were immunized with 200-300ug of conjugated peptide, and boosted at one month intervals. Sera were screened for binding of radioiodinated Bolton-Hunter conjugated peptide tracers,prepared as described (Kream et al., J.Comp. Neurol. 222: 140, 1984). Within 4-6 months sera were obtained that bound 50% of added radioactivity (5 fmol peptide) at final dilutions of 1:25,000 and 1:80,000 for anti-SP-G and anti SP-G-L, respectively. Highly specific radioimmunoassays (RIA) were developed with anti-SP-G-L displaying less than 0.5% and 0.2% cross-reactivity with SP-G and SP, respectively, and anti-SP-G displaying 0.0% cross-reactivity with both SP-G-L and SP. The sensitivity of both RIAs was 8 pg peptide/tube and the 50% displacement was 40-60 pg/tube. Brain stem and spinal cord were quickly excized from twenty male hamsters and frozen on dry ice. After extraction in 2N acetic acid, SP-G-L and SP-G immunoreactivity(I) were respectively approximately 2 pg/ mg tissue and less than 1 pg/ mg tissue. In contrast, the corresponding levels of SP-I were approximately 100-200 pg/ mg tissue and 200-300 pg/ mg tissue. Levels of SP-G-L-I rose 20-50 fold after mild trypsinization (0.5 ug/ml, 16 hr, 22°). Levels of SP-G-I rose 10-20 fold after trypsin followed by CB treatment(1ug/ml, 1hr, 37°). These data indicate that SP precursor exists in central nervous tissues in relatively substantial quantities, as detected by our antibodies following enzymatic conversion. Complementary immunohistochemical analyses to localize immature and processed SP in somata vs. axons are in progress.

201.11 MEASUREMENT OF ENKEPHALIN PEPTIDE AND PROENKEPHALIN mRNA IN THE BASAL GANGLIA: IMPLICATIONS FOR ENKEPHALINERGIC CIRCUITRY AND TURNOVER. M.J. Bannon, P. Giraud*, E. Mezey*, R.E. Siegel*, J.Z. Kiss*, M.J. Brownstein* and L.E. Eiden*. Lab. of Cell Biology, National Institute of Mental Health, Bethesda, MD 20205.

We have measured methionine-enkephalin peptide levels (met-enk) in Cynomologus monkey basal ganglia and in several reference brain areas by radioimmunoassay. Proenkephalin messenger RNA (enk-mRNA) from the same regions was measured as follows: Total RNA from as little as 30 mg tissue was extracted and ethanol-precipitated. Aliquots of total RNA were run on agarose gels, quantitated by ethidium bromide staining and then transfered to nitrocellulose filters. The enkephalin-encoding mRNA on these Northern blots was quantitated by hybridization with a radiolabelled proenkephalin cDNA probe. The results are shown below:

Brain region	met-enk(ng/g)	enk-mRNA(%caudate)	mRNA x 100 / peptide
caudate	1240 ± 330	100	8.1
putamen	280 ± 16	75	26.8
ext. pallidum	1280 ± 370	29	2.3
int. pallidum	1700 ± 200	15	0.9
amygdala	30 ± 3	10	33.3
occip. cortex	19 ± 0.4	<0.2	<1.1
frontal cortex	14 ± 3	<0.2	<1.4

These results suggest that in primate basal ganglia, enkephalin cell bodies (indicated by a high enk-mRNA/met-enk ratio) are localized primarily in the caudate nucleus and putamen, whereas the globus pallidus is rich in nerve terminals but poor in met-enk cell bodies (reflected in a low enk-mRNA/met-enk ratio). Interestingly, the amygdala appears to be relatively rich in enk-mRNA, suggesting that the primate amygdala contains enkephalinergic perikarya. These results suggest that the concomitant measurement of enk-mRNA and met-enk may form a basis for determining the localization and possible function of met-enkephalin in the extrapyramidal and limbic systems. Along these lines, pharmacological studies of rat striatal enkephalin biosynthesis, involving the quantitation of both met-enk and enk-mRNA, are in progress. In addition, preliminary studies of cDNA in situ hybridization in rat striatum will also be presented.

201.12 **KINETIC CHARACTERIZATION OF THE INTERMEDIATE PITUITARY SECRETORY GRANULE-ASSOCIATED αMSH/β-ENDORPHIN ACETYLTRANSFERASE.** **T.R. Gibson* and C.C. Glembotski. (SPON: A. Laties) Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.**

The acetyltransferase from rat and bovine intermediate pituitary secretory granules acetylates both ACTH- and β-endorphin-related peptides. Since α-N-acetylated forms of β-endorphin-related peptides were found to be competitve inhibitors of the acetylation of ACTH-related peptides, it was postulated that both families of peptides bind to the same active site (Glembotski, C.C. (1982) J. Biol. Chem. 257, 10501-10509 and Chappell, M.C., et. al. (1982) Peptides 3, 405-410). To examine the nature of this active site more fully, a more detailed kinetic analysis of the bovine enzyme was performed. When the concentration of ACTH(1-18) was varied at several acetylCoA concentrations, the results suggested that acetylCoA binds first to the active site. Although it is not a substrate, ACTH(1-8) was a competitive inhibitor when both ACTH(1-13NH_2) and β-endorphin(1-31) were used as substrates. This suggests that a common binding domain exists for the NH_2-terminal amino acids of both ACTH- and β-endorphin-related peptides. However, using ACTH(9-13NH_2), inhibition was seen with ACTH(1-13NH_2) as the substrate but not with β-endorphin(1-31). Thus, the first 8 residues of the active site probably comprise the common binding domain, while amino acid residues COOH-terminal to position 9 are bound to separate domains with specificity for either ACTH- or β-endorphin-related peptides. This hypothesis is supported by experiments showing that α- and γ-endorphin (β-endorphin(1-14) and (1-15), respectively), which are not acetylated by the enzyme, inhibit the acetylation of both ACTH(1-13NH_2)and β-endorphin(1-31). Similar results have been found using the rat intermediate pituitary secretory granule acetyltransferase. In conclusion, our data are consistant with the hypothesis that the intermediate pituitary secretory granule acetyltransferase contains a common binding domain for the 8 NH_2-terminal residues of ACTH- and β-endorphin-related peptides, while maintaining separate binding domains for amino acid residues COOH-terminal to that point.

201.13 THE ENKEPHALIN-CONTAINING OCTAPEPTIDE Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu IS DEGRADED PRIMARILY BY AN ANGIOTENSION CONVERTING ENZYME-LIKE ACTIVITY IN SYNAPTIC MEMBRANES. Jon A. Norman and J.Y. Chang*. Research Dept., Pharmaceuticals Div., CIBA-GEIGY Corp., Summit, N.J. 07901 and CIBA-GEIGY Ltd., Basel, Switzerland CH-4002.

The degradation of the enkephalin-containing-octapeptide Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu-(YGGFMRGL) was systematically investigated by incubating the peptide with synaptic membranes from rat striatum or with purified peptidases. The newly formed degradation products were derivatized with dimethylaminoazobenzene isothiocyanate (DABITC) and then analyzed by HPLC and by N-terminal analysis. The incubation of YGGFMRGL with synaptic membranes yielded YGG, YGGF, YGGFM and MR in a manner that was linear with respect to time. The carboxyl terminal fragments FMRGL, MRGL and RGL could not be detected which suggests that the degradation of YGGFMRGL by synaptic membranes occurs by carboxypeptidase activity. The incubation of YGGFMRGL with different purified peptidases produced cleavage patterns unique from that seen with synaptic membranes. Enkephalinase recognized only the gly-phe bond to produce YGG and FMRGL. Thermolysin recognized the gly-phe bond and the phe-met bond to yield YGG, YGGF, FMRGL and MRGL. Angiotensin converting enzyme (ACE) produce primarily YGGF, MR and lesser amounts of YGGFMRF and YG. The formation of YGG, YGGF and YGGFM by synaptic membranes could be stimulated 3 fold by the addition of 30 mM NaCl and inhibited by MK-422, an ACE inhibitor, with an IC_{50} of 3 nM. These data suggest that ACE, a dipeptidyl carboxypeptidase, is the primary enzyme involved in the degradation of YGGFMRGL in brain. ACE apparently works in concert with another carboxypeptidase in brain to yield YGGFM and YGG since the carboxyl terminal peptides RGL and FMRGL could not be detected.

201.14 **REGULATION OF OXYTOCIN AND ENKEPHALIN BIOSYNTHESIS DURING LACTATION** J.D. White and J.F. McKelvy. Dept. of Neurobiology & Behavior, SUNY Stony Brook, Stony Brook, NY 11794

Recent anatomical evidence has suggested that Met-enkephalin peptides co-exist in oxytocin containing neurons in the paraventricular nucleus of the hypothalamus (PVN) and in the terminals of these neurons in the external zone of the median eminence and in the posterior pituitary. To investigate the regulation of neuropeptide biosynthesis and processing, we have begun studies on the in vivo biosynthesis of oxytocin (OXY), vasopressin (AVP), Met^5-enkephalin (MENK), Met^5-Arg^6-Gly^7-Leu^8-enkephalin (MERGL) and Met^5-Arg^6-Phe^7-enkephalin (MERF). As a model for the selective, physiological activation of OXY containing neurons we have used the lactating female rat.

We studied neuropeptide biosynthesis in vivo by delivery of a pulse of radiolabeled amino acid via indwelling cannulae using osmotic minipumps, the extraction of radiolabeled peptides from the terminal fields and their purification in the presence of carrier peptides using sequential HPLC steps and chemical modification of the peptides.

For all the labeling times tested, the ratio of ^{35}S-OXY to ^{35}S-AVP purified from the neural lobe increased from a mean value of 1.10 in normal (diestrous) females to 1.54 in lactating animals, suggesting that OXY biosynthesis is increased approximately 50% during lactation. Similar results were found for OXY vs. AVP in the median eminence. We also found that the radioactivity present in MENK, MERGL and MERF was greater in the median eminence in lactating animals than in normal animals (mean increase of 2.3 fold). The peptides were detectable in the neural lobe, however, too little radioactivity was present to permit accurate quantitation. The ratio of radioactivity present in MENK:MERGL:MERF averaged 3:0.4:1 and appeared to be relatively invariant between normal and lactating animals at any of the pulse and chase times examined.

These studies are the first to examine enkephalin biosynthesis and processing in a defined projection system in the CNS. We have shown that Met-enkephalin peptides are present in the median eminence and that their synthesis can be physiologically stimulated. These data suggest that the enkephalins may play a physiologically relevant role in the neuroendocrine system. (NSF BNS-7684506)

201.15 BIOSYNTHESIS AND PROCESSING OF SOMATOSTATIN-RELATED PEPTIDES BY RAT HYPOTHALAMUS IN VIVO. Kim Stewart and Jeffrey McKelvy. Dept. of Neurobiology and Behavior, SUNY Stony Brook, Stony Brook, NY 11794

Somatostatin, like other neuropeptides, is synthesized as part of a larger molecular weight precursor. To date, three fragments of the somatostatin precursor have been isolated from brain: SS-14, SS-28, and SS-28(1-12). We have developed methods to examine de novo synthesis of somatostatin and its related fragments in awake and unrestrained animals.

Male rats are stereotaxically cannulated in the paraventricular nucleus of the hypothalamus and infused with ^{35}S-cysteine and ^{3}H-proline for a determined pulse time, followed by a chase. The rats are sacrificed and the area of cell bodies (paraventricular nucleus) and nerve terminals (median eminence) dissected. Radiolabeled SS-14, SS-28, and SS-28(1-12) are purified to constant specific activity by three sequential HPLC steps utilizing different buffer and gradient systems. All peptides are subjected to chemical modification and/or enzymatic cleavage, providing evidence of purity. SS-14 and SS-28 are converted to the S-carboxymethyl form, digested with trypsin and mapped on HPLC using uv and fluorescence detection. SS-28(1-12) is first converted to the sulfoxide form and then to the sulfone. Incorporation of radioactive amino acids into all three peptides, and the transport of these peptides to the median eminence, has been observed following total labeling times of 4, 6, and 12 hours.

Using a rat cDNA somatostatin clone, provided by Dr. Richard Goodman, we have also looked at somatostatin mRNA levels. Total cellular RNA and poly(A^+)RNA from various brain regions has been blotted on nitrocellulose paper and hybridized with a ^{32}P-labeled nick-translated somatostatin-coding restriction fragment. The fragment hybridizes with increasing intensity to increasing amounts of RNA from those regions which contain somatostatin cell bodies, while no signal is observed from regions lacking somatostatin. Northern analysis of poly(A^+)RNA from rat hypothalamus reveals one hybridizable band.

We are currently using these techniques to focus on the dynamics of somatostatin biosynthesis and metabolism by examining feedback effects of possible regulatory agents on somatostatin transcription and synthesis. (Supported by NSF BNS 81-11475 and NIH MH 08990).

202.1 PUTATIVE INSECT NEUROTRANSMITTERS EVOKE ELECTROPHYSIOLOGICAL RESPONSES FROM EMBRYONIC COCKROACH NEURONES IN PRIMARY CULTURE. G. LEES*, J.A. BENSON and D.J. BEADLE* School of Biol. Sci., Thames Polytechnic, London, SE18, U.K. and Agricultural Div., Ciba-Geigy Ltd., CH-4002 Basel, Switzerland.

Embroyonic neurones from the brain of the Periplaneta can be maintained in primary culture for periods of four weeks or more. The isolated neuronal somata differentiate in vitro and produce multiple neurites. Morphological evidence suggests that synapse formation occurs in vitro (Beadle, Hicks and Middleton, J. Neurocytol. 11, 611, 1982) and some of the neurones exhibit spontaneous action potentials and excitatory electrical activity which may be of synaptic origin (Lees, Beadle, Botham and Kelly, in prep.). The structural (Beadle et al., 1982) biochemical (Beadle, Lees and Botham, In 'Insect Neurochemistry and Neurophysiology', Plenum Press, New York (in press). and electrical properties of these neurones in vitro are similar to those of insect neurones in vivo. The cholinergic receptors expressed in vitro are indistinguishable from those described in other insect preparations on the basis of both ligand binding and electrophysiological responses (Lees, Beadle and Botham, Brain Res. 288, 49, 1983).

The neuronal monolayer formed in these primary cultures affords unrestricted access for iontophoretic or pressure micropipette application of putative neurotransmitters and other drugs to the extrasynaptic membranes, particularly of the soma, which are largely inaccessible using conventional in vivo techniques. Impaling large numbers of these neurones has made it possible to record several different responses to the putative neurotransmitters tested. For the experiments reported here, all drugs were dissolved in saline at 10^{-3}M and pressure ejected for 200 msec to 2 sec. GABA produced conductance increases in approximately 60% of cells tested. The GABA responses were voltage-dependent, being hyperpolarising in most cells but excitatory in somata with very high resting potentials. The apparent reversal potential was in the range -70 mV to -80 mV. Octopamine evoked hyperpolarisations accompanied by input resistance increases in a small proportion of cells tested. Both GABA and octopamine reversibly inhibited spontaneous spiking. A further subpopulation of neurones exhibited desensitizing excitatory membrane potential changes together with conductance increases in response to serotonin application. Glutamate produced a variety of changes in membrane potential which suggests that further tests will reveal two or more different receptor/channel combinations underlying the response to this compound.

202.2 [^{3}H]MUSCIMOL BINDING TO GABA RECEPTORS IN INSECT CNS. G.G. Lunt*, T.N. Robinson*, Bath University, United Kingdom, T.A. Miller*, University of California, Riverside, W.P. Knowles*, and R.W. Olsen, UCLA School of Medicine and Brain Research Institute, Los Angeles, CA 90024.

Specific binding of the γ-aminobutyric acid (GABA) agonist [^{3}H]muscimol was detected in membrane homogenates from housefly heads and supraesophageal ganglion of locust Shistocerca gregaria. Frozen tissues were homogenized at 0°C in 0.25 M sucrose, filtered, and centrifuged for 10 min at 1000 X g. The pellet was discarded and a crude membrane fraction collected by pelleting for 60 min at 100,000 X g. This particulate fraction was washed twice in water and twice in buffer (0.15 M KCl, 10 mM Tris-HCl, 1 mM EGTA, pH 8.0, no sodium). Binding of [^{3}H]muscimol (19 Ci/mmol, Amersham) was assayed by centrifugation following 30 min incubations at 0°C with 2-4 mg protein/ml. Similar displacement by nonradioactive muscimol and GABA was observed (10-30% but occasionally zero) with IC50 values of 33 nM and 42 nM respectively in housefly. [^{3}H]Muscimol binding was also displaced by micromolar concentrations of GABA receptor-specific analogs 3-aminoprane sulfonate, isoguvacine, and imidazole acetic acid, weakly by bicuculline methiodide (500 μM) and not by uptake-specific analogs nipecotic acid and 2,4-diaminobutyrate (1 mM). There was no effect of pentobarbital (1 mM) in housefly. Binding was proportional to protein concentration, rapid, and reversible, with association complete in under 6 min, and dissociation 63% in 1 minute. Addition of sodium to the assays increased binding to a lower affinity, slowly associating component. Sodium-independant binding was saturable, with Scatchard plots indicating one component: Kd=30 nM, Bmax=30±10 fmol/mg protein (housefly); Kd=10 nM, Bmax=60±10 fmol/mg (locust). This binding appears to label specific GABA receptor sites in insect CNS similar to those we have described in crayfish muscle (Meiners, Kehoe, Shaner, and Olsen, J. Neurochem. 32, 979-990, 1979). GABA receptor binding assays ought to allow biochemical and pharmacological characterization of the function of this neurotransmitter in insects.

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202.3 MAPPING GABA-LIKE IMMUNOREACTIVITY IN ANTENNAL LOBES OF THE MOTH *MANDUCA SEXTA*. S.G. Hoskins, T.G. Kingan, T.A. Christensen, and J.G. Hildebrand. Dept. of Biol. Sci., Columbia University, New York, NY 10027.

The axons of olfactory neurons in the antennae of adult *Manduca sexta* project to the antennal lobes (ALs) of the brain where they synapse upon AL neurons. Previous studies suggested that some of these CNS neurons probably contain GABA [Maxwell et al. *Comp. Biochem. Physiol. 61C*:109, 1978; Kingan & Hildebrand *Soc. Neurosci. Abstr. 8*:988, 1982] and that some AL neurons interact through inhibitory synapses [Harrow & Hildebrand *Soc. Neurosci. Abstr. 8*:528, 1982], possibly using GABA as transmitter. To locate putatively GABAergic neurons, we have begun to study the cellular distribution of GABA-like immunoreactivity in the ALs of mature and metamorphosing, as well as chronically deantennated, moths.

Antisera were raised in rabbits immunized with GABA-BSA conjugates according to the methods of Storm-Mathisen et al. [*Nature 301*:517, 1983]. Antiserum specificity was enhanced by affinity chromatography, after which immunohistochemical staining could be blocked by preadsorption with BSA conjugates of GABA but not those of glutamate, glutamine, or β-alanine. The antisera stain known GABAergic neurons in lobster abdominal ganglia and Purkinje cells in mouse cerebellum.

The neuropil of each AL contains an array of knot-like glomeruli (the sites of all synapses in the AL) and is bordered by 3 groups of neuronal somata. A majority of neurons in the lateral group and a few in the medial group exhibit GABA-like immunoreactivity; cells in the anterior group are not immunoreactive. The antiserum stains most or all "ordinary" glomeruli as well as the male-specific macroglomerular complex. ALs have several types of local and output neurons [Matsumoto & Hildebrand *Proc. Roy. Soc. Lond. B213*:249, 1981]. We are testing for GABA-like immunoreactivity in these various cell types by intracellular physiological characterization and staining of single AL neurons with Lucifer Yellow followed by fixation, sectioning, and staining with GABA-BSA antiserum and then a rhodamine-labeled secondary antibody.

GABA-like immunoreactivity is found in the neuropil of developing ALs (during metamorphosis) by stage 3 (of 18 stages), prior to arrival of antennal axons and the formation of glomeruli and synapses, and also in somata and neurites in the stunted ALs that develop in chronically deantennated animals. These findings suggest that the neurochemical differentiation of some AL elements normally begins before sensory fibers enter the AL and can continue in the absence of those inputs. (Supported by NSF grant BNS 83-12769 and a contract from ARO.)

202.4 DISTRIBUTION OF PROCTOLIN-LIKE AND FMRFAMIDE-LIKE IMMUNOREACTIVITY IN THE STOMATOGASTRIC SYSTEM OF DECAPOD CRUSTACEA. E. Marder, S. L. Hooper, & K. K. Siwicki. Biol., Brandeis Univ., Waltham, MA 02254 & Neurobiol., Harvard Med. Sch., Boston, MA 02115.

The distribution of proctolin and FMRFamide-like immunoreactivities were studied in the stomatogastric systems of three decapods, Cancer irroratus, Homarus americanus and Panulirus interruptus. Stomatogastric ganglia (STG), oesophageal ganglia (OG), commissural ganglia (CG) and connecting nerves were fixed and stained as whole-mount preparations (Beltz & Kravitz, J. Neurosci, 1983). Anti-proctolin antiserum (Kravitz lab) intensely stained the neuropil of the STG but none of the STG somata in all three species. In Cancer and Homarus, stained fibers were seen in the dorsal ventricular nerve (DVN). Stained fibers were seen in the stomatogastric nerves (STNs). In Cancer, a pronounced neuropil was seen at the junction of the STN and the Superior Oesaphageal Nerves (SONs) and three stained somata were seen in the OG. In all three species the CG showed an intensely staining neuropil and several staining somata. When analyzed by reverse-phase liquid chromatography followed by radioimmunoassay of the fractions, a peptide comigrating with authentic proctolin was detected in extracts of stomatogastric systems from each of the three species. Studies on the distribution of FRMFamide-like staining were done in Cancer and Panulirus. Three different anti-FRMFamide antisera (CRB, M. Greenberg, & Watson & O'Donahue) gave essentially the same pattern of staining. In both species, the neuropil of the STG was brightly stained, but none of the STG somata was stained. In Cancer, 3-4 somata were seen in the OG, and an extensive neuropil was seen at the junction of the STN and SONs. In both species somata and a large area of neuropil were stained in the CGs. The proctolin-like staining was blocked by preabsorption with proctolin but not with FMRFamide, and the FMRFamide-like staining was blocked by preabsorption with FMRFamide but not with proctolin. Simultaneous incubation with both anti-proctolin and anti-FMRFamide antibodies stain 6-7 neurons in the OG. Therefore FMRFamide-like staining and the proctolin-like staining appear to define different peptides. These data and physiological experiments (Hooper & Marder, this meeting) suggest that proctolin and a FMRFamide-like peptide are neurotransmitters in the stomatogastric system. Research supported by grants NS-17813 (E.M.) and NS-07848 (E.A. Kravitz).